BEFORE THE HON'BLE NATIONAL GREEN TRIBUNAL
WESTERN ZONE BENCH, PUNE

ORIGINAL APPLICATION NO. 62/2025 (WZ)
EARLIER ORIGINAL APPLICATION NO. 323/2024 (PB)

IN THE MATTER OF: -

NEWS ITEM TITLED "NO PERMISSION PROVIDED FOR USE OF CHEMICALS IN CLEANING WATER BODIES: MPCB" APPEARING IN THE HINDUSTAN TIMES DATED 23.02.2024.

INDEX

8.	**Annexure-VII**: A copy of the CPCB Office Order dated 05/01/2026 constituted to examine the findings of the report submitted by CSIR-IITR	
9.	**Annexure-VIII**: A copy of the CPCB letter dated 22/01/2026 addressed to PMC for seeking the requisite information about the Draynzyme	
10.	**Annexure-IX**: A copy of the information submitted by manufacturer vide email dated 28/01/2026	
11.	**Annexure-X**: A copy of the comments of the subject experts on the Use of "Draynzyme" in Natural Water Bodies	

Date: 20/03/2026
Place: Pune

(Pratik D. Bharne)
Regional Director

क्षेत्रीय निदेशक / Regional Director
केंद्रीय प्रदूषण नियंत्रण बोर्ड
Central Pollution Control Board
क्षेत्रीय निदेशालय, पुणे/Regional Directorate, Pune
पर्यावरण, वन एवं जलवायु परिवर्तन मंत्रालय, भारत सरकार
M/o Envt. Forest & Climate Change, Govt. of India
सर्वे नं. ११०, हीराबाई धनकुडे हॉल, बाणेर रोड, बाणेर, पुणे - 411045
Sr. No. 110, Hirabai Dhankude Hall, Baner Road, Baner, Pune-411045

REPORT OF CPCB IN THE HON'BLE NGT MATTER OF OA. NO. 62/2025 (WZ) IN EARLIER OA. NO. 323/2024 (PB), SUO MOTO MATTER IN RE: NEWS ITEM APPEARING IN THE HINDUSTAN TIMES DATED 23/02/2024 TITLED "NO PERMISSION PROVIDED FOR USE OF CHEMICALS IN CLEANING WATER BODIES: MPCB"

1. BACKGROUND

In OA. No. 323/2024 (PB), Suo Moto matter in "News item titled "No permission provided for use of chemicals in cleaning water bodies: MPCB'' appearing in The Hindustan Times dated 23/02/2024", Hon'ble NGT vide order dated 05/04/2024 directed as follows:

"1. This original application is registered Suo Motu on the basis of the news item titled "No permission provided for use of chemicals in cleaning water bodies: MPCB" appearing in 'The Hindustan Times' dated 23/02/2024.

2. The news item relates to the decision of the Pune Municipal Corporation (PMC) ***to spray bio-enzyme*** *or natural chemicals* ***on rivers and lakes to deal with the problem of water pollution and water hyacinth****. As per the news item, PMC came up with the idea of spraying bio enzymes or natural chemicals on rivers and lakes after the* ***mosquito tornado*** *video went viral on social media. The news item reveals that the PMC has sprayed a bio-enzyme on Ramnadi river of Bavdhan. It also reveals that subsequently* ***the civic body has started using drones to spray insecticide on Mula Mutha river stretch along*** *Keshav Nagar, Kharadi and Mundhwa. The news item also contains the* ***opinion of one of the Professors*** *and Director of Centre for Sustainable Development, Gokhale Institute of Politics and Economics* ***that the PMC's use of enzymes and other additives on Pune's water bodies is like experimenting with the natural ecosystem without scientific proof and that long term results of such use are not known.*** *The report also states that though the PMC claims that these enzymes are natural but are they naturally occurring in the rivers, and if they are* ***foreign matter in the aquatic ecosystem then their use must go through scientific experimentation overtime.***

*5. On advanced notice, report on behalf of the Maharashtra Pollution Control Board (MPCB) has been filed disclosing that the **use of Glyphosate has been restricted**, as it involves health hazard and risk to human beings and that the MPCB has already issued letter dated 30/06/2023 to the PMC.*

*6. A report on behalf of the PMC has also been filed stating that the **PMC has sprayed Draynzyme on water hyacinth on trial basis on an area of 40 sq. feet stagnant water pool along Ramnadi.***

*7. Learned Counsel for the PMC has submitted that **no such experiment will be done in future till the appropriate competent body gives a clearance** in this regard.*

*8. We are of the view that the permissibility of **the spray of Draynzyme on water hyacinth needs to be first examined by the Central Pollution Control Board (CPCB) in coordination with Indian Institute of Toxicology Research (IITR), Lucknow and if it is found that it has no adverse effect on the ecology of the water body, then its use can be permitted in accordance with law.***

*9. Hence, we dispose of the OA directing the **CPCB to do the needful to ascertain the feasibility of use of Draynzyme on water hyacinth/natural water bodies** and submit a report before the Registrar of the Western Zonal Bench of the Tribunal within four months. If found necessary the matter will be listed for consideration before the Western Zonal Bench, Pune. Let the original record of this OA be transferred to the Western Zonal Bench, Pune."*

10. A copy of this order be forwarded to CPCB by e-mail for compliance."

Copy of Hon'ble NGT order dated 05/04/2024 is enclosed as **Annexure-I**.

2. ACTION TAKEN BY CPCB

For ensuring compliance to the Hon'ble NGT directions vide Order date 05/04/2024, **a meeting** was convened with the officials of Pune Municipal Corporation (PMC), Regional Directorate (RD)-Pune, CPCB and Indian Institute of Toxicology Research (IITR), Lucknow (formerly Indian Toxicological Research Centre - ITRC) on

17/05/2024 through video conferencing. It was decided that Toxicity of Draynzyme will be tested by CSIR-IITR, Lucknow which will take around four months to complete. CSIR-IITR, Lucknow will conduct a long-term study that will last approximately seven months, in case Draynzyme shows any potential toxicological impacts during the short-term study and PMC shall provide information about the test item to CSIR-IITR, Lucknow and shall bear all the expenses of the studies to be conducted. (Minutes of the 1st meeting attached enclosed as **Annexure-II**)

In pursuance of the decisions taken, PMC, vide letter dated 13/06/2024, submitted the product sample to CSIR-IITR.

In order to assess the current status of action taken in line with 1st Minutes of the meeting, **2nd meeting** was held on **13/08/2024** through video conferencing with the members PMC, (RD)-Pune, CPCB and IITR, Lucknow. It was reiterated by CSIR-IITR that four tests are required to assess Draynzyme toxicity, to be completed in about four months, a long-term ecosystem impact study will be conducted if any toxic effects are observed during the short-term study and that the sample of Draynzyme has not yet been received from PMC. (Minutes of the 2nd meeting enclosed as **Annexure-III**)

Test report of four test of the Draynzyme were received by CPCB on 30/04/2025 through email. However, the reports submitted by IITR were not conclusive.

In view of above, **3rd meeting** was held on **13/06/2025** through video conferencing with the members PMC, RD-Pune, CPCB and IITR, Lucknow to conclude the findings made by IITR, Lucknow. It was decided that PMC will provide the required information about proposed dose of the substance to CSIR-IITR and CSIR-IITR will provide information on actual tested maximum concentration, interpretation of the test results, safety factors and safe doses in the light of this test results. Further PMC will also obtain information from the supplier regarding the product Draynzyme. Minutes of the 3rd meeting are enclosed as **Annexure-IV**

A reminder email dated 30/10/2025 was subsequently issued to PMC and CSIR-IITR.

In response, PMC, vide e-mail dated 06/11/2025 submitted the details of the product Draynzyme and CSIR-IITR vide e-mail dated 07/11/2025 submitted information on actual tested maximum concentration and interpretation of test results. Information submitted by PMC and IITR are enclosed as **Annexure-V and Annexure-VI.**

Subsequently to examine the information provided by PMC and CSIR-IIT, Central Pollution Control Board (CPCB) vide Office Order dated 05/01/2026 constituted a Committee with members from CSIR-NEERI Delhi Zonal Centre; Department of Biotechnology (DBT), Ministry of Science and Technology; CSIR-Indian Institute of Toxicology Research, Lucknow and DH-Water and Bio Labs, CPCB. The Terms of Reference (ToR) of the Committee are given below: -

‘’To examine the findings of the report submitted by CSIR-IITR and –

i. to ascertain the feasibility of use of bio-enzymes in water bodies to deal with the problem of water pollution and water hyacinth and make necessary recommendations and,

ii. to ascertain no adverse effects on the ecology of the water body in case of the use of bio-enzymes in water bodies to deal with the problem of water pollution and water hyacinth and make necessary recommendations.’’

Copy of Office Order attached as **Annexure-VII**.

1st Meeting of the Committee was held on 17/01/2026 at 11:00 AM with the committee members through Video Conferencing. Accordingly, the Committee recommended that the following information about the product Draynzyme be obtained through the Pune Municipal Corporation (PMC) for further examination:

i. Comprehensive chemical composition and formulation details and characterization of the product Draynzyme, including the nature, source, and concentration of its constituent components.

ii. Relevant scientific documentation, including published literature, peer-reviewed research articles, experimental studies, patent details, and validation reports conducted or sponsored by the manufacturer pertaining to Draynzyme.

iii. Scientific studies and safety assessments, if available, addressing aspects such as bio magnification potential, impacts on microbial ecology, and outcomes of chronic toxicity evaluations.

CPCB vide letter dated 22/01/2026 sought the requisite information as desired by the Committee from PMC. The manufacturer directly communicated the information to CPCB vide E-mail dated 28/01/2026. Copy of CPCB letter and information submitted by the manufacturer is attached as **Annexure-VIII and Annexure-IX.**

Subsequently, 2nd Meeting of the Committee was held on 02/02/2026 at 03:30 PM through Hybrid Mode. The Committee concluded the following:

i. As per information provided by PMC, the product - “Draynzyme”, is an enzyme based formulation comprises consortium of enzymes namely Urease, Exogenous Polysaccharide, Ammonia Monooxygenase, Acid Phosphatase, Lipases catalyze, Proteases, Oxidoreductases and supporting media components of Hydrated Silica etc.

ii. Product appears to be useful for sewerage cleaning.

iii. IITR assessed the product - “Draynzyme” and did not observe any adverse impacts in acute toxicity assessment.

iv. Bio-enzymes concentration and doses with the limits indicated for this product are in general considered safe in nature and may be considered for in-situ sewerage sludge cleaning process.

v. Committee also suggested that the report shared with the Committee may also be reviewed by the subject experts before making the final recommendations for use of this product in wastewater treatment process.

Further, the Committee, CPCB suggested to seek comments from three subject experts from TERI, Delhi University and NEERI. Accordingly, the comments were obtained from three subject experts namely Smt. Atya Kapley (Professor of Practice, Raisoni Group of Institutes, Nagpur; Former Chief Scientist, CSIR-NEERI), Sh.

Banwari Lal (Former Senior Director, TERI, New Delhi) and Sh. Rup Lal (INSA Senior Scientist).

As per the comments of the subject experts,

- The IITR studies primarily focus on acute toxicity endpoints and, while useful as an initial screening, long-term studies are also required to assess ecological risk assessment in complex natural water bodies. Independent biochemical characterisation, enzyme activity validation, and batch-to-batch consistency analysis are required before environmental application can be considered.

- The experts strongly object to the large-scale application of Draynzyme in natural ecosystems for wastewater treatment or water hyacinth, river remediation, control until comprehensive scientific validation is conducted, as large-scale environmental application without proper biochemical characterization and ecological safety assessment poses potential risks to natural ecosystem.

Copy of comments attached as **Annexure-X.**

3. CONCLUSION

- The acute toxicity assessment carried out by IITR did not indicate any adverse impacts on aquatic life and the assessment primarily focused on acute toxicity endpoints. Acute toxicity assessment while useful as an initial screening, long term studies are also required to assess ecological risk. Therefore, application of Dranzyme in natural ecosystems for remediation of rivers/ rivulets is not recommended.

- Based on the case study provided by manufacturer, the product “Draynzyme” has been used for sewerage cleaning. Therefore, such product may be considered only for in-situ bioremediation in wastewater carrying drains with low flow. However, it would necessitate periodic removal of bio-sludge generated from the drains, to avoid addition of pollution load on the receiving water body by transporting the sludge generated, and also periodic testing of

water samples from the drain u/s of addition of such products and d/s of bioremediation zone of the drain, to check effectiveness of the bioremediation and solids/sludge flux.

*****

Annexure-I

Item No. 02 Court No. 1

BEFORE THE NATIONAL GREEN TRIBUNAL
PRINCIPAL BENCH, NEW DELHI

Original Application No. 323/2024

News item titled "No permission provided for use of chemicals in cleaning water bodies: MPCB" appearing in The Hindustan Times dated 23.02.2024.

Date of hearing: 05.04.2024

CORAM: HON'BLE MR. JUSTICE PRAKASH SHRIVASTAVA, CHAIRPERSON
HON'BLE DR. A. SENTHIL VEL, EXPERT MEMBER

Respondent(s): Mr. Mukesh Verma, Adv. for MPCB
Mr. Rahul Garg, Adv. for PMC (Through VC)
Ms. Tanisha Samanta & Mr. Balendu Shekhar, Advs. for CPCB (Through VC)

ORDER

1. This original application is registered *suo motu* on the basis of the news item titled "No permission provided for use of chemicals in cleaning water bodies: MPCB" appearing in 'The Hindustan Times' dated 23.02.2024.

2. The news item relates to the decision of the Pune Municipal Corporation (PMC) to spray bio-enzyme or natural chemicals on rivers and lakes to deal with the problem of water pollution and water hyacinth. As per the news item, PMC came up with the idea of spraying bio enzymes or natural chemicals on rivers and lakes after the mosquito tornado video went viral on social media. The news item reveals that the PMC has sprayed a bio-enzyme on Ramnadi river of Bavdhan. It also reveals that subsequently the civic body has started using drones to spray insecticide on Mula Mutha river stretch along Keshav Nagar, Kharadi and Mundhwa. The news item also contains the opinion of one of the Professors and Director of Centre for Sustainable Development,

Gokhale Institute of Politics and Economics that the PMC's use of enzymes and other additives on Pune's water bodies is like experimenting with the natural ecosystem without scientific proof and that long term results of such use are not known. The report also states that though the PMC claims that these enzymes are natural but are they naturally occurring in the rivers, and if they are foreign matter in the aquatic ecosystem then their use must go through scientific experimentation overtime.

3. The news item raises substantial issue relating to compliance of the environmental norms and implementation of the provisions of Scheduled enactments.

4. Power of the Tribunal to take up the matter *suo-motu* has been recognized by the Hon'ble Supreme Court in the matter of *"Municipal Corporation of Greater Mumbai vs. Ankita Sinha & Ors." reported in 2021 SCC Online SC 897.*

5. On advanced notice, report on behalf of the Maharashtra Pollution Control Board (MPCB) has been filed disclosing that the use of Glyphosate has been restricted, as it involves health hazard and risk to human beings and that the MPCB has already issued letter dated 30.06.2023 to the PMC.

6. A report on behalf of the PMC has also been filed stating that the PMC has sprayed Draynzyme on water hyacinth on trial basis on an area of 40 sq. feet stagnant water pool along Ramnadi.

7. Learned Counsel for the PMC has submitted that no such experiment will be done in future till the appropriate competent body gives a clearance in this regard.

8. We are of the view that the permissibility of the spray of Draynzyme on water hyacinth needs to be first examined by the Central Pollution Control Board (CPCB) in coordination with ITRC, Lucknow and if it is found that it has no adverse effect on the ecology of the water body, then its use can be permitted in accordance with law.

9. Hence, we dispose of the OA directing the CPCB to do the needful to ascertain the feasibility of use of Draynzyme on water hyacinth/natural water bodies and submit a report before the Registrar of the Western Zonal Bench of the Tribunal within four months. If found necessary the matter will be listed for consideration before the Western Zonal Bench, Pune. Let the original record of this OA be transferred to the Western Zonal Bench, Pune.

10. A copy of this order be forwarded to CPCB by e-mail for compliance.

Prakash Shrivastava, CP

Dr. A. Senthil Vel, EM

April 05, 2024
Original Application No. 323/2024
DV

Annexure-II

Central Pollution Control Board

(Ministry of Environment, Forest & Climate Change)

Parivesh Bhawan, East Arjun Nagar,

Delhi – 110032

Minutes of meeting

Introduction

A meeting was held on 17-05-2024 at 4:00 PM through video conferencing with representatives of Pune Municipal Corporation (PMC), CPCB-Regional Directorate (RD)-Pune, CSIR-Indian Institute of Toxicology Research (IITR), Lucknow and CPCB, Head Office for discussion in compliance to direction of Hon'ble NGT in OA No. 323 of 2024, Suo Moto matter In re News item appearing in The Hindustan Times dated 23-02-2024 Titled "No permission provided for use of chemicals in cleaning water bodies: MPCB". List of participants is given at **Annexure-I.**

Sh. R.P. Gurung, Sc- 'E', WQM-I welcomed all the participants and outlined a brief background and purpose of the meeting that Hon'ble NGT took *suo moto* cognizance of the matter via news item titled 'No permission provided for use of chemicals in cleaning water bodies: MPCB' published in 'The Hindustan Times' on 23-02-2024.

Discussions

Sh. Mangesh Dighe, Environment Officer, Pune Municipal Corporation made a presentation on the product 'Draynzyme' used by PMC. It was appraised that bio-enzyme that is applied on water bodies like rivers and lakes to deal with the problem of water pollution and water hyacinth. He further informed that the product 'Draynzyme' has been used for other purposes in the past such as de-clogging drain pipes in restaurants, hotels and STPs. Draynzyme is an 'enzyme' based product in semi liquid form and has been used for the first time on a small puddle near Ramnadi river as a pilot study. He further clarified that no prior permission was obtained from the Maharashtra Pollution Control Board for this pilot study. It was also stated that Draynzyme does not

contain any living organism such as bacteria, rather helps in the growth of bacteria that digest sludge. It has been tested by NABL accredited laboratory for water quality parameters before and after the application of Draynzyme.

In the subsequent discussion, it was decided that toxicity of Draynzyme will be tested by CSIR-IITR, Lucknow which will take around four months to complete. PMC shall provide information about the test item to CSIR-IITR, Lucknow as per the format provided by them as well as the sample of the product.

CSIR-IITR, Lucknow will conduct a long-term study that will last approximately seven months, including testing of water samples to assess the impact on ecosystem in case Draynzyme shows any potential toxicological impacts during the short-term study.

It was also agreed that PMC shall bear all the expenses of the studies to be conducted by CSIR-IITR, Lucknow.

The Meeting ended with thanks to and from the Chair.

-- OO --

Annexure I

List of Participants of the meeting held on 17-05-2024

1. Sh. Shashikant Lokhande, Scientist ‘E’, RD-Pune, CPCB
2. Sh. Vishal Gandhi, Scientist ‘E’, WQM-I, CPCB
3. Sh. R. P. Gurung, Scientist-‘E’,WQM-I, CPCB
4. Dr. Akshay Dwarakanath, Senior Principal Scientist – Regulatory Toxicology, CSIR – Indian Institute of Toxicology Research, Lucknow
5. Sh. Mangesh Dighe, Environment Officer, Pune Municipal Corporation
6. Sh. Partha Pratim Maity, Scientist-‘B’, WQM-I, CPCB
7. Sh. Mahesh Patwardhan, Director, Quinquent India Pvt. Ltd, Pune
8. Sh. Biraj Kukodiya, Technical Director, Dhara Biotech, Gujarat

Annexure-III

Central Pollution Control Board

(Ministry of Environment, Forest & Climate Change)

Parivesh Bhawan, East Arjun Nagar

Delhi-110032

Minutes of meeting

Introduction

A meeting was held on 13-08-2024 at 4:00 PM through video conferencing with representatives of Pune Municipal Corporation (PMC), CPCB-Regional Directorate (RD)-Pune, CSIR-Indian Institute of Toxicology Research (IITR), Lucknow and CPCB, Head Office for discussion in compliance to direction of Hon'ble NGT in OA No. 323 of 2024, Suo Moto matter In re News item appearing in The Hindustan Times dated 23-02-2024 Titled "No permission provided for use of chemicals in cleaning water bodies: MPCB". List of participants is given at **Annexure-I.**

Mr. Partha Pratim Maity, Scientist 'B', WQM-I, welcomed all the participants and provided a brief background mentioning the last meeting on 17-05-2024. He then asked the PMC about the actions taken in accordance with the previous Minutes of Meeting.

Discussions

Mr. Mangesh Dighe, Environment Officer at the Pune Municipal Corporation, enquired about the tests required to test the toxicity of Draynzyme. Dr. Akshay Dwarakanath of CSIR-IITR, Lucknow stated that four tests are required to assess the toxicity of Draynzyme, which was also mentioned at the previous meeting which was held on 17-05-2024. The details of the all four test which are based on short term - study are mentioned below.

1. Freshwater Alga and Cyanobacteria, Growth Inhibition Test
2. Daphnia sp. Acute Immobilisation test
3. Earthworm, Acute Toxicity Test
4. Acute fish embryo toxicity test

He further mentioned that testing of toxicity of Draynzyme will take around four months to complete. CSIR-IITR, Lucknow will conduct a long-term study that will last approximately seven months, including

testing of water samples to assess the impact on ecosystem in case Draynzyme shows any potential toxicological impacts during the short-term study.

CSIR-IITR, Lucknow has also confirmed that they have not yet received the sample of Draynzyme from PMC and that the cost of all four test is Rs. 8,00,000+GST.

Further, it was decided that PMC shall submit confirmation regarding cost bearing of analysis samples of Draynzyme within a period of weeks time. Sh. S. Lokhande, Scientist E, RD, Pune, CPCB mentioned that there has been already considerable delay in conducting the test. Therefore, PMC should confirm regarding bearing the cost of the test without any further delay.

The Meeting ended with thanks to and from the Chair.

--OO--

Annexure I

List of Participants of the meeting held on 13-08-2024

1. Sh. Shashikant Lokhande, Scientist 'E', RD-Pune, CPCB
2. Sh. Vishal Gandhi, Scientist 'E', WQM-I, CPCB
3. Sh. R. P. Gurung, Scientist-'E', WQM-I, CPCB
4. Dr. Akshay Dwarakanath, Senior Principal Scientist – Regulatory Toxicology, CSIR – Indian Institute of Toxicology Research, Lucknow
5. Sh. Mangesh Dighe, Environment Officer, Pune Municipal Corporation
6. Sh. Partha Pratim Maity, Scientist-'B', WQM-I, CPCB
7. Sh. Mahesh Patwardhan, Director, Quinquent India Pvt. Ltd, Pune
8. Sh. Biraj Kukodiya, Technical Director, Dhara Biotech, Gujarat

Annexure-IV

Central Pollution Control Board

(Ministry of Environment, Forest & Climate Change)

Parivesh Bhawan, East Arjun Nagar,

Delhi–110032

Minutes of meeting

Third meeting in the matter O.A. No. 323 of 2024, Suo Moto matter In re News item appearing in The Hindustan Times dated 23-02-2024 Titled "No permission provided for use of chemicals in cleaning water bodies: MPCB", was held on 13-06-2025 at 4:00 PM through video conferencing with representatives of Pune Municipal Corporation (PMC), CPCB-Regional Directorate (RD)-Pune, CSIR-Indian Institute of Toxicology Research (IITR), Lucknow and CPCB, Head Office to discuss the test results of Draynzyme conducted by CSIR-IITR, Lucknow. List of participants is given at **Annexure-I.**

Sh. Vishal Gandhi, Sc- 'E', WQM-I welcomed all the participants and outlined a brief background and purpose of the meeting and requested CSIR-IITR, Lucknow to make a presentation on work carried out as per directions of Hon'ble NGT.

Sh. Anbumani Sadasivam, Principal Scientist, CSIR-IITR, Lucknow made a presentation on the test results of 'Draynzyme'. CSIR-IITR said that information about proposed dose of this substrate has not been provided to them.

It was decided that PMC will provide the required information about proposed dose of the substance to CSIR-IITR and CSIR-IITR will provide information on actual tested maximum concentration, interpretation of the test results, safety factors and safe doses in the light of this test results. Further PMC will also obtain information from the supplier regarding the product Draynzyme.

The Meeting ended with thanks to and from the Chair.

--OO--

Annexure-I

List of Participants of the meeting held on 17-05-2024

1. Nazimuddin, Sc. F & DH, WQM-I, CPCB
2. Sh. Pratik D. Bharne, Sc. E & Incharge RD Pune, CPCB
3. Sh.Shashikant Lokhande, Scientist'E', RD Pune, CPCB
4. Sh.Vishal Gandhi, Scientist'E', WQM-I,CPCB,
5. Dr. Akshay Dwarakanath, Senior Principal Scientist–Regulatory Toxicology, CSIR–Indian Institute of Toxicology Research, Lucknow,
6. Dr. Anbumani Sadasivam, Principal Scientist, CSIR-IITR, Lucknow
7. Dr. Partha Pratim Maity, Scientist-'B',WQM-I,CPCB,
8. Sh.Mangesh Dighe, Environment Officer, Pune Municipal Corporation

QuinQuent INDUSTRIES PVT LTD
A Complete Biological Degradation Technology
Regd Office : S No 131/2A Rajyog Colony Warje Pune - 411052 Maharashtra INDIA

IN-SITU BIOREMEDIATION TREATMENT OF EUTROPHIC WETLAND

Developer brief introduction:
QuinQuent Industries Pvt Ltd, in collaboration with its esteemed partner DHARA BIOTECH, has pioneered a comprehensive array of innovative biological treatment solutions for water, wastewater, and solid waste management.
All technologies and solutions developed are proudly 100% Made in India. Collectively, these advancements represent a significant breakthrough in the treatment and conservation of invaluable resources such as water, land, and air.
"""

The Causes of Wetland Pollution:
Numerous continuous inlets of contaminated water infiltrate a wetland ecosystem. A wetland, located within a densely populated urban catchment area, typically functions as a confluence for stormwater runoff. Due to the relentless influx of polluted water, it becomes saturated with seepage and the discharge of untreated sewage and industrial effluents. These pollutants subsequently induce eutrophication, resulting in significant imbalances within the ecosystem.

Classis Polluted Lake Bellandur at Bengaluru India, with heavy foam & also fire on water.

Water Hyacinth (Eichornia crassipes) covering the water surface.

Algal blooms precipitate the eutrophication of aquatic ecosystems.

The Approach:

Contaminated aquifers and wetlands necessitate distinct approaches and methodologies for treatment. The principle underlying such contaminated aquifer remediation is referred to as bioremediation. Bioremediation constitutes an in-situ treatment employed to purify wastewater-laden wetlands. This aquatic ecosystem is inundated with several million liters of contaminated water, which possesses its own complex supra-system, replete with diverse flora and fauna. The aquatic community is acclimated to diminished levels of dissolved oxygen (DO), elevated oxygen demands due to Total Organic Carbon (TOC), malodorous conditions, excessive silting, the proliferation of water hyacinth, algal blooms, and numerous other related challenges.

Environmental Biotechnology & Bioremediation
Environmental biotechnology isn't new—composting and classic wastewater treatments have harnessed microbes for centuries. Yet advances in molecular biology and microbial ecology now let us tailor biological processes for far greater speed and specificity. One standout success is the targeted cleanup of polluted soils and waters using naturally occurring—or carefully selected—organisms.

Defining Bioremediation
Bioremediation employs living organisms (microbes, plants) or their derivatives (enzymes, bio-products) to transform environmental contaminants into harmless or regulatory-safe forms. These organisms may already inhabit the polluted site or be imported in a process called **bioaugmentation**. Under controlled conditions, their metabolic pathways enzymatically attack organic wastes, heavy metals, or other toxins, converting them to CO_2, water, biomass, or precipitated minerals.

Applying Bioremediation to Sewage-Contaminated Waters
Modern sewage adds organics, nutrients (N, P), pathogens, and sometimes heavy metals into lakes, rivers, and wetlands. Bioremediation strategies fall into two camps:

In situ
- Aeration or nutrient dosing to stimulate indigenous degraders
- Bioaugmentation with specialized strains that thrive only on target pollutants

Ex situ
- Pumping effluent through engineered reactors or **constructed wetlands**
- Optimizing oxygen, temperature, and retention time for maximal microbe activity

Core Mechanisms

1. **Microbial Degradation**
   Organic load (e.g., fecal organics, oils, detergents) becomes microbial "food," reducing BOD as cells oxidize compounds to CO_2, H_2O and new biomass.
2. **Biosorption & Bioaccumulation**
   Cell walls or intracellular processes bind and concentrate metals like Pb, Cd or Cr, simplifying downstream recovery or stabilization.
3. **Enzymatic Transformation**
   In wetland-based systems, specialized enzymes perform frontline detoxification:

   - Laccases (EC 1.10.3.2), secreted by white-rot fungi and select bacterial species, catalyze the oxidation of phenolic dyes and pesticides into less soluble, polymeric forms that subsequently precipitate.
   - Peroxidases – lignin peroxidase (EC 1.11.1.14) and manganese peroxidase (EC 1.11.1.13) utilize hydrogen peroxide (H_2O_2) to decompose polycyclic aromatic hydrocarbons (PAHs) or azo dyes into smaller molecular constituents.
   - Monooxygenases – Toluene-4-monooxygenase (EC 1.14.13.9) derived from Pseudomonas species catalyzes the incorporation of molecular oxygen into phenol or toluene, thereby rendering them amenable to subsequent ring-cleavage reactions.
   - Dioxygenases – Naphthalene dioxygenase (EC 1.14.12.12) catalyzes the incorporation of two molecular oxygen atoms to cleave aromatic rings, such as naphthalene and anthracene, resulting in the formation of cis-diols that subsequently integrate into central metabolic pathways.
   - Microbial Phosphatases – Alkaline phosphatase (EC 3.1.3.1) catalyzes the liberation of inorganic phosphate from organophosphate residues, thereby mitigating the risks of eutrophication and supplying essential phosphorus to wetland flora.

Integration of Drynzyme into Wetland Bioremediation

Bioremediation thrives only if microbes can grow and remain active. Engineers routinely adjust pH, temperature, dissolved oxygen, and retention times—and in constructed wetlands, select plant–microbe partnerships—to accelerate degradation and ensure pollutant levels fall below regulatory limits. This integrated approach turns sewage-laden waters into self-purifying ecosystems, marrying time-tested practices with cutting-edge molecular insights.

As per the Wetlands (Conservation & Management) Rules 2017 under the Environmental Protection Act 1986 and its implementation guidelines:

- Introduction of invasive species—microbial cultures or living plants—is prohibited.
- Use of hazardous or genetically engineered microorganisms is banned (Rule 1989).
- Dumping pesticides, chemical weedicides or other hazardous substances in wetlands is forbidden.
- A mix of mechanical and biological methods must control species invasion.

Hence, wetland conservation and remediation must avoid live non-native microbes, pesticides, herbicides and similar substances. Our methodologies fully comply with these rules.

Aquifer & Wetland Water Treatment Modes

a) In-Situ Treatment
Direct dosing of harmless chemicals, enzymes, natural reagents or bio-cultures into the water body—no pumping required.

b) Ex-Situ Treatment

Wastewater is piped to a treatment plant (sump), dosed with requisite agents, and processed to remove impurities.

Introducing Dryzyme™ for Compliant Enzyme-Only Treatment

Product: Dryzyme™ (enzyme pack for sewage treatment)

- Contains pre-formulated waste-digesting enzymes—no live bacteria or fungi
- Formaldehyde-fumigated to denature all DNA/RNA, ensuring zero viable organisms
- Acts as a biological catalyst, accelerating native microbial breakdown of organics
- Fully compliant: no invasive species, no GMOs, no hazardous chemicals

Recommended Dosage

- **Typical**: 5–6 ppm
- **Maximum**: 10 ppm

(1 ppm = 1 mg enzyme per liter of water)

Treatment Capacity per 1 kg of Dryzyme

Dosage (ppm)	Enzyme Required (mg/L)	Water Treated per 1 kg Dryzyme	Volume (kiloliters)
5 ppm (Minimum dose)	5 mg/L	1,000,000 mg ÷ 5 mg/L	200 kL
6 ppm (Recommended dose)	6 mg/L	1,000,000 mg ÷ 6 mg/L	166.7 kL
10 ppm (Maximum recommended dose)	10 mg/L	1,000,000 mg ÷ 10 mg/L	100 kL

At 5–6 ppm, 1 kg Dryzyme treats **166–200 kiloliters** of wetland or aquifer water.

Application Scenarios

- **In-Situ**: Float or anchor Dryzyme packs directly in sewage-impacted wetlands; enzymes release upon contact with flowing water.
- **Ex-Situ**: Add measured Dryzyme doses in bioreactors or constructed-wetland influent channels.

The enzyme-only approach ensures rapid reduction of biochemical oxygen demand (BOD), removal of odors and sludge prevention—without violating wetland conservation rules.

Our Natural in-situ Bio Re-mediation Treatment Methodology:

Draynzyme™ ILD (In line Digestion) Enzymes: used to accelerate sewage and other effluent waste digestion in waterways and sewage networks.

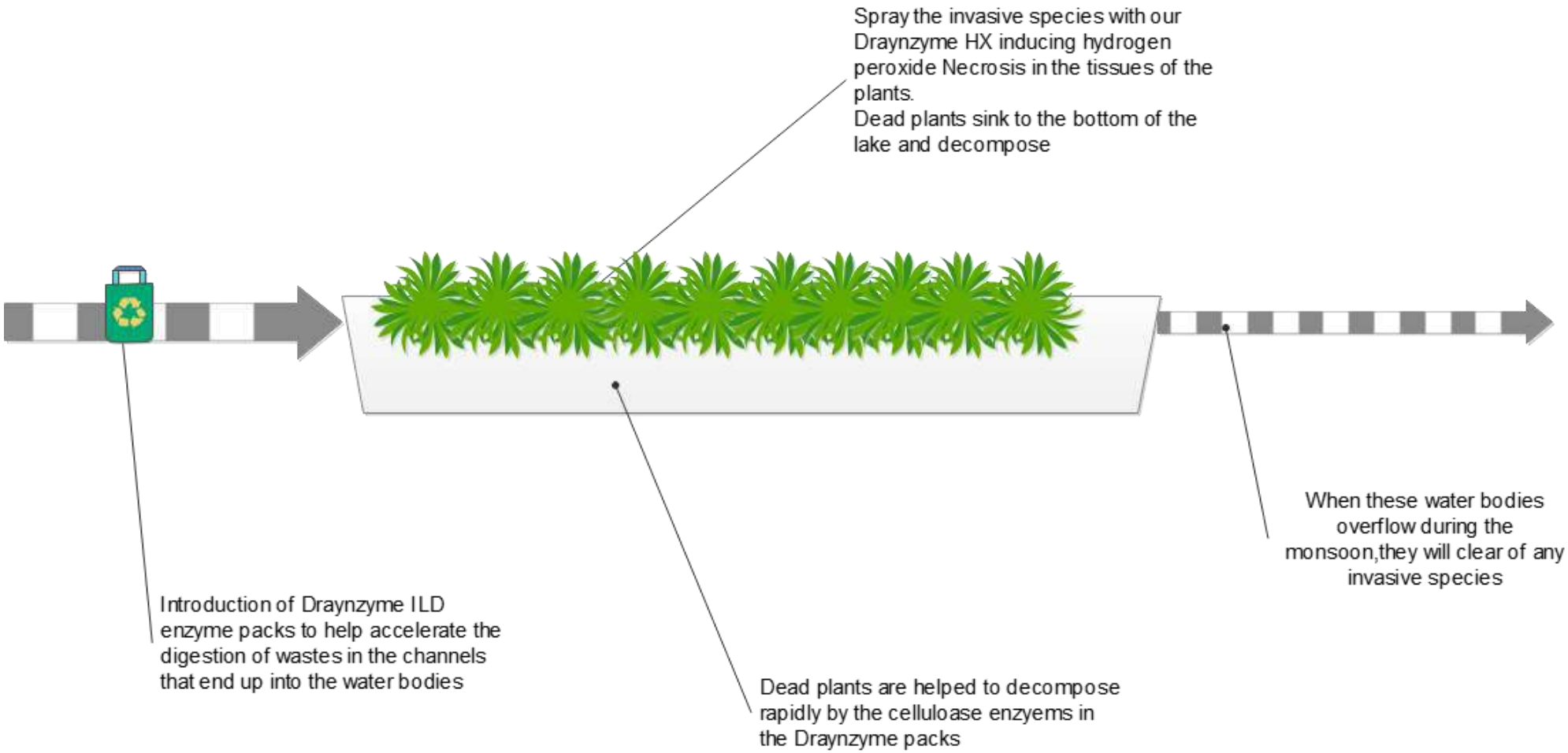
Typical Flow of water in lakes and water bodies in India

Water from Storm water channels drain into lakes
These channels due to lack of education and poor infrastructure are contaminated by sewage, industrial effluents and leachates containing ferilizers from farm lands

As the water collects in the water bodies, bacteria grow and try to digest the wastes. However, if a threshold is reached and the bacteria are unable to meet the time frame of digestion, other opportunistic organisms will take advantage of the situtation and florish. These organisms are usually invasive species and disturb the natural order of things.
Water Hyacinth is one such example of an invasive species which thrives in sewage water contaminated water bodies.

When these water bodies overflow during the monsoon, they carry the seeds of these invasive species to other water bodies

Proposed processing of the Flow of water in lakes and water bodies



Initially when the invasive plants are killed using Draynzyme HX, there will be a spike in BOD and COD values of the water, but as they die and decompose, the BOD and COD values will return back to normal.
Close monitoring the COD values of the lake on a Bi-monthly rate will allow close monitoring of the status of the water body.
Introduction of fish spicies can take place when the COD values return back to normal values.

Long Term planning:
Entry points of water bodies should have constructed wet lands which act as filters of the water entering the water body so as to avoid repeatation of the current situation.

Annexure-VI

Ecotoxicology Studies with Draynzyme Product

Safety assessment of Draynzyme product was carried out as per OECD test guidelines on aquatic and terrestrial organisms in the Ecotoxicology Laboratory of CSIR-Indian Institute of Toxicology Research (CSIR-IITR) GLP Test Facility. The following studies were conducted as per Standard Operating Procedures (SOPs) and mutually agreed (between Study Sponsor and Test Facility) Study Plans:

1. Algal Growth Inhibition Test (OECD Test Guideline 201)
2. Daphnia sp. Acute Immobilization Test (OECD Test Guideline 202)
3. Acute Fish Embryo Toxicity Test (OECD Test Guideline 236)
4. Acute Earthworm Toxicity Test (OECD Test Guideline 207)

The results of the studies are summarized below:

S. No.	Study Title	Endpoint	Toxicological Dose Descriptor	Value
1.	Algal Growth Inhibition Test	Growth rate and Yield	ErC_{50} and EyC_{50}	>100 mg/L
2.	Daphnia sp. Acute Immobilization Test	Immobilization	EC_{50}	>100 mg/L
3.	Acute Fish Embryo Toxicity Test	Lethality and Developmental Defects	LC_{50}	>100 mg/L
4.	Acute Earthworm Toxicity Test	Survival	LC_{50}	>1000 mg/Kg

ErC_{50} – Concentration resulting in 50% inhibition of growth rate
EyC_{50} – Concentration resulting in 50% inhibition of yield
EC_{50} – Effective Concentration
LC_{50} – Lethal Concentration

As per the manufacturer (Dhara Biotech) the recommended dosage at which the Draynzyme product is to be used is 5-6 ppm (mg/L) and maximum dosage of the product will not exceed 10 ppm (mg/L) respectively (Ref: Page 3/6 in the In-situ Bioremediation Treatment of Eutrophic Wetland Document). Thus, the Draynzyme product was tested at 10-20 times higher concentration than the recommended dosage, yet it did not induce any adverse impacts on the aquatic and terrestrial organisms. Based on the acute toxicity assessment, Draynzyme product was found to be non-toxic to the aquatic and terrestrial non-target organisms at the tested doses.

Annexure-VII

Central Pollution Control Board
(Ministry of Environment, Forest & Climate Change, Govt. of India)
Parivesh Bhawan, East Arjun Nagar,
Delhi – 110032

F No- 14011/WQM-I/2026 /5 **Date: 05.01.2026**

OFFICE ORDER

Constitution of Committee for ensuring compliance to Hon'ble NGT order dated 05.04.2024 in O.A No. 323/2024

Hon'ble NGT passed order dated 05.04.2024 in O.A No. 323/2024, Suo Moto matter: News item appearing in The Hindustan Times dated 23.02.2024 titled "No permission provided for use of chemicals in cleaning water bodies: MPCB" (Annexure-I), giving following directions:

> *"8. We are of the view that the permissibility of the spray of Draynzyme on water hyacinth needs to be first examined by the Central Pollution Control Board (CPCB) in coordination with ITRC, Lucknow and if it is found that it has no adverse effect on the ecology of the water body, then its use can be permitted in accordance with law.*
> *9. Hence, we dispose of the OA directing the CPCB to do the needful to ascertain the feasibility of use of Draynzyme on water hyacinth /natural water bodies and submit a report before the Registrar of the Western Zonal Bench of the Tribunal within four months. If found necessary the matter will be listed for consideration before the Western Zonal Bench, Pune. Let the original record of this OA be transferred to the Western Zonal Bench, Pune."*

In above reference, Pune Municipal Corporation (PMC) submitted the details of the product Draynzyme vide e-mail dated 06.11.2025 (Annexure-II) and Indian Institute of Toxicological Research, Lucknow (CSIR-IITR) submitted information on actual tested maximum concentration and interpretation of test results vide e-mail dated 07.11.2025 (Annexure-III).

In pursuance to Hon'ble NGT order dated 05.04.2024 in O.A No. 323/2024, Suo Moto matter In re: News item appearing in The Hindustan Times dated 23.02.2024 titled "No permission provided for use of chemicals in cleaning water bodies: MPCB", Central Pollution Control Board (CPCB) is hereby constituting a Committee to examine the information provided by PMC and CSIR-IITR with following members:

S.No.	Ministry/Organization/Name of the official
1.	Representative from CSIR-NEERI Delhi Zonal Centre
2.	Representative from Department of Biotechnology (DBT), Ministry of Science and Technology
3.	Representative from CSIR-Indian Institute of Toxicology Research, Lucknow
4.	DH-Water and Bio Labs, CPCB
5.	Scientist-E, WQM-I - Convener

The Terms of Reference (ToR) of the Committe :-
To examine the findings of the report submitted by CSIR-IITR and -

i. to ascertain the feasibility of use of bio-enzymes in water bodies to deal with the problem of water pollution and water hyacinth and make necessary recommendations and,

ii. to ascertain no adverse effects on the ecology of the water body in case of the use of bio-enzymes in water bodies to deal with the problem of water pollution and water hyacinth and make necessary recommendations.

Tenure of the Committee : Tenure of the Committee is 15 days and the same shall be extended if required.

This issues with the approval of 'Competent Authority, Central Board'.

(Nazimuddin)
DH, WQM-I Division

To: o/c

1.	The Chief Scientist, CSIR-NEERI Delhi Zonal Centre, CSIR R&D Centre (1st Floor), A-93/94, Phase -1, Naraina Industrial Area, New Delhi-110028. sk_goyal@neeri.res.in
2.	The Secretary, Department of Biotechnology, 6th-8th Floor, Block 2 and 4th-5th Floor, Block 3 CGO Complex, Lodhi Road New Delhi - 110 003 Secy.dbt@nic.in
3.	The Director, CSIR-Indian Institute of Toxicology Research Sarojani Nagar Industrial Area, Gheru Lucknow - 226 008, Uttar Pradesh, India director@iitrindia.org
4.	DH-Water and Bio Labs, CPCB

Copy to:

1. PS to MS : for information of 'MS', please

(Nazimuddin)
o/c

Speed Post / E-mail

Annexure-VIII

F No. A-14011/1/2026 – WQM - I /28 **22.01.2026**

To

Municipal Commissioner,
Pune Municipal Corporation,
PMC Main Building, Shivajinagar,
Pune-411005

Sub: Hon'ble NGT order dated 05.04.2024 in O.A No. 323/2024, Suo Moto matter In re: News item appearing in The Hindustan Times dated 23.02.2024 titled "No permission provided for use of chemicals in cleaning water bodies: MPCB"–reg.

Sir,

In pursuance to Hon'ble NGT order dated 05.04.2024 in the above-mentioned matter, CPCB vide office order dated 05.01.2026 constituted a Committee to examine the information provided by PMC and CSIR-IITR with members from CSIR-NEERI Delhi Zonal Centre; Department of Biotechnology (DBT), Ministry of Science and Technology; CSIR-Indian Institute of Toxicology Research, Lucknow and DH-Water and Bio Labs, CPCB. 1st Meeting of the Committee constituted was held on 17th January, 2025 at 11:00 AM with the committee members through Video Conference.

Accordingly, the Committee recommended that the following information about the product Draynzyme needs to be obtained through the Pune Municipal Corporation (PMC) for further examination:

I. Comprehensive chemical composition and formulation details and characterization of the product Draynzyme, including the nature, source, and concentration of its constituent components.
II. Relevant scientific documentation, including published literature, peer-reviewed research articles, experimental studies, patent details, and validation reports conducted or sponsored by the manufacturer pertaining to Draynzyme.
III. Scientific studies and safety assessments, if available, addressing aspects such as biomagnification potential, impacts on microbial ecology, and outcomes of chronic toxicity evaluations.

In this regard, it is requested to provide the requisite information urgently, as CPCB is to file appropriate reply in compliance to the above- mentioned Hon'ble NGT matter.

Yours faithfully,
22/1/2026
(Nazimuddin)
Divisional Head, WQM -I Div.

Copy to:

d/c

The Regional Director,
Regional Directorate-Pune, CPCB,
Survey No. 110, Dhankude Multi Purpose Hall,
Baner Road, Baner, Pune - 411045

: For follow-up with PMC please.

22/1/2026
(Nazimuddin)

d/c

Comprehensive chemical composition and formulation details, and characterization of the product Draynzyme, including the nature, source, and concentration of its constituent components.

What is Draynzyme:

?

… an innovation designed to enzymatically manipulate bacterial behaviour and economically deliver numerous benefits …

It is an organic product meticulously formulated to achieve the desired outcomes naturally, devoid of side effects or residual pollutants commonly associated with synthetic chemicals.

Nature of action:

This innovative and indigenous enzyme-based treatment is designed to engage local bacteria present in sewage networks and treatment facilities to expeditiously decompose biodegradable waste and industrial effluents, effectively mitigating microbiologically induced corrosion in pipes and processing equipment.
Our proprietary methodology employs enzymes cultivated in controlled fermenters from heterotrophic, non-pathogenic decomposing bacteria. Our enzymes represent a bespoke treatment process characterized by the continuous release of specific enzymes from specially engineered sustained-release packs, ensuring that the discharged water adheres to the requisite norms and standards.
Our products contain no live bacteria; it is our social and environmental commitment to refrain from introducing foreign bacteria into the ecosystem.

Enzymes are intricate proteins that function as biological catalysts (biocatalysts). Catalysts expedite chemical reactions by facilitating the transformation of substrates—the molecules upon which enzymes act—into distinct molecules known as products. Nearly all metabolic processes within cells necessitate enzyme catalysis to occur at rates sufficient to sustain life. Enzymes are biodegradable and are synthesized by various bacteria and fungi to enhance their survival. The production of enzymes is an energy-intensive process; consequently, bacteria and fungi tend to favor the most efficient and economical pathways for survival. However, we can alter this dynamic by providing alternative pathways through pre-prepared enzymes, thereby directing bacteria and fungi to utilize otherwise neglected food sources. Enzymes, being biodegradable, will ultimately be assimilated by bacteria and fungi.

Draynzyme is a product specifically designed to function within sewage systems that are already abundant with bacteria; it does not require the introduction of new bacterial strains to fulfill its purpose. Rather, it necessitates the acceleration of existing microbial activity through a natural catalyst that enhances sewage degradation and mitigates the presence of bacteria considered detrimental to human health.

How it is manufactured (Source):

Draynzyme final product contains no live bacteria, fungi, or plant matter. During production, selected bacteria, fungi, and plant materials are used only in the fermentation stage to produce enzymes. Those enzymes are extracted and purified; after extraction each batch is fumigated with formaldehyde to denature nucleic acids and inactivate any remaining microorganisms. As a result, **no viable cells, spores, or intact plant tissue are present in the finished, packaged product** and the product does not contain live bacteria, fungi, or plant material.

Constituent Components (Formulation detials):

DraynZyme comprises the following Enzymes and Componenets:

- **Urease**: Catalyzes the conversion of urea to ammonia.
- **Exogenous Polysaccharide-Degrading Enzymes**: This category includes xylanase, carboxymethylcellulase, amylase, lignocellulases, and alpha/beta-glucosidases. These enzymes are utilized by bacteria, fungi, and protozoa to decompose starches and cellulose into their constituent sugar units.

- **Ammonia Monooxygenase**: Facilitates the transformation of ammonia into nitrate under both aerobic and anaerobic conditions. The Anammox reaction facilitated by this enzyme converts ammonia and nitrate into nitrogen gas in anaerobic environments.
- **Acid Phosphatase**: Liberates phosphate groups that are covalently bonded to other molecules.
- **Lipases catalyze** the hydrolysis of lipids and are classified as a subset of esterases.
- **Proteases**: Enzymes that initiate proteolysis, thereby commencing protein catabolism through the hydrolysis of peptide bonds that connect amino acids within the polypeptide chain that constitutes the protein.
- **Oxidoreductases**: These enzymes facilitate redox (oxidation-reduction) reactions, promoting the transfer of electrons between molecules (the reductant and the oxidant). This process is vital for a myriad of biological functions, including metabolism, detoxification, and environmental remediation.
- Hydrated Silica: Media to hold and maintain sustained release of enzymes
- Lignocellulose: Media to hold and maintain sustained release of enzymes

The concentrations of each enzyme in pack as follows:

	Enzyme/Constituent	% (weight)
1	Sustained release media - Hydrated silica	Upto 70%
2	Lignocellulose	Upto 29%
3	Enzymes	Upto 15%

How do these Enzymes work?

1. Urease:

Ureases (EC 3.5.1.5), functionally, belong to the superfamily of amidohydrolases and phosphotriesterases.[2] Ureases are found in numerous bacteria, fungi, algae, plants, and some invertebrates, as well as in soils, as a soil enzyme. They are nickel-containing metalloenzymes of high molecular weight.

These enzymes catalyze the hydrolysis of urea into carbon dioxide and ammonia:

$(NH_2)_2CO + H_2O$ urease$\rightarrow CO_2 + 2NH_3$

The hydrolysis of urea occurs in two stages. In the first stage, ammonia and carbamate are produced.
The carbamate spontaneously and rapidly hydrolyzes to ammonia and carbonic acid. Urease activity increases the pH of its environment as ammonia is produced, which is basic.

Activity

The k_{cat}/K_m of urease in the processing of urea is 10^{14} times greater than the rate of the uncatalyzed elimination reaction of urea. There are many reasons for this observation in nature. The proximity of urea to active groups in the active site along with the correct orientation of urea allow hydrolysis to occur rapidly. Urea alone is very stable due to the resonance forms it can adopt. The stability of urea is understood to be due to its resonance energy, which has been estimated at 30–40 kcal/mol. This is because the zwitterionic resonance forms all donate electrons to the carbonyl carbon making it less of an electrophile making it less reactive to nucleophilic attack.

Proposed mechanisms By Blakeley/Zerner

One mechanism for the catalysis of this reaction by urease was proposed by Blakely and Zerner. It begins with a nucleophilic attack by the carbonyl oxygen of the urea molecule onto the 5-coordinate Ni (Ni-1). A weakly coordinated water ligand is displaced in its place. A lone pair of electrons from one of the nitrogen atoms on the Urea molecule creates a double bond with the central carbon, and the resulting NH_2^- of the coordinated substrate interacts with a nearby positively charged group. Blakeley and Zerner proposed this nearby group to be a Carboxylate ion, although deprotonated carboxylates are negatively charged.

A hydroxide ligand on the six coordinate Ni is deprotonated by a base. The carbonyl carbon is subsequently attacked by the electronegative oxygen. A pair of electrons from the nitrogen-carbon double bond returns to the nitrogen and neutralizes the charge on it, while the now 4-coordinate carbon assumes an intermediate tetrahedral orientation.

The breakdown of this intermediate is then helped by a sulfhydryl group of a cysteine located near the active site. A hydrogen bonds to one of the nitrogen atoms, breaking its bond with carbon, and releasing an NH3 molecule. Simultaneously, the bond between the oxygen and the 6-coordinate nickel is broken. This leaves a carbamate ion coordinated to the 5-coordinate Ni, which is then displaced by a water molecule, regenerating the enzyme.

The carbamate produced then spontaneously degrades to produce another ammonia and carbonic acid.

By Hausinger/Karplus

The mechanism proposed by Hausinger and Karplus attempts to revise some of the issues apparent in the Blakely and Zerner pathway, and focuses on the positions of the side chains making up the urea-binding pocket. From the crystal structures from K. aerogenes urease, it was argued that the general base used in the Blakely mechanism, His^{320}, was too far away from the Ni2-bound water to deprotonate in order to form the attacking hydroxide moiety. In addition, the general acidic ligand required to protonate the urea nitrogen was not identified. Hausinger and Karplus suggests a reverse protonation scheme, where a protonated form of the His^{320} ligand plays the role of the general acid and the Ni2-bound water is already in the deprotonated state. The mechanism follows the same path, with the general base omitted (as there is no more need for it) and His^{320} donating its proton to form the ammonia molecule, which is then released from the enzyme. While the majority of the His^{320} ligands and bound water will not be in their active forms (protonated and deprotonated, respectively,) it was calculated that approximately 0.3% of total urease enzyme would be active at any one time. While logically, this would imply that the enzyme is not very efficient, contrary to established knowledge, usage of the reverse protonation scheme provides an advantage in increased reactivity for the active form, balancing out the disadvantage. Placing the His^{320} ligand as an essential component in the mechanism also takes into account the mobile flap region of the enzyme. As this histidine ligand is part of the mobile flap, binding of the urea substrate for catalysis closes this flap over the active site and with the addition of the hydrogen bonding pattern to urea from other ligands in the pocket, speaks to the selectivity of the urease enzyme for urea.

By Ciurli/Mangani

The mechanism proposed by Ciurli and Mangani is one of the more recent and currently accepted views of the mechanism of urease and is based primarily on the different roles of the two nickel ions in the active site. One of which binds and activates urea, the other nickel ion binds and activates the nucleophilic water molecule. With regards to this proposal, urea enters the active site cavity when the mobile 'flap' (which allows for the entrance of urea into the active site) is open. Stability of the binding of urea to the active site is achieved via a hydrogen-bonding network, orienting the substrate into the catalytic cavity. Urea binds to the five-coordinated nickel (Ni1) with the carbonyl oxygen atom. It approaches the six-coordinated nickel (Ni2) with one of its amino groups and subsequently bridges the two nickel centers. The binding of the urea carbonyl oxygen atom to Ni1 is stabilized through the protonation state of $His^{\alpha 222}$ Nε. Additionally, the conformational change from the open to closed state of the mobile flap generates a rearrangement of $Ala^{\alpha 222}$ carbonyl group in such a way that its oxygen atom points to Ni2. The $Ala^{\alpha 170}$ and $Ala^{\alpha 366}$ are now oriented in a way that their carbonyl groups act as hydrogen-bond acceptors towards NH_2 group of urea, thus aiding its binding to Ni2. Urea is a very poor chelating ligand due to low Lewis base character of its NH_2 groups. However the carbonyl oxygens of $Ala^{\alpha 170}$ and $Ala^{\alpha 366}$ enhance the basicity of the NH_2 groups and allow for binding to Ni2. Therefore, in this proposed mechanism, the positioning of urea in the active site is induced by the structural features of the active site residues which are positioned to act as hydrogen-bond donors in the vicinity of Ni1 and as acceptors in the vicinity of Ni2. The main structural difference between the Ciurli/Mangani mechanism and the other two is that it incorporates a nitrogen, oxygen bridging urea that is attacked by a bridging hydroxide.

2. Exogenous Polysacceride degrading Enzymes

A. Xylanase

Xylanase (EC 3.2.1.8) is any of a class of enzymes that degrade the linear polysaccharide xylan into xylose, thus breaking down hemicellulose, one of the major components of plant cell walls.

As such, it plays a major role in micro-organisms thriving on plant sources for the degradation of plant matter into usable nutrients. Xylanases are produced by fungi, bacteria, yeast, marine algae, protozoans, snails, crustaceans, insect, seeds, etc.; mammals do not produce xylanases. However, the principal commercial source of xylanases is filamentous fungi.

Commercial applications for xylanase include the chlorine-free bleaching of wood pulp prior to the papermaking process, and the increased digestibility of silage (in this aspect, it is also used for fermentative composting).

Apart from its use in the pulp and paper industry, xylanases are also used as food additives to poultry; in wheat flour for improving dough handling and quality of baked products ; for the extraction of coffee, plant oils, and starch; in the improvement of nutritional properties of agricultural silage and grain feed; and in combination with pectinase and cellulase for clarification of fruit juices and degumming of plant fiber sources such as flax, hemp, jute, and ramie. A good quantity of scientific literature is available on key features of xylanase enzymes in biotechnology ranging from their screening in microbial sources to production methods, characterization, purification and applications in commercial sector.

Additionally, it is the key ingredient in the dough conditioners s500 and us500 manufactured by Puratos [nl]. These enzymes are used to improve the dough's workability and absorption of water.

In the future, xylanase may be used for the production of biofuel from unusable plant material.

B. Amylase

An **amylase** is an enzyme that catalyses the hydrolysis of starch (Latin amylum) into sugars. Amylase is present in the saliva of humans and some other mammals, where it begins the chemical process of digestion. Foods that contain large amounts of starch but little sugar, such as rice and potatoes, may acquire a slightly sweet taste as they are chewed because amylase degrades some of their starch into sugar. The pancreas and salivary gland make amylase (alpha amylase) to hydrolyse dietary starch into disaccharides and trisaccharides which are converted by other enzymes to glucose to supply the body with energy. Plants and some bacteria also produce amylase. Specific amylase proteins are designated by different Greek letters. All amylases are glycoside hydrolases and act on α-1,4-glycosidic bonds.

α-Amylase

The α-amylases (EC 3.2.1.1) (CAS 9014-71-5) (alternative names: 1,4-α-D-glucan glucanohydrolase; glycogenase) are calcium metalloenzymes. By acting at random locations along the starch chain, α-amylase breaks down long-chain saccharides, ultimately yielding either maltotriose and maltose from amylose, or maltose, glucose and "limit dextrin" from amylopectin. They belong to glycoside hydrolase family 13.
Because it can act anywhere on the substrate, α-amylase tends to be faster-acting than β-amylase. In animals, it is a major digestive enzyme, and its optimum pH is 6.7–7.0.
In human physiology, both the salivary and pancreatic amylases are α-amylases.
The α-amylase form is also found in plants, fungi (ascomycetes and basidiomycetes) and bacteria (*Bacillus*).

β-Amylase

Another form of amylase, β-amylase (EC 3.2.1.2) (alternative names: 1,4-α-D-glucan maltohydrolase; glycogenase; saccharogen amylase) is also synthesized by bacteria, fungi, and plants. Working from the non-reducing end, β-amylase catalyzes the hydrolysis of the second α-1,4 glycosidic bond, cleaving off two glucose units (maltose) at a time. During the ripening of fruit, β-amylase breaks starch into maltose, resulting in the sweet flavor of ripe fruit. They belong to glycoside hydrolase family 14.

Both α-amylase and β-amylase are present in seeds; β-amylase is present in an inactive form prior to germination, whereas α-amylase and proteases appear once germination has begun. Many microbes also produce amylase to degrade extracellular starches. Animal tissues do not contain β-amylase, although it may be present in microorganisms contained within the digestive tract. The optimum pH for β-amylase is 4.0–5.0.

γ-Amylase

γ-Amylase (EC 3.2.1.3) (alternative names: Glucan 1,4-a-glucosidase; amyloglucosidase; *exo*-1,4-α-glucosidase; glucoamylase; lysosomal α-glucosidase; 1,4-α-D-glucan glucohydrolase) will cleave α(1–6) glycosidic linkages, as well as the last α-1,4 glycosidic bond at the nonreducing end of amylose and amylopectin, yielding glucose. The γ-amylase has most acidic optimum pH of all amylases because it is most active around pH 3. They belong to a variety of different GH families, such as glycoside hydrolase family 15 in fungi, glycoside hydrolase family 31 of human MGAM, and glycoside hydrolase family 97 of bacterial forms.

C. Cellulase

Cellulase is any of several enzymes produced chiefly by fungi, bacteria, and protozoans that catalyze cellulolysis, the decomposition of cellulose and of some related polysaccharides. The name is also used for any naturally occurring mixture or complex of various such enzymes, that act serially or synergistically to decompose cellulosic material.
Cellulases break down the cellulose molecule into monosaccharides ("simple sugars") such as beta-glucose, or shorter polysaccharides and oligosaccharides. Cellulose breakdown is of considerable economic importance, because it makes a major constituent of plants available for consumption and use in chemical reactions. The specific reaction involved is the hydrolysis of the 1,4-beta-D-glycosidic linkages in cellulose, hemicellulose, lichenin, and cereal beta-D-glucans. Because cellulose molecules bind strongly to each other, cellulolysis is relatively difficult compared to the breakdown of other polysaccharides such as starch. Several different kinds of cellulases are known, which differ structurally and mechanistically. Synonyms, derivatives, and specific enzymes associated with the name "cellulase" include endo-1,4-beta-D-glucanase (beta-1,4-glucanase, beta-1,4-endoglucan hydrolase, endoglucanase D, 1,4-(1,3,1,4)-beta-D-glucan 4-glucanohydrolase), carboxymethyl cellulase (CMCase), avicelase, celludextrinase, cellulase A, cellulosin AP, alkali cellulase, cellulase A 3, 9.5 cellulase, and pancellase SS. Enzymes that cleave lignin have occasionally been called cellulases, but this old usage is deprecated; they are lignin-modifying enzymes.
Types and action
Five general types of cellulases based on the type of reaction catalyzed:

- **Endocellulases** (EC 3.2.1.4) randomly cleave internal bonds at amorphous sites that create new chain ends.
- **Exocellulases or cellobiohydrolases** (EC 3.2.1.91) cleave two to four units from the ends of the exposed chains produced by endocellulase, resulting in tetrasaccharides or disaccharides, such as cellobiose. Exocellulases are further classified into type I, that work processively from the reducing end of the cellulose chain, and type II, that work processively from the nonreducing end.
- **Cellobiases** (EC 3.2.1.21) or beta-glucosidases hydrolyse the exocellulase product into individual monosaccharides.
- **Oxidative cellulases** depolymerize cellulose by radical reactions, as for instance cellobiose dehydrogenase (acceptor).
- **Cellulose phosphorylases** depolymerize cellulose using phosphates instead of water.

Some Notable Cellulases used in the product:

- Carboxymethyl Cellulase
- Ligno Cellulase: An enzyme that hydrolyses lignocellulose.
- Alpha - glucosidase:
  Alpha-glucosidase (EC 3.2.1.20, *maltase*, *glucoinvertase*, *glucosidosucrase*, *maltase-glucoamylase*, *alpha-glucopyranosidase*, *glucosidoinvertase*, *alpha-D-glucosidase*, *alpha-glucoside hydrolase*, *alpha-1,4-glucosidase*, *alpha-D-glucoside glucohydrolase*). Alpha-glucosidase breaks down starch and disaccharides to glucose. Maltase, a similar enzyme that cleaves maltose, is nearly functionally equivalent.
- Beta - glucosidase : **Beta-glucosidase** is an enzyme that catalyzes the hydrolysis of the glycosidic bonds to terminal non-reducing residues in beta-D-glucosides and oligosaccharides, with release of glucose.

3. Ammonia monooxygenase

Ammonia monooxygenase (EC 1.14.99.39, *AMO*) is an enzyme, which catalyses the following chemical reaction

$$\text{Ammonia} + AH_2 + O_2 \rightarrow NH_2OH + a + H_2O$$

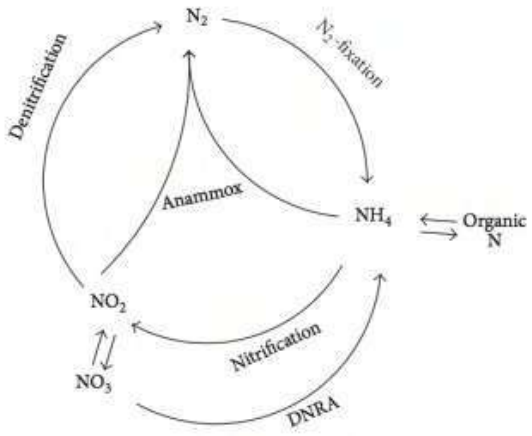
Ammonia monooxygenase contains copper and possibly nonheme iron. AMO is the first enzyme in ammonia oxidation. Aerobic oxidation of ammonia to hydroxylamine via AMO is an endergonic reaction. So, all aerobic ammonia oxidizing organisms conserve energy by further oxidizing hydroxylamine. It was believed that aerobic ammonia-oxidizing bacteria oxidize hydroxylamine to nitrite using octahaem hydroxylamine oxidoreductase (HAO). Recently, it was shown that the product of HAO is not nitrite but nitric oxide, which is further oxidized to nitrite by an unknown enzyme.

Anammox

Anammox, an abbreviation for **anaerobic ammonium oxidation**, is a globally important microbial process of the nitrogen cycle that takes place in many natural environments. The bacteria mediating this process were identified in 1999, and were a great surprise for the scientific community. In the anammox reaction, nitrite and ammonium ions are converted directly into diatomic nitrogen and water.

In this biological process, which is a comproportionation reaction, nitrite and ammonium ions are converted directly into diatomic nitrogen and water.

$$NH^+_4 + NO^-_2 \rightarrow N_2 + 2H_2O$$



Possible reaction mechanisms:

According to ^{15}N labeling experiments carried out in 1997, ammonium is biologically oxidized by hydroxylamine, most likely derived from nitrite, as the probable electron acceptor. The conversion of hydrazine to dinitrogen gas is hypothesized to be the reaction that generates the electron equivalents for the reduction of nitrite to hydroxylamine. In general, two possible reaction mechanisms are addressed:

- One mechanism hypothesizes that a membrane-bound enzyme complex converts ammonium and hydroxylamine to hydrazine first, followed by the oxidation of hydrazine to dinitrogen gas in the periplasm. At the same time, nitrite is reduced to hydroxylamine at the cytoplasmic site of the same enzyme complex responsible for hydrazine oxidation with an internal electron transport (Figure 3a).
- The other mechanism postulates the following: ammonium and hydroxylamine are converted to hydrazine by a membrane-bound enzyme complex, hydrazine is oxidized in the periplasm to dinitrogen gas, and the generated electrons are transferred via an electron transport chain to nitrite reducing enzyme in the cytoplasm where nitrite is reduced to hydroxylamine (Figure 3b).

Whether the reduction of nitrite and the oxidation of hydrazine occur at different sites of the same enzyme or the reactions are catalyzed by different enzyme systems connected via an electron transport chain remains to be investigated. In microbial nitrogen metabolism, the occurrence of hydrazine as an intermediate is rare. Hydrazine has been proposed as an enzyme-bound intermediate in the nitrogenase reaction. Recently, using detailed molecular analyses and combining complementary methods,

Kartal and coworkers published strong evidence supporting the latter mechanism. Furthermore, the enzyme producing hydrazine, hydrazine synthase was purified and shown to produce hydrazine from NO and ammonium. The production of hydrazine from ammonium and NO was also supported by the resolution of the crystal structure of the enzyme hydrazine sythase.

A possible role of nitric oxide (NO) or nitroxyl (HNO) in anammox was proposed by Hooper et al. by way of condensation of NO or HNO and ammonium on an enzyme related to the ammonium monooxygenase family. The formed hydrazine or imine could subsequently be converted by the enzyme hydroxylamine oxidase to dinitrogen gas, and the reducing equivalents produced in the reaction are required to combine NO or HNO and ammonium or to reduce nitrite to NO. Environmental genomics analysis of the species *Candidatus Kuenenia stuttgartiensis*, through a slightly different and complementary metabolism mechanism, suggested NO to be the intermediate instead of hydroxylamine (Figure 4). However, this hypothesis also agreed that hydrazine was an important intermediate in the process. In this pathway (Figure 4), there are two enzymes unique to anammox bacteria: hydrazine synthase (hzs) and hydrazine dehydrogenase (hdh). The HZS produces hydrazine from nitric oxide and ammonium, and HDH transfer the electrons from hydrazine to ferredoxin. Few new genes, such as some known fatty acid biosynthesis and S-adenosylmethionine radical enzyme genes, containing domains involved in electron transfer and catalysis have been detected. Anammox microorganisms can also directly couple NO reduction to ammonia oxidation, without the need for nitrite supply.

Another, still unexplored, reaction mechanism involves anaerobic ammonium oxidation on anodes of bio-electrical systems. Such systems can be microbial fuel cells or microbial electrolysis cells. In the absence of dissolved oxygen, nitrite, or nitrate, microbes living in the anode compartment are able to oxidize ammonium to dinitrogen gas (N_2) just as in the classical anammox process. At the same time, they unload the liberated electrons onto the anode, producing electrical current. This electrical current can be used either directly in fuel cell mode or for hydrogen and methane gas production in electrolysis mode. While there is no clarity on the reaction mechanism behind, one hypothesis is that nitrite, nitrate, or dinitrogen oxide play a role as intermediates. However, since the process occurs at very low electrochemical potentials, other, more speculative, reaction mechanisms seem possible as well.

Application in Wastewater Treatment:
The application of the anammox process lies in the removal of ammonium in wastewater treatment and consists of two separate processes. The first step is partial nitrification (nitritation) of half of the ammonium to nitrite by ammonia oxidizing bacteria:

$$2NH^+_4 + 3O_2 \rightarrow 2NO^-_2 + 4H^+ + 2H_2O$$

The resulting ammonium and nitrite are converted in the anammox process to dinitrogen gas and ~15% nitrate (not shown) by anammox bacteria:

$$NH^+_4 + NO^-_2 \rightarrow N_2 + 2H_2O$$

Both processes can take place in 1 reactor.

The cost reduction compared to conventional nitrogen removal is considerable; the technique is still young but proven in several fullscale installations.

4. Acid Phosphatase

Acid phosphatase (EC 3.1.3.2, *acid phosphomonoesterase*, *phosphomonoesterase*, *glycerophosphatase*, *acid monophosphatase*, *acid phosphohydrolase*, *acid phosphomonoester hydrolase*, *uteroferrin*, *acid nucleoside diphosphate phosphatase*, *orthophosphoric-monoester phosphohydrolase (acid optimum)*) is a phosphatase, a type of enzyme, used to free attached phosphoryl groups from other molecules during digestion.

Acid phosphatase catalyzes the following reaction:

$$\text{Orthophosphoric monoester} + H_2O \rightarrow \text{alcohol} + H_3PO_4$$

Phosphatase enzymes are also used by soil microorganisms to access organically bound phosphate nutrients. An assay on the rates of activity of these enzymes may be used to ascertain biological demand for phosphates in the soil.

Some plant roots, especially cluster roots, exude carboxylates that perform acid phosphatase activity, helping to mobilise phosphorus in nutrient-deficient soils.

5. Lipase

A **lipase** are a family of enzymes that catalyzes the hydrolysis of fats. Some lipases display broad substrate scope including esters of cholesterol, phospholipids, and of lipid-soluble vitamins. and sphingomyelinases; however, these are usually treated separately from "conventional" lipases. Unlike esterases, which function in water, lipases "are activated only when adsorbed to an oil–water interface". Lipases perform essential roles in digestion, transport and processing of dietary lipids in most, if not all, organisms.

6. Protease

A **protease** (also called a **peptidase** or **proteinase**) is an enzyme that catalyzes (increases reaction rate or "speeds up") proteolysis, breaking down proteins into smaller polypeptides or single amino acids, and spurring the formation of new protein products.[1] They do this by cleaving the peptide bonds within proteins by hydrolysis, a reaction where water breaks bonds. Proteases are involved in many biological functions, including digestion of ingested proteins, protein catabolism (breakdown of old proteins),[2][3] and cell signaling.

In the absence of functional accelerants, proteolysis would be very slow, taking hundreds of years.[4] Proteases can be found in all forms of life and viruses. They have independently evolved multiple times, and different classes of protease can perform the same reaction by completely different catalytic mechanisms.

Bacteria secrete proteases to hydrolyse the peptide bonds in proteins and therefore break the proteins down into their constituent amino acids. Bacterial and fungal proteases are particularly important to the global carbon and nitrogen cycles in the recycling of proteins, and such activity tends to be regulated by nutritional signals in these organisms. The net impact of nutritional regulation of protease activity among the thousands of species present in soil can be observed at the overall microbial community level as proteins are broken down in response to carbon, nitrogen, or sulfur limitation.

Bacteria contain proteases responsible for general protein quality control (e.g. the AAA+ proteasome) by degrading unfolded or misfolded proteins.

7. Oxidoreductases:

Oxidoreductases are enzymes that catalyze redox (oxidation-reduction) reactions. They facilitate the transfer of electrons from one molecule (the reductant) to another (the oxidant), which is essential in numerous biological processes such as metabolism, detoxification, and environmental remediation.

Extraction Sources:

These enzymes can be extracted from diverse sources. Many oxidoreductases are found in:

- **Fungi:** White-rot fungi are well-known producers of laccases and peroxidases, which play significant roles in nature by degrading lignin in wood.
- **Bacteria:** Certain bacterial species produce oxidoreductases that help in soil and water remediation.
- **Plants and Animals:** Plants and even some animal tissues contain oxidoreductases, including enzymes like tyrosinase and peroxidases, which have roles in pigmentation and cellular defense.

How They Work:

At the molecular level, oxidoreductases function by accepting or donating electrons through their active sites. For example:

- **Laccases** use molecular oxygen to oxidize phenolic substrates.
- **Peroxidases** like manganese peroxidase (MnP) produced by white-rot fungi, lignin peroxidase (LiP) (plant origin), and horseradish peroxidase, use hydrogen peroxide induced by their biochemical reactions to oxidize complex organic compounds.
- **Tyrosinases** catalyze the formation of reactive quinones from phenols, which then spontaneously polymerize. **This oxidation reaction leads to the formation of free radicals, which couple together to produce larger, insoluble polymers - thus leading to the effect of coagulation.**

Inducing Coagulation in Sewage Treatment:

Unlike traditional chemical coagulants such as iron or aluminum salts—which primarily work by neutralizing charges and aggregating particles—oxidoreductases chemically modify organic compounds. As they oxidize these substrates, the resulting free radicals undergo polymerization, generating high-molecular-weight aggregates (or flocs) that settle out of solution. This biological pathway not only facilitates clearer separation of solids but also avoids introducing additional metal contaminants into the water.

Facilitating Bacterial Digestion of Organic Waste:

The action of oxidoreductases can transform complex and sometimes recalcitrant organic matter into forms that are more amenable to bacterial metabolism. By pre-oxidizing and polymerizing dissolved organic components, these enzymes effectively "pre-condition" the organic load, making it easier for bacteria to break down the waste. This contrasts with iron or aluminum salts, which do not chemically modify the organic materials and may even create environments less favorable for microbial digestion.

This integrated approach not only enhances coagulation efficiency but also supports a more biological treatment route that can lead to a cleaner, more sustainable process in sewage management.

Dranyme modes of delivery:

Draynzyme is available from the factory as a dry powder that is packaged in two different formats:

1. The Tea-bag: encased in a polyester bag that allows for the enzymes to gradually seep out of the bag over a period of 1-3 months depending on the application. To help with the dispersement of the enzyme in a controlled manner over the duration of the dosing period, a clay is used to envelope the enzymes. These clays absorb water and swell, and as running water flows over these bags, the clay erodes and thus releases the enzymes into the water.

2. The loose pack: this packing is intened to be used to dilute the enzymes into a water base that can then be used to dosing a more stronger dose of enzymes into more contaminated locations where rapid cleanup is required. These enzymes will first induce a coagulation type of effect that entrapes the organic waste into clumps making it easier for bacteria to access nutrients and thus degrade and consume the organic wastes.

Product MSDS:

Material Safety Data Sheet

Section 1: MATERIAL IDENTIFICATION

Product Number/Size:

DWE/2005A-1Kg ILD Draynzyme in line digestion

Trade Name: Draynzyme™

Description: Waste Water treatment Enzymes

Chemical Composition: Enzymes embedded in a Hydrogel /
for (BAC/M only) - Bacteria embedded in hydrogel mix

Section 2: COMPOSITION/INGREDIENTS

Hazardous Ingredient (s)*	% BY Volume	CAS #
Non Hazardous Ingredient (s)		
Sustained release media *Hydrated Silica* *Lignocellulose*	Upto 99%	 1332-58-7 11132-73-3
Organic waste digestive enzymes	Upto 15%	9015-70-7 9029-60-1 9002-13-5 9037-40-5

Section 3: FIRE AND EXPLOSION HAZARD DATA

Flash Point (method): None
Hazardous Products of Combustion: None
Auto-ignition Temp.: None
Extinguishing Material: None

HEALTH HAZARD DATA

Potential Acute Health Effects: Nil
Eye contact: Not likely to occur under normal condition use.
Skin contact: Non/ No ill effects.
Inhalation: No ill effects
Toxicity to Animals: None
LD50: Not available.
LC50: Not available.
Chronic Effects on Humans: None

DHARA BIOTECH SANGLI

Section 4: FIRST AID MEASURES

Eye contact: Immediately flush eyes with water for 10-15 minutes. If irritation persists, obtain medical attention.

Skin Contact: If irritation develops, wash skin thoroughly with soap and water. If irritation persists seek medical attention.

Inhalation:	Not likely to under normal conditions.
Ingestion:	Consult a physician or seek medical advice, otherwise Not toxic.

Section 5: FIRE FIGHTING MEASURES

Flash point:	Not applicable
Flammable limits:	Not determined
Auto Ignition Temperature:	Not applicable
Fire Extinguishing materials:	Use extinguishing media suitable for materials supporting combustion such as water fog, CO_2 foam, or dry chemicals
Fire fighting procedure:	Use self-contained breathing apparatus in confined spaces or as otherwise needed.

Section 6: ACCIDENTAL SPILLS OR LEAK PROCEDURES

Steps to be taken if material is released or spilled:
Pick up and dispose.
Waste Disposal Method: Be aware that the waste owner has responsibility for final disposal. Regulations may also apply to empty containers, liners, or rinsate. Laws may change or be reinterpreted; state and local regulations may be different from Federal regulations. This information applies to materials as manufactured; contamination or processing may change waste characteristics and requirements.

Section 7: HANDLING AND STORAGE

Storage and Handling: Store away from heat, out of reach of children. Do not contaminate water, food or feed by storage or disposal. Do not reuse container.

Section 8: EXPOSURE CONTROLS/PERSONAL PROTECTION

Engineering Controls: None
Personal Protection: None required
Personal Protection in Case of a Large Spill: None required
Exposure Limits: Not available.

Section 9: PHYSICAL DATA

Physical State: Hydrogel media (solid), packed in polyester sacks
Boiling Point: Not Available
Vapor Pressure (mm Hg): Not Determined
Freezing Point: $0^{o}c$
Solubility in Water: Slowly dissolve into flowing water over a long period of time
Appearance: Mustard Yellow gel encapsulated inside polyester sack
Odor: Mild musty
Specific Gravity (Water=1): 1.2
Volatile by Volume %: Nil.
pH: 7
Danger of explosion: Product is not explosive

Section 10: REACTIVITY DATA

Conditions to avoid: Product denatures rapidly in environments outside of ph 5 – 8.5
Materials to avoid: None
Hazardous Decomposition: None
Hazardous Polymerization: None Will Occur
Stability: The product is stable.

DHARA BIOTECH SARSA 368365

Instability Temperature: Not available.
Conditions of Instability: Not available.
Incompatibility with various substances: Not available.
Corrosivity: Non-corrosive in presence of glass.
Special Remarks on Reactivity: Not available.
Special Remarks on Corrosivity: Not available.
Polymerization: No.

Section 11: TOXICOLOGICAL INFORMATION

MATERIAL IS NOT TOXIC

Section 12: ECOLOGICAL INFORMATION

Eco-toxicity: Non-Toxic as per tests for release into Water bodies.
Non toxicity report generated by Charotar University of science & technology
Dated: 20th Dec. 2022

Section 13: Disposal Consideration

Product Disposal Method: Wastes resulting from use of this product may be disposed of on site or at an approved waste disposal facility.
CONTAINER DISPOSAL:
If Empty: Do not reuse this container. Place in trash or offer for recycling if available.
If Partly Filled: Call your local solid waste agency for disposal instructions.

Section 14: TRANSPORTATION INFORMATION

DOT Classification: Not a DOT controlled material (United States).
Identification: Not applicable.
Special Provisions for Transport: Not applicable.

Section 15: REGULATORY INFORMATION

TSCA (Toxic substances Control Act):
Components of this product are not listed on the TSCA Inventory.
CERCLA (Comprehensive Environmental Response, Compensation, and Liablity Act.):
This product contains no components subject to reporting or notification requirements.
SARA title III (Super Amendments and Reauthorization Acts):
Reportable ingredients: None

Section 16: OTHER INFORMATION

Memo: While this information and recommendations set forth are believed to be accurate as of the date hereof, "M/S DHARA BIOTECH" makes no warranty with respect hereto and disclaims all liability from reliance thereon.
Date MSDS Prepared/updated: 15th October, 2024
Contact: Ms. Pratibha Patel / Mr. Kirit Bhoi

DHARA BIOTECH SARSA 388265

The Ethics of the Product:

Subject:
Ethics of why we use Enzymes in Draynzyme and not live bacteria.

Invasive (Not native to region) bacteria, fungi and parasites can affect native plants, animals and agricultural crops.
As they (invasive bacteria and fungi) are introduced into any new environment to do a particular task such as digest waste, they will interact with their surrounding bacteria and fungi, and destroy an existing balance of nature on which natural order of life is dependent on. The new (introduced) bacteria and fungi being more competitive will out grow existing bacterial and fungal species buy consuming their food and force them to either die or adapt. Many times these introduced species will also breed with existing species and create new variants, which may by chance emerge as a new disease in plants or animals or humans.
As an ethical view point, Dhara Biotech and QuinQuent Industries Pvt. Ltd., product "Draynzyme" is made of specifically chosen enzymes and does not contain any bacteria or fungi.
Enzymes are proteins that act as biological catalysts (biocatalysts). Catalysts accelerate chemical reactions. The molecules upon which enzymes may act are called substrates, and the enzyme converts the substrates into different molecules known as products. Almost all metabolic processes in the cell need enzyme catalysis in order to occur at rates fast enough to sustain life.
They are bio- degradable and eventually will be consumed by the local bacteria and fungi.
Draynzyme is a product targeted to work in sewage waters which is already teaming with alot of bacteria, does not need new bacteria to do the work, they only need to be speed up using a natural catalyst which promotes sewage degradation and reduction in bacteria deemed harmful for humans.
In order to ensure all Draynzyme products are free of Bacteria and fungi, they are all fumigated using Formaldehyde prior to packaging. This ensures that all viable DNA and RNA in live bacteria and fungi are denatured (scrambled) rendering them dead.

Copy of Certificate of Analysis:

DHARA BIOTECH

Sarsa - Valad Chowkdi, Bhalej Rd, SARSA - 388 365 (Guj.)
Ph. (02692) 272419

Certificate of Analysis

This is acknowledge the need for the user of the product Draynzyme Sewage Aid (DWE/2005A - 1Kg ILD Draynzyme) that contain sewage treatment enzymes embedded onto a Hydrated silicate material and then packed into Polyester cloth to aid in the deployment of the product.

Please find each 100gm of product contains the following:
1. Organic matter which will constitute proteins (enzymes) extracted from fermentation processes via industrial refinning: up to 5% by weight

2. Hydrated Silicate with the following mineral constituents: up to 98% by weight

Constituent	% per wt
SiO_2	50-60%
Fe_2O_3	7-9%
Al_2O_3	4.5-6%
Na	0.01-0.05%
Cl	0.1-0.2%
Mn	0.05-0.1%

For Dhara Biotech,

4/10/2022

DHARA BIOTECH SARSA 388365

Page 1 of 1

Website: www.dharabiotech.com
Email:

Copy of Certificate of live Bacteria in Finished product:

Test Report
Water Sample Analysis Report

Client's Name & Address: **M/s. Dhara Bio-tech** Near SarasChokdi, Near Gaushala, Kunjrav Road, Sarsa, Anand 388365, Gujarat. **Contact Person:** Mr. Vasantlal Patel	**Report No:** GLEPL/240124/01 **Issue Date:** 01/02/2024

Lab ID Code	:	GLEPL/240124/WL_1			
Sample Description	:	Product Sample	Purpose	:	As per Client requirement
Date of Sampling	:	Submitted by Client (Dhara Bio-tech)	Sample collected/Submitted by	:	Submitted by Client (Dhara Bio-tech)
Date of Sample Received	:	24/01/2024	Test Parameters	:	As per Client requirement
Date of starting Analysis	:	25/01/2024	Quantity/No. of sample	:	1No. Batch No. 01/01/24
Date of completion Analysis	:	31/01/2024	Packed/Seal	:	Sealed (13/01/24)

Result Table

Sr. No.	Test Parameters	Test Method	Unit	Results
1	Draynzyme for Bacteria Count	ISO 16649-3	MPN/100 mL	Absent

Remark: In accordance to G.S.R 613(E)-This product does not contain any Bacteria, thus does not require 'Appraisal by genetic Engineering Appraisal Committee).

Chemist

Authorized Signatory
Rekha Dare

Notes: (1) The results pertain to tested items only.
(2) This report shall not be reproduced, except in full, without written approval of the laboratory.
(3) Authenticity of this Report could be validated with office copy at Greenleaf Evirotech Pvt. Ltd.
(4) Perishable samples will be destroyed after testing, others after 7 days from the date of issue of the report, unless otherwise agreed with the customer or as required by the applicable regulations.

CIN: U74140GJ2010PTC059798

Greenleaf EnvirotechPvt. Ltd., Nr. RangoliFlats,Radhanpur Road, Mehsana – 384002. Gujarat, India.
Tel : +91-9725519974, E-mail: info@glepl.com, Web: www.glepl.com
Branch Office: 304, Kankavati Complex, Singanpor-Cauzway Road, Katargam, Surat– 395004

Relevant scientific documentation, including published literature, peer-reviewed research articles, experimental studies, patent details, and validation reports conducted or sponsored by the manufacturer pertaining to Draynzyme

Patent Details:

Draynzyme is not patented because the enzymes themselves are established commercial products and therefore lack the novelty required for patent protection; instead, Dhara Biotech and Quinquents rely on confidentiality for the formulation and proprietary process protection for the manufacturing method.

Why enzymes are not a patentable basis: Enzymes are widely produced and used in industry (food, detergents, pharmaceuticals, specialty applications) both in India and globally; a mere use or extraction of such known enzymes generally fails the novelty and inventive-step requirements for patentability. Indian patent law and commentary treat naturally occurring biological substances and mere discoveries of natural products as limited in patentability unless modified or shown to have an inventive technical contribution.

Why the company chose secrecy over patenting:
Formulation: The exact mixture and proportions are maintained as a closely guarded trade secret to avoid public disclosure that a patent would require.
Production methodology: The competitive advantage arises from proprietary fermentation and extraction processes; these process details are kept confidential because process patents require disclosure and have finite terms, whereas trade secrets can protect know-how indefinitely if kept secure.

Experimental studies:

Chemical Oxygen Demand of Waste in Water under Controlled Environment:

Gram : UNIVERSITY
Telephone : (02692)-30355
FAX No. : (02692)-36475

DEPARTMENT OF BIOSCIENCES

SARDAR PATEL UNIVERSITY, Vallabh Vidyanagar-388120, Gujarat (India)

Chemical Oxygen Demand of Waste in Water under controlled environment:

Test start date: 25th July, 2011
Report date: 8th Aug, 2011
Test protocol followed: ASTM D1252-01

1. **Scope**
   1.1. These test methods cover the determination of the quantity of oxygen that certain impurities in water will consume, based on the reduction of a dichromate solution under specified conditions.
   1.2. These test methods are limited by the reagents employed to a maximum chemical oxygen demand (COD) of 800 mg/L.

2. **Terminology**
   2.1. *Definitions*—For definitions of other terms used in these test methods, refer to Terminology D 1129.
   2.2. The term "oxygen demand" (COD) in these test methods is defined in accordance with Terminology D 1129 as follows:
      2.2.1. *oxygen demand*—the amount of oxygen required under specified test conditions for the oxidation of water borne organic and inorganic matter.

3. **Summary of Test Methods**
   3.1. Most organic and oxidizable inorganic substances present in water are oxidized by a standard potassium dichromate solution in 50 % (vol/vol) sulfuric acid.
   3.2. The oxidation of many otherwise refractory organics is facilitated by the use of silver sulfate that acts as a catalyst in the reaction.
   3.3. These test methods provide for combining the reagents and sample in a manner that minimizes the loss of volatile organic materials, if present.
   3.4. The oxidation of up to 1000 mg/L of chloride ion is inhibited by the addition of mercuric chloride to form stable and soluble mercuric sulfate complex.
   3.5. The chemical reaction involved in oxidation of materials by dichromate is illustrated by the following reaction with potassium acid phthalate ($KC_8H_5O_4$):

$$41\ H_2SO_4 + 10\ K_2Cr_2O_7 + 2\ KC_8H_5O_4 \rightarrow 10\ Cr_2(SO_4)_3 + 11\ K_2SO_4 + 16\ CO_2 + 46\ H_2O$$

   Since 10 mol of potassium dichromate has the same oxidation power as 15 mol of oxygen, the equivalent reaction is:

$$2\ KC_8H_5O_4 + 15\ O_2 + H_2SO_4 \rightarrow 16\ CO_2 + 6\ H_2O + K_2SO_4$$

   Thus 2 mol of potassium acid phthalate consumes 15 mol of oxygen. The theoretical COD of potassium acid phthalate is 1.175g of oxygen per gram of potassium acid phthalate.

4. **Significance and Use**
   4.1. These test methods are used to chemically determine the maximum quantity of oxygen that could be consumed by biological or natural chemical processes due to impurities in water. Typically this measurement is used to monitor and control oxygen-consuming pollutants, both inorganic and organic, in domestic and industrial wastewaters.

TEST RESULTS:

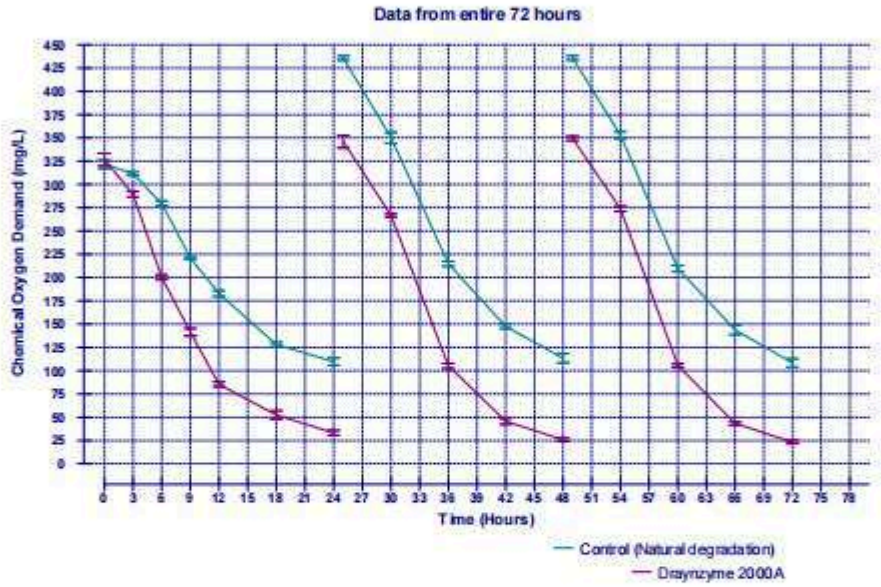
1. **Objective:** To simulate and understand effectiveness of Dhara Biotech product - "***Draynzyme***" sustained release enzyme packs to digest organic waste in a controlled experiment.
2. **Test Setup:** Two sets of Anaerobic digesters that simulate septic tanks were setup – First set was used to understand natural degradation of milk wastes, second set was setup with the apt quantity of Draynzyme 2000A sustained release enzyme pack. Before beginning the tests, each digester was allowed to develop a basal micro flora: in control digesters the micro flora was dictated by degradation of milk solids, whereas in Draynzyme digesters, DWE-2000A was allowed to release enzymes for 24 hours prior to starting tests. Approximately 320mg/L of predefined COD value organic waste consisting of milk wastes was added to digesters. The COD (mg/L) were observed on the first day at 0 hrs, 3 hrs, 6 hrs, 12 hrs, 18 hrs, and 24 hrs. Thereafter fresh organic waste equivalent to approximately 320mg/L of COD value was added to the digesters to simulate continuous (same as in septic tanks) and again the COD values were monitored at time of fresh feed, and thereafter at 30 hrs, 36 hrs, 42 hrs, 48 hrs. Again fresh organic waste equivalent to 320mg/L of COD value was fed into the digesters and COD values were monitored at time of adding (49 hrs), and thereafter at 54 hrs, 60 hrs, 66 hrs, and 72 hrs. All tests were triplicates to verify reproducibility of results.

Results:

Time (Hours)	Control (Natural degradation) avg. COD values (mg/L)	Draynzyme 2000A influenced avg. COD values (mg/L)
0	321.67± 7.64	326.67 ± 11.72
3	311.33± 3.51	289.33 ± 5.13
6	279.67± 4.51	200.33 ± 4.51
9	221.00± 2.65	141.33 ± 7.09
12	182.67± 5.51	85.33 ±4.16
18	128.00± 4.00	52.33 ±7.51
24	109.67± 6.81	33.00 ±4.58
25	435.67± 4.04	346.00 ± 11.53
30	350.00± 10.00	266.67 ± 2.89
36	214.67± 4.51	105.00 ±4.58
42	147.67± 4.51	45.67 ±5.51
48	113.33± 8.33	25.67 ±3.06
49	435.67± 4.04	349.33 ± 4.04
54	353.00± 6.24	273.67 ± 4.73
60	209.67± 5.51	105.67 ± 5.51
66	143.67± 8.62	43.00 ±3.06
72	108.33± 7.64	23.33 ±3.21

	Control (Natural degradation)	Draynzyme 2000A
Best-fit values		
Slope	-9.909 ± 0.5203	-12.82 ± 1.073
Y-intercept when X=0.0	323.9 ± 6.726	293.1 ± 13.87
X-intercept when Y=0.0	32.69	22.86
1/slope	-0.1009	-0.07800
95% Confidence Intervals		
Slope	-11.00 to -8.820	-15.07 to -10.57
Goodness of Fit		
r^2	0.9502	0.8825
Sy.x	18.67	38.52
Is slope significantly non-zero?		
F	362.7	142.7
DFn, DFd	1.000, 19.00	1.000, 19.00
P value	< 0.0001	< 0.0001
Deviation from zero?	Significant	Significant
Data		
Number of X values	7	7
Maximum number of Y replicates	3	3
Total number of values	21	21
Number of missing values	0	0

Are the slopes equal?
F = 5.96369. DFn=1 DFd=38
P=0.01937
If the overall slopes were identical, there is a 1.9% chance of randomly choosing data points with slopes this different. You can conclude that the differences between the slopes are significant.
Because the slopes differ so much, it is not possible to test whether the intercepts differ significantly.



Interpretation: It can be seen that Draynzyme influenced digesters process more waste in the same unit time.

V.R.Thakkar
(This is a digital signature)
Report by: Dr. Vasudev Thakkar.

Certification of Products' Anti-Corrosive Nature:

TEST REPORT

MSME Regd. No. 24619-2-0281 & VUDA REGD. NO. UDA/PLAN-052/AV2005

Test Report No.: GES/SOIL/VAD-141120-02/01 **Date : 19-11-2014**

Name of Customer	:	Dhara Biotech, Sarsa, Dist.: Vadodara
Name of Owner	:	---
Consultants	:	---
Name of Project	:	General inspection
Material Received	:	Drayn zyme sewage aid pack
Date of Sample receipt	:	Date: 10-11-2014
Condition of Sample when Received	:	Soil Cakes wrapped in cloth
Date of Sample Testing	:	Dt: 11-11-2014 to 19-11-2014
Letter Reference No.	:	Letter Dated: 10-11-2014
Source of Sample & Sample ID	:	Sample 1

RESULTS:

Sr. No.	Paramters	Result	Unit
1	Silica, SiO_2	55.8	%
2	Iron, Fe_2O_3	8.5	%
3	Alluminium, Al_2O_3	5.2	%
4	Sodium, Na	0.04	%
5	Chloride, Cl	0.16	%
6	Mangenese, Mn	0.069	%

Remark: The sample tested comprises of major Silica. Soluble Chlorides causing corrosiveness is less than 0.5%. Hence, the sample is mild towards corrosiveness to concrete elements in contact.

FOR GEO ENGINEERING SERVICES, VADODARA

Authorized Signatory
Name: Abhay Dubey
Designation: (Reporting In Charge)

NOTE:

1. Sample have been submitted by customer
2. This Report shall not be reproduced except in full, without approval of the laboratory.
3. Results given above refer only to the samples submitted for testing.

Page 01 of 01

CSIR - National Environmental Engineering Research Institute (NEERI) Test Report:

सी.एस.आई.आर. – राष्ट्रीय पर्यावरण अभियांत्रिकी अनुसंधान संस्थान
CSIR - National Environmental Engineering Research Institute
(वैज्ञानिक तथा औद्योगिक अनुसंधान परिषद, / Council of Scientific & Industrial Research)
(वैज्ञानिक तथा औद्योगिक अनुसंधान परिषद, विज्ञान एवं प्रौद्योगिकी मंत्रालय, भारत सरकार के अंतर्गत स्वायत्त संगठन)
(Autonomous Organisation under the Dept. of Scientific and Industrial Research, Ministry of Science & Technology, Govt. of India)
NEERI

परिष्कृत पर्यावरणीय विश्लेषणात्मक सुविधा
Sophisticated Environmental Analytical Facility

Date	15/06/2023	Test Report No.	SEAF / JUNE 2023-24/ 03

TEST REPORT

Name and Address of the Customer:	**To** **The Chief Engineer (Electrical)** **Malni: Saaran Department,** **Pune Municipal Corporation (PMC), Pune, Maharashtra**
Customer References	(1) Letter No. 323 dt. 19.05.2023 addressed to Director, CSIR-NEERI, Nagpur from Pune Municipal Corporation (2) Letter No. DO-5907/SJM-Mum/2023 dated 11.05.2023 addressed to Director, CSIR-NEERI, Nagpur through Office of Hon'ble Shri Ramdas Athawale Ji, Minister of State for Social Justice & Empowerment, Govt. of India.

Sample Particulars:	
Sample Received & Date	Through Courier on dated 23.05.2023
Materials to be tested	Water Samples
Sampling Done By	Client, PMC (Samples received from Quinquent Industries Pvt. Ltd. Through PMC)
Number of Samples	2
Date of Start of Analysis	09.06.2023
Date of Completion of Analysis	15.06.2023

ANALYSIS RESULTS

Sr. No.	Parameters	Water Sample (Before Treatment)	Water Sample (After Treatment)	Test Method
1.	pH	6.7	6.8	APHA 23rd Edition-2017, 4500-H+ Electrometric Method
2.	Total Suspended Solids, mg/L	98	18	APHA 23rd Edition-2017, 2540 D: Gravimetric Method
3.	COD, mg/L	62.08	23.28	APHA 23rd Edition-2017, 5220 B: Open Reflux Method

Contd.....

Page 1/2

नेहरू मार्ग, नागपुर 440 020 भारत - Nehru Marg, Nagpur 440 020 INDIA
Ph (O) : +91-712-2249885-88, Ext. No. : 323, Direct No. : +91-712-2249764, Mobile No. : +91-9422304282
Email : skr_singh@neeri.res.in, URL : www.neeri.res.in
सतत विकास की ओर / Towards Sustainable Development

सी.एस.आई.आर. - राष्ट्रीय पर्यावरण अभियांत्रिकी अनुसंधान संस्थान
CSIR - National Environmental Engineering Research Institute
वैज्ञानिक तथा औद्योगिक अनुसंधान परिषद, / Council of Scientific & Industrial Research)
(वैज्ञानिक तथा औद्योगिक अनुसंधान परिषद, विज्ञान एवं प्रौद्योगिकी मंत्रालय, भारत सरकार के अंतर्गत स्वायत संघटन)
(Autonomous Organisation under the Dept. of Scientific and Industrial Research, Ministry of Science & Technology, Govt. of India)
NEERI

परिष्कृत पर्यावरणीय विश्लेषणात्मक सुविधा
Sophisticated Environmental Analytical Facility

Sr. No.	Parameters	Water Sample (Before Treatment)	Water Sample (After Treatment)	Test Method
4.	BOD, mg/L	BDL	BDL	APHA 23rd Edition-2017, 5210 B: 3 Day BOD Test
5.	Sulphide (S^{2-}), mg/L	18.6	3.2	APHA 23rd Edition-2017, 4500-S2- F. Iodometric Method
6.	E. Coli (CFU/100 ml)	60	10	APHA 23rd Edition-2017, 9222 H: EC-MUG Broth Method

NOTE:

*Indicates information supplied by the customer for which the laboratory has no control.

- SAMPLING NOT DONE BY THE TESTING LABORATORY & TEST(S) CONDUCTED ON SAMPLE(S) AS RECEIVED.
- RESULT(S) RELATE TO THE PARTICULAR SAMPLE(S) RECEIVED FOR TESTING
- ANY CORRECTION TO THIS REPORT INVALIDATES THIS REPORT.
- THE CONTENTS OF THE REPORT SHALL NOT BE REPRODUCED EITHER IN FULL OR IN PART FOR ARBITRATION, PUBLICITY AND AS AN EVIDENCE IN LEGAL DISPUTE WITHOUT PRIOR WRITTEN CONSENT OF THE ISSUING AUTHORITY
- ALL DISPUTES ARE SUBJECTED TO NAGPUR JURISDICTION.

15/06/2023

Scientist and Head, SEAF

डॉ. संजीव कुमार सिंग / Dr. Sanjeev Kumar Singh
वरिष्ठ प्रधान वैज्ञानिक एवं प्रमुख / Sr. Principal Scientist & Head
परिष्कृत पर्यावरणीय विश्लेषणात्मक सुविधा / Sophisticated Environmental Analytical Facility
सी.एस.आई.आर.–राष्ट्रीय पर्यावरण अभियांत्रिकी अनुसंधान संस्थान
CSIR-National Environmental Engineering Research Institute
नेहरू मार्ग, नागपुर-440020(भारत) / Nehru Marg, Nagpur-440020(INDIA)

नेहरू मार्ग, नागपुर 440 020 भारत - Nehru Marg, Nagpur 440 020 INDIA
Ph (O) : +91-712-2249885-88, Ext. No. : 323, Direct No. : +91-712-2249764, Mobile No. : +91-9422304282
Email : skr_singh@neeri.res.in, URL : www.neeri.res.in
सतत विकास की ओर / Towards Sustainable Development

Field Test Reports:

Panaji PWD testing for Sewage Pipeline cleaning:

No. 2/01/13-14/WDIII(PHE)/PWD/ADM/1693
Government of Goa,
Office of the Executive Engineer,
Works Division III(PHE-N)
P.W.D., St-Inez,
Panaji-Goa.

Dated:- 13/01/2014.

To,
ECO TECHNOLOGIES
G-12, Angariki Bldg, Opp fire station,
Ponda Goa.

Sub:- confirmation on usage and the effectivity of DraynZyme

Sir,

We confirm having used "DraynZyme Sewage Aid" an In-Line Digestion (ILD) Enzyme based sewage treatment solution in a section of our sewage network to verify the publicised performance.

Preliminary experience during initial tests using DraynZyme Sewage Aid have shown:

a. Elimination of foul odour
b. Clearing of blockages in our 6" pipe lines
c. Digestion of sludge in chambers, manholes and grease traps

Satisfied with the interim results we propose to use DraynZyme ILD packs in a larger section of our sewage network to further substantiate and document its utility in the sewage network maintenance.

Yours faithfully

(Dileep M. Dhavalikar)
Executive Engineer, III, PWD

No. F 31 /13-14/PWD/WDIII SDII JE/ 120

Government of Goa,
Office of the Assistant Engineer,
Sub-Div-II,WDIII (PHE)
Public Works Department,
Tonca, Caranzalem - Goa

Dated: 28/11/2013.

REPORT

Name of Work :- Dousing of Sewage chambers with ILD (in line digestion) draynzyme pack under pilot project.

Estimated Cost :- Rs. 4,95,600/- (Rupees Four lakhs Ninety five thousand Six hundred only) including 5% contingency.

Budget Head :- 2215 – Water Supply & Sanitation,
02 – Sewage & Sanitation,
107 – Sewage Service,
01 – Sewage Treatment Plant,
27 – Maintenance (N.P)

History :- This office is looking after the maintenance work of the sewer lines of Panaji city & surrounding areas. A couple of months back Eco Technologies approached this office stating that they have developed some enzymes which help in dissolving the FOG (Fats, Oils, Grease). Trail packs were installed at various points and it was found to be quiet effective. A demonstration / conference was also held at the Head Office regarding this product.

Eco Technologies are dealers in septic management products & solutions who have submitted a proposal to douse around 32 manholes upstream with these enzymes pack. This office was directed to take up the scheme as a Pilot project in which these enzyme packs have been developed as a standardized product considering the maximum flow rate through a 6" diameter pipe line to suffice for 3months. Based on the above this office is forwarding the estimate for dousing 32 manholes with Draynzyme ILD Aid (DWE-2005) with the rates submitted by the company "Eco Technologies" which seem to be a proprietary product.

Time :- 60 (15) days (considering dousing of around 3 manholes per day).

Method :- By call of Tender.

Submitted for necessary perusal please.

Assistant Engineer – II

Pr perusal immediately

E.E. III

ASW 28/11/13

No. PWD/D.III/PHE/ASW/F 42/2013-2014/ 8244
Government of Goa,
Office of the Executive Engineer,
Division-III(PHE), P.W.D.,
St.Inez, Panaji-Goa

Dated :24 /12/ 2013.

NOTE

Sub: Dousing of Sewage chambers with ILD (in line digestion) draynzyme pack under pilot project

With reference to order No: 7-5-2012/Fin (Exp)-III dated: 23.05.2012 from the Finance Department, necessary Administrative and Financial sanction may be obtained from the Principal Chief Engineer, PWD to take up the following work under maintenance Budget Head: 2215/01/101/01/27-Maintenance works, Also approval may be accorded to tender the work as per provisions of CPWD Manual 2007.

Sr.No.	Name of work	Estimate cost
1.	Dousing of Sewage chambers with ILD (in line digestion) draynzyme pack under pilot project	Rs. 4,95,600.00

Submitted for obtaining Administrative and Financial sanction from the Principal Chief Engineer ,PWD.

(Executive Engineer- III

SE.V Submitted for approval
30/12/

CE(A)

4089 2/1/14

GOVERNMENT OF GOA
OFFICE OF THE ASSISTANT ENGINEER
SUB-DIV-II, WDIII (PHE)
PUBLIC WORKS DEPARTMENT
TONCA CARANZALEM GOA

ABSTRACT

Name of Work :- Dousing of Sewage chambers with ILD (in line digestion) draynzyme pack under pilot project

Item No.	Description of item	Unit	Qty	Rate	Amount
1	Draynzyme ILD Aid (DWE-2005). In Line Digestion Enzyme pack for Treating maximum flow rates in 6" diameter metro municipal sewage pipes Designed to last/provide sustained release of Enzymes for a period of upto 90 days Approx. weight of each ILD Aid Enzyme pack is 3 6 kgs., Contents: Agar Gum Gel containing Enzymes packed in a polyester sack with 15 ft string.	Nos.,	32 00	14250 00	456000 00
2	Cost of Dousing per Manhole	Nos	32 00	500 00	16000 00
				Total ..... Rs.	**472000.00**
				Add 5% contigencies	23600 00
				Total ..... Rs.	**495600.00**
				Say..... Rs.	**4,95,600.00**

(Rupees - Four lakhs Ninety five thousand Six hundred only)

Junior Engineer

Assistant Engineer-II

Executive Engineer-III

ANNEXURE - II
Letter for Commencement of Work/Work Order

Registered A/D

No. 3-6/PWD/PHE/WDIII/Accts/ WOT-351 /2013-2014.
Government of Goa,
Office of the Executive Engineer,
Works Division III (PHE), P.W.D.,
St. Inez Panaji Goa.

Dated: 24/02/2014

To,

ECO Technologies,
G-12, 'AGARKI' Bldg.,
Opp. Fire Station,
Ponda – Goa.

Sub: Dousing of sewage chambers with ILD (in line digestion) draynzyme pack under Pilot project.

Ref:.1.Performance Bank Guarantee submitted by you vide your letter dt 24-2-2014.

2. This Office letter of intent/acceptance of your tender.

No. 3-6/PWD/PHE/WDIII/Accts/1990//2013-14 dated 21-2-2014.

Tendered Cost: Rs. 4,95,364.00

Estimated Cost: Rs.4,72,000.00

Sir,

You are requested to contact the Asst. Engineer, Sub. Div. II for taking possession of site and starting the work at once.

In continuation to the letters referred to above, you are requested to attend this office to complete the formal agreement within fifteen days from the date of this letter.

Date of commencement: 11/03/2014

Date of completion: 10/05/2014

Expenditure is debatable to

Budget Head: 2215/02/107/01/27-Minor works.

Yours faithfully,

Executive Engineer-III
FOR AND ON BEHALF OF GOVERNOR OF GOA

ECO
TECHNOLOGIES
G-12. "ANGARIKI" Bldg, Opp Fire Station, Ponda, GOA-403401.
Email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

To, 10/05/2014

The Exe Engineer,
Works Division III (PHE), P.W.D.,
St. Inez Panji, GOA.

SUB: Report Submission on Dousing of Enzymes in 32 manholes.

Respected Sir,

As per the order we had received No 3-6/PWD/PHE/WDIII/Accts/WOT-357/2013-14
We have completed the dousing of 32 manholes in and around Panjim City. We found some manholes to be critical in their respective underground sewage lines and few clear since these manholes were cleaned for clogging's.

In the adjoining pages we have mentioned the details of location and the condition of the manhole at the time of dousing.

Please find the details in our next pages.

Thanking you,
Warm Regards,

For Eco Technologies.

CC: To, Asst Engineer, SUB Div II. , along with soft copy.

ECO
TECHNOLOGIES
G-12. "ANGARIKI" Bldg, Opp Fire Station, Ponda, GOA-403401.
Email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

Place 1:Opp Hotel Solmar.

Condition: Drain Pit filled with F(fats) O(oils) G(grease)

ECO
TECHNOLOGIES
G-12. "ANGARIKI" Bldg, Opp Fire Station, Ponda, GOA-403401.
Email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

Place 2:Opp Pan Asia.

Condition: Drain Pit filled with F(fats) O(oils) G(grease)

ECO
TECHNOLOGIES
G-12. "ANGARIKI" Bldg, Opp Fire Station, Ponda, GOA-403401.
Email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

Place 3: Food Land (Miramar Circle).

Condition: Drain Pit filled with F(fats) O(oils) G(grease)

ECO
TECHNOLOGIES
G-12. "ANGARIKI" Bldg, Opp Fire Station, Ponda, GOA-403401.
Email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

Place4:Health Directorate.

Condition: Drain Pit filled with F(fats) O(oils) G(grease)

ECO
TECHNOLOGIES
G-12. "ANGARIKI" Bldg, Opp Fire Station, Ponda, GOA-403401.
Email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

Place5: Market Parking Area

Condition: Drain Pit was clear with Sludge due to recent cleanings

ECO
TECHNOLOGIES
G-12. "ANGARIKI" Bldg, Opp Fire Station, Ponda, GOA-403401.
Email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

Place6: Inside Market.

Condition: Drain Pit was clear with Sludge due to recent cleanings

ECO
TECHNOLOGIES
G-12. "ANGARIKI" Bldg, Opp Fire Station, Ponda, GOA-403401.
Email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

Place7: Palacio De Goa

Condition: Drain Pit was clear with Sludge due to recent cleanings

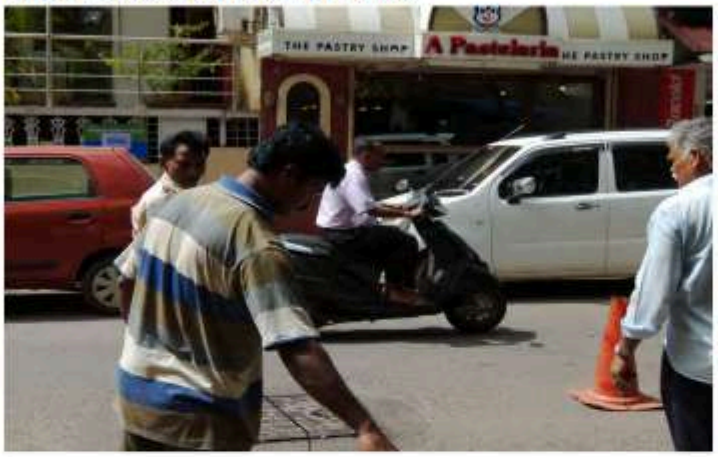
ECO
TECHNOLOGIES
G-12. "ANGARIKI" Bldg, Opp Fire Station, Ponda, GOA-403401.
Email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

Place8: Next to GOENCHIN.

Condition: Drain Pit was with Sludge mainly Fats Oils & Grease from kitchen.

ECO
TECHNOLOGIES
G-12. "ANGARIKI" Bldg, Opp Fire Station, Ponda, GOA-403401.
Email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

Place9: Next to PASTELARIA.



Condition: Drain Pit was with Sludge mainly Fats Oils & Grease from kitchen.

ECO
TECHNOLOGIES
G-12. "ANGARIKI" Bldg, Opp Fire Station, Ponda, GOA-403401.
Email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

Place 10:Opp to Redtape.

Condition: Drain Pit was with Sludge mainly Fats Oils & Grease from kitchen.

ECO
TECHNOLOGIES
G-12. "ANGARIKI" Bldg, Opp Fire Station, Ponda, GOA-403401.
Email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

Place 11: Near Sai Baba Temple.

Condition: Drain Pit was with clear with Sludge due to rescent removal.

ECO
TECHNOLOGIES
G-12. "ANGARIKI" Bldg, Opp Fire Station, Ponda, GOA-403401.
Email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

Place 12: Behind Fidalgo(oppSamrat).

Condition: Drain Pit was filled with Sludge due to Fats Oils & Grease.

ECO
TECHNOLOGIES
G-12. "ANGARIKI" Bldg, Opp Fire Station, Ponda, GOA-403401.
Email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

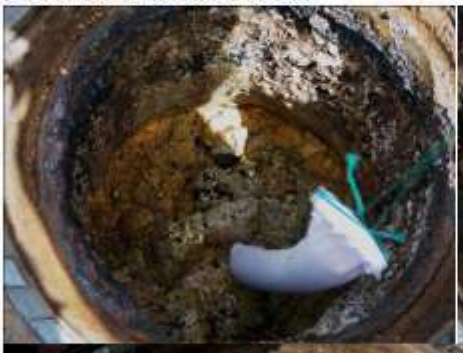
Place 13: Behind Fidalgo(opp Annapurna).

Condition: Drain Pit was filled with Sludge due to Fats Oils & Grease and was solidified.

ECO
TECHNOLOGIES
G-12. "ANGARIKI" Bldg, Opp Fire Station, Ponda, GOA-403401.
Email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

Place 14:In front of World Of Titan.

Condition: Drain Pit was filled with Sludge due to Fats Oils & Grease and was solidified hard.



ECO
TECHNOLOGIES
G-12. "ANGARIKI" Bldg, Opp Fire Station, Ponda, GOA-403401.
Email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

Place 15:In front of Urban Health.

Condition: Drain Pit was filled with Sludge due to Fats Oils & Grease.

ECO
TECHNOLOGIES
G-12. "ANGARIKI" Bldg, Opp Fire Station, Ponda, GOA-403401.
Email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

Place 16:In front of Bharne& Co near National Theater.

Condition: Drain Pit was clear with Sludge due to recent cleanings.

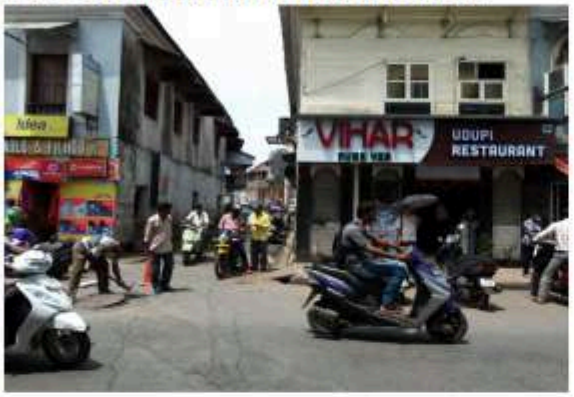
ECO
TECHNOLOGIES
G-12. "ANGARIKI" Bldg, Opp Fire Station, Ponda, GOA-403401.
Email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

Place 17: In front of Lawrence & Mayo near National Theater.

Condition: Drain Pit was clear with Sludge due to recent cleanings.

ECO
TECHNOLOGIES
G-12. "ANGARIKI" Bldg, Opp Fire Station, Ponda, GOA-403401.
Email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

Place 18: In front of Hotel VIHAR.



Condition: Drain Pit was clear with Sludge due to recent cleanings.

ECO
TECHNOLOGIES
G-12. "ANGARIKI" Bldg, Opp Fire Station, Ponda, GOA-403401.
Email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

Place 19: In front of Hotel VIVA Panjim.

Condition: Drain Pit was clear with Sludge due to recent cleanings.

ECO
TECHNOLOGIES
G-12. "ANGARIKI" Bldg, Opp Fire Station, Ponda, GOA-403401.
Email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

Place20: In front of IOB near Hotel VIVA Panjim.

Condition: Drain Pit was clear with Sludge due to recent cleanings.

ECO
TECHNOLOGIES
G-12. "ANGARIKI" Bldg, Opp Fire Station, Ponda, GOA-403401.
Email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

Place20: In front of IOB near Hotel VIVA Panjim.

Condition: Drain Pit was clear with Sludge due to recent cleanings.

ECO
TECHNOLOGIES
G-12. "ANGARIKI" Bldg, Opp Fire Station, Ponda, GOA-403401.
Email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

Place 22: Near Kamat Hotel next to Panjim Church.

Condition: Drain Pit was found with Sludge due to Fats Oils & Grease.

ECO
TECHNOLOGIES
G-12. "ANGARIKI" Bldg, Opp Fire Station, Ponda, GOA-403401.
Email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

Place 23:Opp Mitsubishi Electric near National Theater.

Condition: Drain Pit was found with Sludge due to Fats Oils & Grease.

ECO
TECHNOLOGIES
G-12. "ANGARIKI" Bldg, Opp Fire Station, Ponda, GOA-403401.
Email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

Place 24:Opp Goa Urban bank behind Kamat hotel.

Condition: Drain Pit was found with Sludge due to Fats Oils & Grease.

ECO
TECHNOLOGIES
G-12. "ANGARIKI" Bldg, Opp Fire Station, Ponda, GOA-403401.
Email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

Place 25: Near Marigold hotel at Patto.

Condition: Drain Pit was found with Sludge due to Fats Oils & Grease.

ECO
TECHNOLOGIES
G-12. "ANGARIKI" Bldg, Opp Fire Station, Ponda, GOA-403401.
Email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

Place 26: Next to PILAR Office behind Hotel Ruchi.

Condition: Drain Pit was found with Sludge due to Fats Oils & Grease.

ECO
TECHNOLOGIES
G-12. "ANGARIKI" Bldg, Opp Fire Station, Ponda, GOA-403401.
Email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

Place 27:Opp PAULO Travels.

Condition: Drain Pit was found with Sludge due to Fats Oils & Grease.

ECO
TECHNOLOGIES
G-12. "ANGARIKI" Bldg, Opp Fire Station, Ponda, GOA-403401.
Email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

Place 28: at PATTO PLAZA.

Condition: Drain Pit was found with Sludge due to Fats Oils & Grease.

ECO
TECHNOLOGIES
G-12. "ANGARIKI" Bldg, Opp Fire Station, Ponda, GOA-403401.
Email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

Place 29: at SULABH Complex in KTC.

Condition: Drain Pit was found with Sludge.

ECO
TECHNOLOGIES
G-12. "ANGARIKI" Bldg, Opp Fire Station, Ponda, GOA-403401.
Email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

Place30: at DEMPO TOWERS.

Condition: Drain Pit was found with Sludge full of kitchen waste.

ECO
TECHNOLOGIES
G-12. "ANGARIKI" Bldg, Opp Fire Station, Ponda, GOA-403401.
Email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

Place 31: at KTC Kamat Hotel.

Condition: Drain Pit was found with Sludge full of kitchen waste.

ECO
TECHNOLOGIES
G-12. "ANGARIKI" Bldg, Opp Fire Station, Ponda, GOA-403401.
Email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

Place 32:Near Hotel Mandovi.

Condition: Drain Pit was clear of sludge due to recent cleanings.

ECO TECHNOLOGIES

G-12, "ANGARIKI" Bldg, Opp Fire Stn.,
Ponda, GOA-403401.
email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

To,

05/07/2014

The Exe Engineer,
Works Division III (PHE), P.W.D.,
St. Inez Panji, GOA.

SUB: Report Submission on Observation of 5 manholes found critical during the dousing .

Respected Sir,

With reference to the order received No 3-6/PWD/PHE/WDIII/Accts/WOT-357/2013-14 for dousing of Enzymes in 32 Manholes around Panjim we made a follow up study on 5 manholes found critical during the time of dousing. The details is as follows:

- In all these manholes either soft sludge or No Sludge was found.
- Sludge found was fresh.
- The level of the previously found sludge had receded.

Conclusion after 45 days period:

- The Enzymes have been found doing its work as desired.
- Due to enzyme pack's presence; the sludge has softened up allowing it to flow further without getting hardened in the manholes.
- We feel the clogging in the pipelines is also getting cleared, since only fresh sludge is visible.
- In this period of 45 days; if at all the concerned department had got clogging complaints in the respective doused manhole areas then it is because of the chunk of the hardened sludge which got released at a time causing temporary block in the system.

The details in picture form with analysis is explained on individual manholes found critical during the time of dousing.

Warm Regards,

For Eco Technologies.
CC: To, Asst Engineer, SUB Div II.

ECO TECHNOLOGIES

G-12. "ANGARIKI" Bldg, Opp Fire Stn.,
Ponda, GOA-403401.
email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

Place: Behind Fidalgo (opp Annapurna).

Observations on the day of dousing:

1. As we see in the picture this drain pit is filled with Sludge with Oil and Grease. We can see the sludge been hardened due to the binding property of the Oil and Grease.
2. We can see the surrounding layer of the sludge around the Enzyme pack in black n grey shade due the hardness of it.

Observation made after 45 days from the dousing:

1. Clear water is been seen.
2. Either the sludge has been softened and flown further down the line or the softened sludge must have chocked the line due to which physical removal of it must have happened.
3. Due to clearance of the hardened sludge fresh sewage is flowing un notices as it is.

ECO TECHNOLOGIES

G-12. "ANGARIKI" Bldg, Opp Fire Stn.,
Ponda, GOA-403401.
email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

Place: Behind Fidalgo (opp Samrat).

Observations on the day of dousing:

1. Drain pit (manhole) is filled with thick sludge.
2. This manhole is located next to the previous observed manhole where we had found hardened sludge.
3. Here the sludge is not hardened but neither it is fresh or watery, its found to be thick.
4. This can happen because its not getting to flow as fresh to further down the line.

Observation made after 45 days from the dousing:

1. The picture itself is self explanatory.
2. No deposited sludge been found.
3. All visible is smoothly flowing fresh sludge.
4. This is a clear condition when the further pipeline and manholes are not blocked due the sedimentation of the sludge.
5. Seen in the picture is the bottom of the pit.

ECO TECHNOLOGIES

G-12. "ANGARIKI" Bldg, Opp Fire Stn.,
Ponda, GOA-403401.
email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

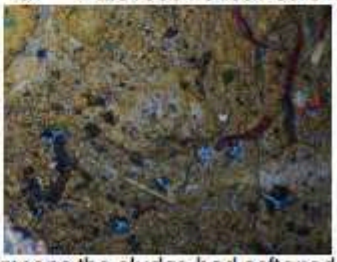
Place: In front of World Of Titan.

Observations on the day of dousing:

1. We can see in this picture that the sludge has hardened due to Fats Oils and Grease.
2. Hardened so much that the enzyme pack is not getting in it.
3. On the day of dousing we had to dig the enzyme pack in to the sludge hard enough.

Observation made after 45 days from the dousing:

1. sludge is found to be fresh even red chilly flown from kitchen discharge is visible .
2. Water is seen at the top.
3. Water been observed on top



means the sludge had softened enough to flow further is journey to get treated.

ECO TECHNOLOGIES

G-12. "ANGARIKI" Bldg, Opp Fire Stn.,
Ponda, GOA-403401.
email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

Place: Next to PASTELARIA.

Observations on the day of dousing:

1. accumulated sludge mainly of commercial discharge with Fats, Oil, Grease was found.
2. Found sludge was in semisolid condition not hardened though.

Observation made after 45 days from the dousing:

1. Manhole is found clear of sludge.
2. As seen in the picture, the drain pipelines are visible.
3. The conclusion here is that the sludge is flowing as fresh as it is without getting blocked even in the pipelines.

ECO TECHNOLOGIES

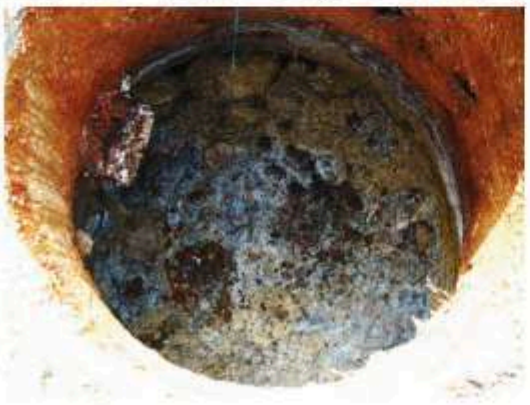
G-12. "ANGARIKI" Bldg, Opp Fire Stn.,
Ponda, GOA-403401.
email: support@eco-tech.in
Ph: +91 9823352268 / +91 9326127275

Place: In front of Hotel VIHAR.

Observations on the day of dousing:

1. sludge from commercial kitchen was found hardened with cracks formed in the top layer.
2. This hardening is mainly due to the Oils and Grease discharge from the kitchens which acts as a binding agent for the sewage.

Observation made after 45 days from the dousing:



1. Water is found on the top.
2. The sludge is found softened and disintegrated.

3. Since the sludge is found softened it should be free flowing.

Military Engineering Services, Indian Army, Pune Cantonement:

Tele: 26331157

Military Engineer Services
Headquarters
Chief Engineer Pune Zone
Dakshin Kaman Marg
Pune- 411 001

46032 / 104 /E4

05 Nov 2014

CWE Kirkee Pune
CWE Pune

EFFLUENT MANAGEMENT BY IN LINE DIGESTION (ILD) PROCESS

1. M/S Dharastan Tech Pvt Ltd has introduced Effluent Management by ILD Process and has approached HQ Chef Engineer Southern Command and this office for Conduct of trial on Septic Tank / STP on **NO COST BASIS**.

2. In view of the above the reps of the firm are directed to your office and concerned GEs for the purpose of carrying out ibid trial at the following locations :-

(a) Septic Tank behind Maitri shopping Complex under GE (North) Pune.

(b) One of the Septic Tank inside BEG Complex which is also receiving organic food waste from a cook house under GE (Central) Kirkee.

(c) One of the Septic Tank inside Aundh Complex under GE (MH & RH) Pune.

3. The Literature and necessary tech in put shall be provided by the firm to the executives. The performance report / feedback on the trials carried out at above locations (Pre & Post Trial Test report) should be submitted to this HQ for information of Chief Engineer and for onward transmission to HQ Chief Engineer Southern Command.

4. This has the approval of Chief Engineer Pune Zone.

(NK Sharma)
SE (NFG)
Jt Dir (U)
for Chief Engineer

Copy to :

HQ CESC Pune - for info Please. W.r.t your HQ letter No 400000/WWR/46/E4 dated 31 Oct 2014.

GE (Central) Pune
GE (North) Pune
GE (MH & RH) Pune

Internal

E2 (Plg) - for info please.

CC

GreenDHARA
info@dharastan.in
www.dharastan.in

Ref.: DT/150107/SC-MES

7th January, 2015

MES – IDSE,
Chief Engineer - Pune Zone,
Pune 411 001

Kind attn.: Shri. S. K. Jain - IDSE, Chief Engineer Pune Zone

Sub.: Trial Report – Effluent Management - DraynZyme ILD Process
Enzyme based treatment solutions for Sewage Networks

Dear Sir,

We attach herewith our detailed analysis report for "DraynZyme - In-Line Digestion" treatment process trials conducted in the presence of respective MES representatives for the sanctioned sites ...

1. The treatment process was initiated on the 29th of November 2104 when effluent (waste-water) samples from the sites were collected and submitted to an accredited and approved laboratory for an evaluation (before treatment) test report.

2. Challenges to overcome ... objectives of the treatment.
   a. Elimination of foul odour caused by sulfur based gases and precipitate a bioprocess to release safe gases.
   b. Liquefy the existing sludge that has accumulated and solidified over time.
   c. Once softened... Revitalize existing microbes to aid digestion to prevent sediments.
   d. Digestion of fats and oils binding the organic sludge to remove blockages and facilitate easy flow.
   e. Treated effluent to conform to MPCB discharge norms for recycling or disposal.

3. The appropriate sites were doused with recommended dosage of "DraynZyme" treatment packs and the outcome monitored on a weekly basis.

4. Effluent (treated water) samples were once again collected on the 29th of December 2014 (30 days later) and submitted for test analysis at the same accredited laboratory.

GreenDHARA
info@dharastan.in
www.dharastan.in

Ref.: DT/150107/SC-MES

7th January, 2015

MES – IDSE,
Chief Engineer - Pune Zone,
Pune 411 001

Kind attn.: Shri. S. K. Jain - IDSE, Chief Engineer Pune Zone

Sub.: Trial Report – Effluent Management - DraynZyme ILD Process
Enzyme based treatment solutions for Sewage Networks

Dear Sir,

We attach herewith our detailed analysis report for "DraynZyme - In-Line Digestion" treatment process trials conducted in the presence of respective MES representatives for the sanctioned sites ...

1. The treatment process was initiated on the 29th of November 2104 when effluent (waste-water) samples from the sites were collected and submitted to an accredited and approved laboratory for an evaluation (before treatment) test report.

2. Challenges to overcome ... objectives of the treatment.
   a. Elimination of foul odour caused by sulfur based gases and precipitate a bioprocess to release safe gases.
   b. Liquefy the existing sludge that has accumulated and solidified over time.
   c. Once softened... Revitalize existing microbes to aid digestion to prevent sediments.
   d. Digestion of fats and oils binding the organic sludge to remove blockages and facilitate easy flow.
   e. Treated effluent to conform to MPCB discharge norms for recycling or disposal.

3. The appropriate sites were doused with recommended dosage of "DraynZyme" treatment packs and the outcome monitored on a weekly basis.

4. Effluent (treated water) samples were once again collected on the 29th of December 2014 (30 days later) and submitted for test analysis at the same accredited laboratory.

GreenDHARA
info@dharastan.in
www.dharastan.in

5. To summarize ... the results observed are detailed hereunder for your perusal:
   a. No foul odour experienced in manholes, sump or anywhere near open channel, verbally confirmed by canteen staff.
   b. Sludge accumulated over time was liquefied and degradation process activated and accelerated.
   c. No line choking observed since implementation of the "DraynZyme" treatment process.
   d. Oil and grease layer broke easily to allow visual observation of water below oil layer. Manholes with sludge found to be rock hard during the dousing of "DraynZyme" packs were found clean of scum layers with clear water visible.
   e. Lab analysis of the effluent post treatment shows marked improvement in performance, stability and conforming towards MPCB discharge norms.

The "DraynZyme" treatment process program has been successfully and convincingly demonstrated as documented and confirmed by the attached field trial reports.

To maintain continuity of treatment and prevent the recurrence of sludge buildup and reclogging of the manholes, chambers and pipe networks ... periodic, timely and sustained re-dousing of "DraynZyme" treatment packs is recommended without occurring delays.

We remain at your entire disposal for any further information, clarification or assistance that you may require.

Yours sincerely,

Capt. Pratap Bhonsle
Director
DHARASTAN Technologies

Dr. Urvik Patel
Executive Director
DHARASTAN Technologies

GreenDHARA
info@dharastan.in
www.dharastan.in

SITE 2.
Septic Tank - Depot Langar Area inside BEG complex:
Visit under supervision of: Mr. Prabhakar Shetty.

Initial water testing from the site shows the following reports collected on 29th November, 2014:
Report Number: HTL/14-15/1211
Sample Id: BEG CANTEEN INTERNAL WASHING: RAW WATER
Effluent sample collected from manhole chamber prior to outdoor dish and pot washing point which receives water from any washings taking place inside the canteen premise.
Report Number: HTL/14-15/1210
Sample Id: BEG (SUMP) CANTEEN WASHING: RAW WATER
Effluent sample collected from channel at the point of discharge, 2 feet from sump which receives fresh water from outdoor dish and pot washing area.

BEG COMPLEX CANTEEN – Depot Langar Area				
Effluent sample collected and submitted for analysis on ... 29.11.2014				
	REPORT NUMBER / DATE	INTERNAL WASH RAW WATER HTL/14-15/1211 05.12.2014	SUMP WASHING RAW WATER HTL/14-15/1210 05.12.2014	
SR.	PARAMETERS	VALUES		MPCB LIMITS
1.	pH	3.55	4.24	5.50 – 9.00
2.	Total Suspended Solids (ppm)	100.29	109.20	LESS THAN 100 ppm
3.	Chemical Oxygen Demand (mg O_2 per Liter)	4480.00	5800.00	LESS THAN 250 ppm
4.	Biological Oxygen Demand (mg O_2 per Liter) (3 days @ 27° C)	2900.00	3500.00	LESS THAN 100 ppm
5.	Oil and Grease (ppm)	18.00	26.00	LESS THAN 10 ppm
6.	Total Dissolved Solids (ppm)	1350.00	1200.00	LESS THAN 2100 ppm
7.	Chlorides as Cl (ppm)	567.20	531.75	LESS THAN 600 ppm
8.	Sulphates as SO_4 (ppm)	118.27	100.02	LESS THAN 1000 ppm

Note: As it can be noticed, both waters have very similar characteristics, with the BEG canteen washing samples showing increase in COD and BOD values as expected when fresh grey water from canteen is added to existing stream already containing very high COD and BOD values.

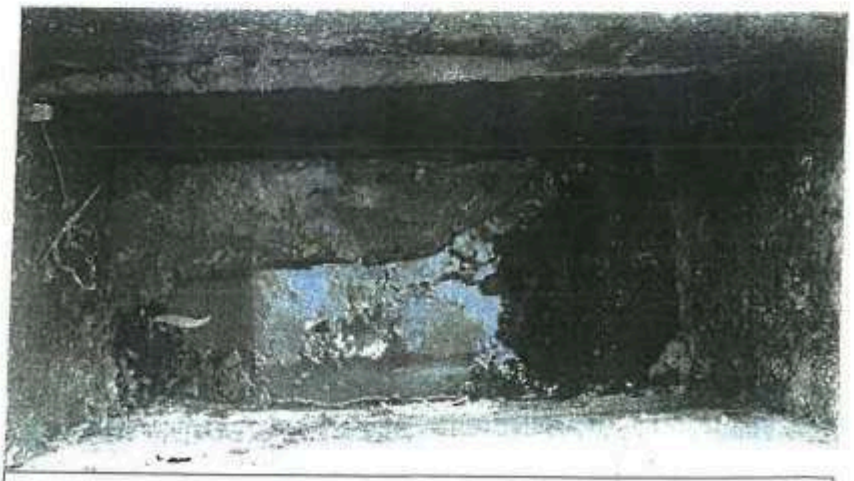
Other than high COD and BOD values, it was observed that:

1. **High levels of foul odour was experienced due to the conversion of Sulphates to Sulphides at both collection points, complaint verbally communicated by the canteen staff.**
2. **Collection Sump had very high levels of FOG layer on the surface not allowing water to be visually observed even when trying to partition it with a stick, thickness of oil and grease layer being approximately 10mm on the inlet side of sump.**
3. **No fermentation / bacterial degradation activity seen in the sump.**
4. **Canteen staff was complaining of frequent line choking by food particles.**

GreenDHARA
info@dharastan.in
www.dharastan.in

BEG Sump showing oil layer on the 29th of November, 2014

DraynZyme enzyme packs and were doused to match the load and flow rates on the 29th of November, 2014.



BEG Canteen manhole where choking is frequent

GreenDHARA
info@dharastan.in
www.dharastan.in

Second water testing on 15th December, 2014f shows the following reports:
Report Number: HTL/14-15/1329
Sample Id: BEG SUMP: WASHING
Effluent sample collected from sump on the inlet side where all washing water is collected, water from here leaches into the ground and also has to be pumped out for discharge.

BEG COMPLEX CANTEEN – Depot Langar Area			
Effluent sample collected and submitted for analysis on ... 15.12.2014			
	REPORT NUMBER / DATE	BEG SUMP - RAW WATER HTL/14-15/1329 20.12.2014	
SR.	PARAMETERS		MPCB LIMITS
1.	pH	6.02	5.50 – 9.00
2.	Total Suspended Solids (ppm)	23.22	LESS THAN 100 ppm
3.	Chemical Oxygen Demand (mg O_2 per Liter)	1185.00	LESS THAN 250 ppm
4.	Biological Oxygen Demand (mg O_2 per Liter) (3 days @ 27° C)	400.00	LESS THAN 100 ppm
5.	Oil and Grease (ppm)	10.92	LESS THAN 10 ppm
6.	Total Dissolved Solids (ppm)	350	LESS THAN 2100 ppm
7.	Chlorides as Cl (ppm)	93.94	LESS THAN 600 ppm
8.	Sulphates as SO_4 (ppm)	10.00	LESS THAN 1000 ppm

Observations:

1. There is an increase in the pH values increasing from the 3.5-4 range towards 6, allowing better bacterial growth.
2. Total suspended solids are much lower, probably due to the large settling time the chamber would provide.
3. Chemical oxygen demand is lower, assuming input COD values from earlier samples of 4500 mg/l, there is a 70% reduction in COD values
4. Biological oxygen demand is lower, there is a 85% reduction in BOD values.
5. Oil and grease values are marginally lower, probably due to fresh daily input, however, thick oil and grease layer observed on the 29th of November is now only a thin layer of approximately 4-5mm thick.
6. Total dissolved salts and Chloride concentrations are not affected by our enzyme or bacterial activities, any change is values reflected are due to daily fluctuations in water quality.
7. Sulphates are lower by 12%, normally indicates good bacterial activity as healthy bacterial activity will consume sulphates as secondary nutrient to make proteins.

Other observations:

a. No foul odour experienced in manholes, sump or anywhere near open channel, verbally confirmed by canteen staff.
b. No line choking observed since dousing of DraynZyme.
c. Oil and grease layer broke easily to allow visual observation of water below oil layer.

Copies of all original reports ... appended in Annexure-1 attached.

GreenDHARA
info@dharastan.in
www.dharastan.in

SITE 4.
Aundh - Old Married Quarters Septic tanks
Visit under supervision of: Mr. Rajesh

DraynZyme dousing done in chambers connected to the sewerage network and at the septic tanks on the 9th of December, 2014.
Quality of water in the system: At the Chambers level:
Report Number: HTL/14-15/1256
Sample Id: AUND 2: RAW WATER CHAMBERS
Report Number: HTL/14-15/1256
Sample Id: AUND 1: RAW WATER SEPTIC TANK

AUNDH – Old Married Quarters Septic Tanks				
Effluent sample collected and submitted for analysis on ... 09.12.2014				
	REPORT NUMBER / DATE	RAW WAER CHAMBERS HTL/14-15/1256 14.12.2014	RAW WATER SEPTIC TANK HTL/14-15/1255 14.12.2014	
SR.	PARAMETERS	VALUES		MPCB LIMITS
1.	pH	7.02	7.35	5.50 – 9.00
2.	Total Suspended Solids (ppm)	190.00	78.00	LESS THAN 100 ppm
3.	Chemical Oxygen Demand (mg O_2 per Liter)	293.33	215.80	LESS THAN 250 ppm
4.	Biological Oxygen Demand (mg O_2 per Liter) (3 days @ 27° C)	95.00	70.00	LESS THAN 100 ppm
5.	Oil and Grease (ppm)	3.00	Nil	LESS THAN 10 ppm
6.	Total Dissolved Solids (ppm)	580	650	LESS THAN 2100 ppm
7.	Chlorides as Cl (ppm)	177.25	248.15	LESS THAN 600 ppm
8.	Sulphates as SO_4 (ppm)	32.22	17.11	LESS THAN 1000 ppm

Observations on the 9th of December, 2014:

1. Foul odour at chamber level around the residences
2. Water in Chambers was reasonably high and even overflowing at lowest chamber due to blockages in pipeline
3. Very foul odour at the septic tank region due to overflow of water from septic tanks.
4. Septic tanks showing no signs of fermentation, water is black.

Observations on the 15th of December, 2014:

1. Foul odour at chamber level around the residences was not experienced.
2. Foul odour at Septic tank level also showed marked reduction in foul odour experiences.
3. Water in Septic tank started showing some signs of fermentation, water color is now dull gray.

We recommended and awaited the removal/clearing of blockage in pipeline network leading to the septic tanks which caused the overflowing at thus contaminating the water exiting the septic tank.

Once blockage was cleared and enough we will start taking observation of water quality.

Picture of overflow from manhole bypassing the septic tanks

Quality of water in the system:
At the Chambers level:
Report Numbers: HTL/14-15/1406 & HTL/14-15/1405

AUNDH – Old Married Quarters Septic Tanks				
Effluent sample collected and submitted for analysis on ... 29.12.2014				
	REPORT NUMBER / DATE	RAW WATER CHAMBER HTL/14-15/1406 03.01.2015	SEPTIC TANK HTL/14-15/1405 03.01.2015	
SR.	PARAMETERS	VALUES		MPCB LIMITS
1.	pH	6.12	7.40	5.50 – 9.00
2.	Total Suspended Solids (ppm)	189.20	19.20	LESS THAN 100 ppm
3.	Chemical Oxygen Demand (mg O_2 per Liter)	1660.00	100.00	LESS THAN 250 ppm
4.	Biological Oxygen Demand (mg O_2 per Liter) (3 days @ 27° C)	570.00	31.00	LESS THAN 100 ppm
5.	Oil and Grease (ppm)	11.20	7.88	LESS THAN 10 ppm
6.	Total Dissolved Solids (ppm)	1150.00	700.00	LESS THAN 2100 ppm
7.	Chlorides as Cl (ppm)	638.11	283.60	LESS THAN 600 ppm
8.	Sulphates as SO_4 (ppm)	80.20	24.32	LESS THAN 1000 ppm

Observations on the 29th December, 2014:

1. The Septic tanks shows stability and is also conforming to discharge norms.

Copies of all original reports ... appended in Annexure-1 attached.

GreenDHARA
info@dharastan.in
www.dharastan.in

Annexure-1

HYDROTECH LABORATORY REPORTS

HTL/14-15/1210 ... 05.12.2014

HTL/14-15/1211 ... 05.12.2014

HTL/14-15/1212 ... 05.12.2014

HTL/14-15/1255 ... 14.12.2014

HTL/14-15/1256 ... 14.12.2014

HTL/14-15/1329 ... 15.12.2014

HTL/14-15/1405 ... 03.01.2015

HTL/14-15/1406 ... 03.01.2015

HTL/14-15/1407 ... 03.01.2015

HYDROTECH LABORATORY

Drinking Water Analysis of : Borewell Water Corporation Water Aquaguard Water Water Purifiers Reverse Osmosis (RO) Systems
• Demineralized Water (DM) Analysis • Industrial Effluent Analysis
• Sewage Treatment Plant (STP) Analysis • Swimming Pool Analysis • Construction Water Analysis
Email : hydrotechlaboratory@gmail.com, hydrotechlab@rediffmail.com
Mobile : 9970213615

Date of Issue: 05.12.2014

Report Number: HTL/14-15/1210

Customer Name: DHARASTAN PVT.LTD.

Sample Id: BEG CANTEEN WASHING:RAW WATER

Sample Details

Receipt date	29.11.2014	Type	WATER
No.	01	Collected by	PARTY

Sr. No.	Parameters	VALUE	MPCB LIMITS
1.	pH	4.24	5.5-9.00
2.	Total Suspended Solids (ppm)	109.20	LESS THAN 100 ppm
3.	Chemical Oxygen Demand (mg O_2 per liter)	5800.00	LESS THAN 250 ppm
4.	Biological Oxygen Demand (mg O_2 per liter) (3days@ 27 degree C)	3500.00	LESS THAN 100 ppm
5.	Oil and Grease (ppm)	26.00	LESS THAN 10 ppm
6.	Total Dissolved Solids (ppm)	1200.00	LESS THAN 2100 ppm
7.	Chlorides as Cl (ppm)	531.75	LESS THAN 600 ppm
8.	Sulphates as SO4(ppm)	100.02	LESS THAN 1000 ppm

FOR HYDROTECH LABORATORY

Note: This analysis report is extended as technical assistance only & Hydrotech Laboratory will not be involved in any legal dispute arising out of this report.

HYDROTECH LABORATORY
Office No.4, Surya Tower,
2nd Floor, CTS No.50A,
Near Mhatre Bridge, Erandwane,
Gaothan, Pune - 411004
Mobile No.: 9970213615
email : hydrotechlab@rediffmail.com

HYDROTECH LABORATORY

Drinking Water Analysis of : Borewell Water Corporation Water Aquaguard Water Water Purifiers Reverse Osmosis (RO) Systems
• Demineralized Water (DM) Analysis • Industrial Effluent Analysis
• Sewage Treatment Plant (STP) Analysis • Swimming Pool Analysis • Construction Water Analysis
Email : hydrotechlaboratory@gmail.com, hydrotechlab@rediffmail.com
Mobile : 9970213815

Date of Issue: 05.12.2014

Report Number: HTL/14-15/1211

Customer Name: DHARASTAN PVT.LTD.

Sample Id: BEG CANTEEN INTERNAL WASHING:RAW WATER

Sample Details

Receipt date	29.11.2014	Type	WATER
No.	01	Collected by	PARTY

Sr. No.	Parameters	VALUE	MPCB LIMITS
1.	pH	3.55	5.5-9.00
2.	Total Suspended Solids (ppm)	100.29	LESS THAN 100 ppm
3.	Chemical Oxygen Demand (mg O_2 per liter)	4480.00	LESS THAN 250 ppm
4.	Biological Oxygen Demand (mg O_2 per liter) (3days@ 27 degree C)	2900.00	LESS THAN 100 ppm
5.	Oil and Grease (ppm)	18.00	LESS THAN 10 ppm
6.	Total Dissolved Solids (ppm)	1350.00	LESS THAN 2100 ppm
7.	Chlorides as Cl (ppm)	567.20	LESS THAN 600 ppm
8.	Sulphates as SO4(ppm)	118.27	LESS THAN 1000 ppm

FOR HYDROTECH LABORATORY

Note: This analysis report is extended as technical assistance only & Hydrotech Laboratory will not be involved in any legal dispute arising out of this report.

HYDROTECH LABORATORY
Office No.4, Surya Tower,
2nd Floor, CTS No.50A,
Near Mhatre Bridge, Erandwane,
Gaothan, Pune - 411004
Mobile No.: 9970213815
email : hydrotechlab@rediffmail.com

HYDROTECH LABORATORY

Drinking Water Analysis of : Borewell Water Corporation Water Aquaguard Water Water Purifiers Reverse Osmosis (RO) Systems
• Demineralized Water (DM) Analysis • Industrial Effluent Analysis
• Sewage Treatment Plant (STP) Analysis • Swimming Pool Analysis • Construction Water Analysis
Email : hydrotechlaboratory@gmail.com, hydrotechlab@rediffmail.com
Mobile : 9970213615

Date of Issue: 05.12.2014

Report Number: HTL/14-15/1212

Customer Name: DHARASTAN PVT.LTD.

Sample Id: MAITRAIYEE CANTEEN SEWAGE TANK WATER

Sample Details

Receipt date	29.11.2014	Type	WATER
No.	01	Collected by	PARTY

Sr. No.	Parameters	VALUE	MPCB LIMITS
1.	pH	6.70	5.5-9.00
2.	Total Suspended Solids (ppm)	368.00	LESS THAN 100 ppm
3.	Chemical Oxygen Demand (mg O_2 per liter)	1216.00	LESS THAN 250 ppm
4.	Biological Oxygen Demand (mg O_2 per liter) (3days@ 27 degree C)	480.00	LESS THAN 100 ppm
5.	Oil and Grease (ppm)	13.27	LESS THAN 10 ppm
6.	Total Dissolved Solids (ppm)	600.00	LESS THAN 2100 ppm
7.	Chlorides as Cl (ppm)	354.60	LESS THAN 600 ppm
8.	Sulphates as SO4(ppm)	57.60	LESS THAN 1000 ppm

FOR HYDROTECH LABORATORY

Note: This analysis report is extended as technical assistance only & Hydrotech Laboratory will not be involved in any legal dispute arising out of this report.

HYDROTECH LABORATORY
Office No.4, Surya Tower,
2nd Floor, CTS No.50A,
Near Mhatre Bridge, Erandwane,
Geothan, Pune - 411004
Mobile No.: 9970213615
email : hydrotechlab@rediffmail.com

HYDROTECH LABORATORY

Drinking Water Analysis of : Borewell Water Corporation Water Aquaguard Water Water Purifiers Reverse Osmosis (RO) Systems
• Demineralized Water (DM) Analysis • Industrial Effluent Analysis
• Sewage Treatment Plant (STP) Analysis • Swimming Pool Analysis • Construction Water Analysis
Email : hydrotechlaboratory@gmail.com, hydrotechlab@rediffmail.com
Mobile : 9970213615

Date of Issue: 14.12.2014

Report Number: HTL/14-15/1255

Customer Name: DHARASTAN PVT.LTD

Sample Id: AUNDH-1

Sample Details

Receipt date	09.12.2014	Type	WATER
No.	01	Collected by	PARTY

Sr. No.	Parameters	VALUE	MPCB LIMITS
1.	pH	7.35	5.5-9.00
2.	Total Suspended Solids (ppm)	78.00	LESS THAN 100 ppm
3.	Chemical Oxygen Demand (mg O_2 per liter)	215.80	LESS THAN 250 ppm
4.	Biological Oxygen Demand (mg O_2 per liter) (3days@ 27 degree C)	70.00	LESS THAN 100 ppm
5.	Oil and Grease (ppm)	Nil	LESS THAN 10 ppm
6.	Total Dissolved Solids (ppm)	650.00	LESS THAN 2100 ppm
7.	Chlorides as Cl (ppm)	248.15	LESS THAN 600 ppm
8.	Sulphates as SO4(ppm)	32.22	LESS THAN 1000 ppm

FOR HYDROTECH LABORATORY

Note: This analysis report is extended as technical assistance only & Hydrotech Laboratory will not be involved in any legal dispute arising out of this report.

HYDROTECH LABORATORY
Office No.4, Surya Tower,
2nd Floor, CTS No.50A,
Near Mhatre Bridge, Erandwane,
Gaothan, Pune - 411004
Mobile No.: 9970213615
email : hydrotechlab@...

HYDROTECH LABORATORY

Drinking Water Analysis of : Borewell Water Corporation Water Aquaguard Water Water Purifiers Reverse Osmosis (RO) Systems
• Demineralized Water (DM) Analysis • Industrial Effluent Analysis
• Sewage Treatment Plant (STP) Analysis • Swimming Pool Analysis • Construction Water Analysis
Email : hydrotechlaboratory@gmail.com, hydrotechlab@rediffmail.com
Mobile : 9970213615

Date of Issue: 14.12.2014

Report Number: HTL/14-15/1256

Customer Name: DHARASTAN PVT.LTD

Sample Id: AUNDH 2

Sample Details

Receipt date	09.12.2014	Type	WATER
No.	01	Collected by	PARTY

Sr. No.	Parameters	VALUE	MPCB LIMITS
1.	pH	7.02	5.5-9.00
2.	Total Suspended Solids (ppm)	190.00	LESS THAN 100 ppm
3.	Chemical Oxygen Demand (mg O_2 per liter)	293.33	LESS THAN 250 ppm
4.	Biological Oxygen Demand (mg O_2 per liter) (3days@ 27 degree C)	95.00	LESS THAN 100 ppm
5.	Oil and Grease (ppm)	3.00	LESS THAN 10 ppm
6.	Total Dissolved Solids (ppm)	580.00	LESS THAN 2100 ppm
7.	Chlorides as Cl (ppm)	177.25	LESS THAN 600 ppm
8.	Sulphates as SO4(ppm)	17.11	LESS THAN 1000 ppm

FOR HYDROTECH LABORATORY

Note: This analysis report is extended as technical assistance only & Hydrotech Laboratory will not be involved in any legal dispute arising out of this report.

HYDROTECH LABORATORY
Office No.4, Surya Tower,
2nd Floor, CTS No.50A,
Near Mhatre Bridge, Erandwane,
Gaothan, Pune - 411004
Mobile No.: 9970213615
email : hydrotechlab@rediffmail.com

HYDROTECH LABORATORY

Drinking Water Analysis of : Borewell Water Corporation Water Aquaguard Water Water Purifiers Reverse Osmosis (RO) Systems
• Demineralized Water (DM) Analysis • Industrial Effluent Analysis
• Sewage Treatment Plant (STP) Analysis • Swimming Pool Analysis • Construction Water Analysis
Email : hydrotechlaboratory@gmail.com, hydrotechlab@rediffmail.com
Mobile : 9970213615

Date of Issue: 20.12.2014

Report Number: HTL/14-15/1329

Customer Name: DHARASTAN PVT.LTD.

Sample Id: BEG SUMP: WASHING

Sample Details

Receipt date	15.12.2014	Type	WATER
No.	01	Collected by	PARTY

Sr. No.	Parameters	VALUE	MPCB LIMITS
1.	pH	6.02	5.5-9.00
2.	Total Suspended Solids (ppm)	23.22	LESS THAN 100 ppm
3.	Chemical Oxygen Demand (mg O_2 per liter)	1185.00	LESS THAN 250 ppm
4.	Biological Oxygen Demand (mg O_2 per liter) (3days@ 27 degree C)	400.00	LESS THAN 100 ppm
5.	Oil and Grease (ppm)	10.92	LESS THAN 10 ppm
6.	Total Dissolved Solids (ppm)	350.00	LESS THAN 2100 ppm
7.	Chlorides as Cl (ppm)	93.94	LESS THAN 600 ppm
8.	Sulphates as SO4(ppm)	10.00	LESS THAN 1000 ppm

Sunaskam

FOR HYDROTECH LABORATORY

Note: This analysis report is extended as technical assistance only & Hydrotech Laboratory will not be involved in any legal dispute arising out of this report.

HYDROTECH LABORATORY
Office No.4, Surya Tower,
2nd Floor, CTS No.50A,
Near Mhatre Bridge, Erandwane,
Gaothan, Pune - 411004
Mobile No.: 9970213615
email : hydrotechlab@rediffmail.com

HYDROTECH LABORATORY

Drinking Water Analysis of : Borewell Water Corporation Water Aquaguard Water Water Purifiers Reverse Osmosis (RO) Systems
• Demineralized Water (DM) Analysis • Industrial Effluent Analysis
• Sewage Treatment Plant (STP) Analysis • Swimming Pool Analysis • Construction Water Analysis
Email : hydrotechlaboratory@gmail.com, hydrotechlab@rediffmail.com
Mobile : 9970213615

Date of Issue: 03.01.2015

Report Number: HTL/14-15/1405

Customer Name: DHARASTAN PVT.LTD.

Sample Id: [redacted] AUNDH :RAW CHAMBER

Sample Details

Receipt date	29.12.2014	Type	WATER
No.	01	Collected by	PARTY

Sr. No.	Parameters	VALUE	MPCB LIMITS
1.	pH	6.12	5.5-9.00
2.	Total Suspended Solids (ppm)	189.20	LESS THAN 100 ppm
3.	Chemical Oxygen Demand (mg O_2 per liter)	1660.00	LESS THAN 250 ppm
4.	Biological Oxygen Demand (mg O_2 per liter) (3days@ 27 degree C)	570.00	LESS THAN 100 ppm
5.	Oil and Grease (ppm)	11.20	LESS THAN 10 ppm
6.	Total Dissolved Solids (ppm)	1150.00	LESS THAN 2100 ppm
7.	Chlorides as Cl (ppm)	638.11	LESS THAN 600 ppm
8.	Sulphates as SO4(ppm)	80.20	LESS THAN 1000 ppm

FOR HYDROTECH LABORATORY

Note: This analysis report is extended as technical assistance only & Hydrotech Laboratory will not be involved in any legal dispute arising out of this report.

HYDROTECH LABORATORY
Office No.4, Surya Tower,
2nd Floor, CTS No.50A,
Near Mhalsa Bridge, Erandwane,
Gaothan, Pune - 411004
Mobile No.: 9970213615
email : hydrotechlab@rediffmail.com

HYDROTECH LABORATORY

Drinking Water Analysis of : Borewell Water Corporation Water Aquaguard Water Water Purifiers Reverse Osmosis (RO) Systems
• Demineralized Water (DM) Analysis • Industrial Effluent Analysis
• Sewage Treatment Plant (STP) Analysis • Swimming Pool Analysis • Construction Water Analysis
Email : hydrotechlaboratory@gmail.com, hydrotechlab@rediffmail.com
Mobile : 9970213615

Date of Issue: 03.01.2015

Report Number: HTL/14-15/1406

Customer Name: DHARASTAN PVT.LTD.

Sample Id: [redacted] AUNDH :SEPTIC TANK

Sample Details

Receipt date	29.12.2014	Type	WATER
No.	01	Collected by	PARTY

Sr. No.	Parameters	VALUE	MPCB LIMITS
1.	pH	7.40	5.5-9.00
2.	Total Suspended Solids (ppm)	19.20	LESS THAN 100 ppm
3.	Chemical Oxygen Demand (mg O_2 per liter)	100.00	LESS THAN 250 ppm
4.	Biological Oxygen Demand (mg O_2 per liter) (3days/@ 27 degree C)	31.00	LESS THAN 100 ppm
5.	Oil and Grease (ppm)	7.88	LESS THAN 10 ppm
6.	Total Dissolved Solids (ppm)	700.00	LESS THAN 2100 ppm
7.	Chlorides as Cl (ppm)	283.60	LESS THAN 600 ppm
8.	Sulphates as SO4(ppm)	24.32	LESS THAN 1000 ppm

Subashkan

FOR HYDROTECH LABORATORY

Note: This analysis report is extended as technical assistance only & Hydrotech Laboratory will not be involved in any legal dispute arising out of this report.

HYDROTECH LABORATORY
Office No.4, Surya Tower,
2nd Floor, CTS No.50A,
Near Mhatre Bridge, Erandwane,
Gaothan, Pune - 411004
Mobile No.: 9970213615
email : hydrotechlab@rediffmail.com

HYDROTECH LABORATORY

Drinking Water Analysis of : Borewell Water Corporation Water Aquaguard Water Water Purifiers Reverse Osmosis (RO) Systems
• Demineralized Water (DM) Analysis • Industrial Effluent Analysis
• Sewage Treatment Plant (STP) Analysis • Swimming Pool Analysis • Construction Water Analysis
Email : hydrotechlaboratory@gmail.com, hydrotechlab@rediffmail.com
Mobile : 9970213615

Date of Issue: 03.01.2015

Report Number: HTL/14-15/1407

Customer Name: DHARASTAN PVT.LTD.

Sample Id: MAITRAIYEE :SEPTIC TANK

Sample Details

Receipt date	29.12.2014	Type	WATER
No.	01	Collected by	PARTY

Sr. No.	Parameters	VALUE	MPCB LIMITS
1.	pH	6.62	5.5-9.00
2.	Total Suspended Solids (ppm)	5.00	LESS THAN 100 ppm
3.	Chemical Oxygen Demand (mg O_2 per liter)	530.00	LESS THAN 250 ppm
4.	Biological Oxygen Demand (mg O_2 per liter) (3days@ 27 degree C)	170.00	LESS THAN 100 ppm
5.	Oil and Grease (ppm)	6.00	LESS THAN 10 ppm
6.	Total Dissolved Solids (ppm)	520.00	LESS THAN 2100 ppm
7.	Chlorides as Cl (ppm)	148.89	LESS THAN 600 ppm
8.	Sulphates as SO4(ppm)	16.22	LESS THAN 1000 ppm

FOR HYDROTECH LABORATORY

Note: This analysis report is extended as technical assistance only & Hydrotech Laboratory will not be involved in any legal dispute arising out of this report.

HYDROTECH LABORATORY
Office No.4, Surya Tower,
2nd Floor, CTS No.56A,
Near Mhatre Bridge, Erandwane,
Gaothan, Pune 411004
Mobile No. 9970213615
email hydrotechlab@rediffmail.com

Hydrabad Metropolitan Water and Sewerage Board:

HYDERABAD METROPOLITAN WATER SUPPLY AND SEWERAGE BOARD

Notice No. GM(E)/O&MDn.VII//DB-IV/2015-16/ 1634 **Date:** 17 /12/2015

From:
Er. B.Mahesh Kumar, B.*Tech*
General Manager (E)
O&Maint. Division No.VII,
Marredpally,
SECUNDERABAD.

To:
The General Manager
South India, MAKS. Bio Tech.
H.No: #9 Jhaver Plaza,
#1 Nungambakkam High Road,
CHENNAI, 600 034.
TAMILNADU.

Sir,

Sub: - HMWS&SB – O&M Div-VII - Performance of ILD Enzyme packs in Sewer lines during Pilot project study at Rezimental Bazaar, Marredpally Section, O&M Division VII, –Secunderabad.

Ref: - Lr.No: MACK BIO/HYD/SSR/HMWSSB/001, Dt: 27-08-15.

*****

Our Engineering Team with DGM and field staff had witnessed and confirm having used DraynZyme In line Digestion in Sewer lines network with 45 Main manholes with interconnected grid of 250mm mm dia SWG sewer line for a length of 1100 M.

We are satisfied with the results, we highly recommend to use ILD Enzyme packs for avoiding clogging, chowkage of manholes.

Free from odor and smell and no pollution risk for Line men and O&M Work force. We recommend for use of dousing of ILD Enzyme for sewer lines.

Yours faithfully,

17/12

General Manager (Engg),
O&M Dn.No.VII,
Marredpally, Sec'Bad

Pune Municiple Corporation:

PMC First trails:

रिठे आनंद रमेश
अध्यक्ष,
शहर सुधारणा समिती,
सभासद,
पुणे महानगरपालिका.

मोबाईल : ९८२२६६३६३६
ई-मेल : anandrithe805@gmail.com
ऑफीस : ५५८, साई मंदिरासमोर,
दत्तवाडी, पुणे ३०.

दिनांक 13 | 05 | २०२१

प्रति,
Quinquent Industries Pvt. Ltd.
स. नं. १३१/२अ, राजयोग कॉलनी,
वारजे, पुणे - ४११०५१.

-: दाखला :-

प्रभाग क्र. ३० मधील सावित्रीबाई फुले वसाहत (पु. ल. देशपांडे उद्यानजवळ) येथे फेब्रुवारी २०२१ महिन्यात प्रायोजिक तत्वावर Quinquent Industries Pvt. Ltd. या कंपनीमार्फत गल्ली बोळातील चेंबर्स, ड्रेनेज चोकअप चाचणी करण्यात आली. त्यामध्ये Draynzyme हे बायोडिग्री ड्रेनेज चोकअप काढणारे प्रॉडक्ट वापरून ड्रेनेज चोकअप ची समस्या दूर होवून दुर्गंधीसुध्दा आजपर्यंत दूर झाली आहे. तरी वरील कंपनीने चांगल्याप्रकारे प्रॉडक्ट निर्माण करून ड्रेनेज समस्या दूर होणेस व नागारिकांचे आरोग्य रक्षण होणेस चांगल्याप्रकारे मदत होणार आहे. तरी सदरचा दाखला Quinquent Industries Pvt. Ltd. या कंपनीने मागणी केलेनुसार देण्यात येत आहे.

मा. स. कळावे,

अध्यक्ष,
शहर सुधारणा समिती,
पुणे महानगरपालिका.

महापालिका सहाय्यक आयुक्त कार्यालय, सिंहगड रोड, पुणे महानगरपालिका
कार्यालयाचा पत्ता:- राजमाता जिजाऊ बहुउद्देशीय केंद्र, वडगांव बु. पुणे-४११०४१

कार्यालयाचा संपर्क क्र. 020 24606300, Email - sinhgadroad@punecorporation.org

जा.क्र.:- २७६६
दिनांक :- 23/08/2029

प्रति,
Quinquent Industries Pvt Ltd
स.नं.१३१/२अ, राजयोग कॉलनी,
वारजे, पुणे - ४११०५२.

दाखला

प्रभाग क्र.३० मधील सावित्रीबाई फुले वसाहत (पु.ल.देशपांडे उद्यानाजवळ) येथे फेब्रुवारी २०२१ या महिन्यात प्रायोजिक तत्वावर Quinquent Industries Pvt Ltd या कंपनीमार्फत गल्ली बोळातील चौकोनी चेंबर्स ड्रेनेज चोकअप चाचणी करण्यात आली त्यामध्ये Draynzyme हे बायोडिग्री ड्रेनेज चोकअप काढणारे प्रॉडक्ट वापरून ड्रेनेज चोकअप ची समस्या दूर होऊन दुर्गंधीसुध्दा आजपर्यंत दूर झाली आहे. तरी वरील कंपनीने चांगल्या प्रकारे प्रॉडक्ट निर्माण करून ड्रेनेज समस्या दूर होणेस व नागरिकांचे आरोग्य रक्षण होणेस चांगल्या प्रकारे मदत होणार आहे. तरी सदरचा दाखला Quinquent Industries Pvt Ltd या कंपनीने मागणी केलेनुसार देण्यात येत आहे.

मा.स.कळावे,

डॉ.जयश्री कात्कर-बोराडे
महापालिका सहा.आयुक्त
२/महा.सहा.आयुक्त कार्यालय
सिंहगडरोड, पुणे महानगरपालिका

महापालिका सहाय्यक आयुक्त कार्यालय, सिंहगड रोड, पुणे महानगरपालिका
कार्यालयाचा पत्ता:- राजमाता जिजाऊ बहुउद्देशीय केंद्र, वडगांव बु. पुणे-४११०४१

कार्यालयाचा संपर्क क्र. 020 24605300, Email - sinhgadroad@punecorporation.org

जा.क्र.:- २७६८
दिनांक :- 23/02/2021

प्रति,
Quinquent Industries Pvt Ltd
स.नं.१३१/२अ, राजयोग कॉलनी,
वारजे, पुणे - ४११०५२.

दाखला

प्रभाग क्र.३० मधील सावित्रीबाई फुले वसाहत (पु.ल.देशपांडे उद्यानाजवळ) येथे फेब्रुवारी २०२१ या महिन्यात प्रायोजिक तत्वावर Quinquent Industries Pvt Ltd या कंपनीमार्फत गल्ली बोळातील चौकोनी चेंबर्स ड्रेनेज चोकअप चाचणी करण्यात आली.त्यामध्ये Draynzyme हे बायोडिग्री ड्रेनेज चोकअप काढणारे प्रॉडक्ट वापरून ड्रेनेज चोकअप ची समस्या दूर होऊन दुर्गंधीसुध्दा आजपर्यंत दूर झाली आहे. तरी वरील कंपनीने चांगल्या प्रकारे प्रॉडक्ट निर्माण करून ड्रेनेज समस्या दूर होणेस व नागरिकांचे आरोग्य रक्षण होणेस चांगल्या प्रकारे मदत होणार आहे. तरी सदरचा दाखला Quinquent Industries Pvt Ltd या कंपनीने मागणी केलेनुसार देण्यात येत आहे.

मा.स.कळावे,

Kakar

डॉ.जयश्री काटकर-भोराडे
महापालिका सहा.आयुक्त
23/2/21महा.सहा.आयुक्त कार्यालय
सिंहगडरोड, पुणे महानगरपालिका

महानगरपालिका मालकीच्या जागेवर
जाहिरातीचे परवानगीसाठी
पुणे महानगरपालिका
क्र.अ. ८००७
दिनांक २७/१/२२

प्रति,
Quiquent Industries Pvt Ltd
स न १३१/२ अ, राजयोग कॉलनी
वाघोली पुणे - ४१२२०७

दाखला

प्रभाग क्र.३६ मधील प्रेमनगर वसाहत येथे २६ ऑक्टोबर २०२१ रोजी प्रायोगिक तत्वावर Quiquent Industries Pvt Ltd या कंपनीमार्फत गल्लीबोळातील गोल चेंबर्स ड्रेनेज चोकअप चाचणी करण्यात आली. त्यामध्ये Draynzyme हे द्रावणाद्वारे ड्रेनेज चोकअप काढण्याचे प्रोडक्ट वापरून ड्रेनेज चोकअप ची समस्या दूर होऊन दुर्गंधीसुध्दा आजपर्यंत दूर झाली आहे.

तरी वरील कंपनीने प्रकारे प्रोडक्ट निर्माण करून ड्रेनेज समस्या दूर होऊन व नागरीकांचे आरोग्य रक्षण होणेस चांगल्या प्रकारे मदत होणार आहे. तरी सदरचा दाखला Quiquent Industries Pvt Ltd या कंपनीने मागणी केलेनुसार देण्यात येत आहे.

कळावे,

महानगरपालिका सहाय्यक आयुक्त
विश्रामबाग कार्यालय
पुणे महानगरपालिका

रामदास आठवले
RAMDAS ATHAWALE

सामाजिक न्याय और अधिकारिता राज्य मंत्री
भारत सरकार
MINISTER OF STATE FOR
SOCIAL JUSTICE & EMPOWERMENT
GOVERNMENT OF INDIA

जा.क्र.५३७/सा.न्या./मु.ऑ./२०२१

दिनांक:-२५.१०.२०२१

विषय:-Quinquent Industries Pvt. Ltd. या कंपनीचे Draynzyme हे बायोडिग्री ड्रेनेज चोकअप काढणारे प्रॉडक्ट वापरणेबाबत.

महोदय,

विषयांकीत Quinquent Industries Pvt. Ltd. या कंपनीने Draynzyme हे बायोडिग्री ड्रेनेज चोकअप काढणारे प्रॉडक्ट तयार केलेले आहे. तसेच कंपनी मार्फत पुणे महानगरपालिका हद्दीमध्ये प्रभाग क्र. ३० मधील सावित्रीबाई फुले वसाहत (पु.ल. देशपांडे उद्यानजवळ) येथे फेब्रुवारी २०२१ महिन्यात प्रायोजिक तत्वावर गल्ली बोळातील चेंबर्स, ड्रेनेज चोकअपची यशस्वी चाचणी करण्यात आली. त्यामध्ये Draynzyme हे बायोडिग्री ड्रेनेज चोकअप काढणारे प्रॉडक्ट वापरल्याने ड्रेनेज चोकअप ची समस्या दूर होवून दुर्गंधी दूर झाली आहे. सदर कंपनीचे प्रॉडक्ट चांगले असून त्याने ड्रेनेज समस्या दूर होण्यात मदत होईल.

तरी उपरोक्त विषयान्वये विशेषबाब म्हणून लक्ष घालून Quinquent Industries Pvt. Ltd. या कंपनीचे Draynzyme हे बायोडिग्री ड्रेनेज चोकअप काढणारे प्रॉडक्ट वापरुन सदर कंपनीला पुणे महानगरपालिकेत काम करण्याची संधी देण्यात यावी.

आपला,

25/10/2021

(रामदास आठवले)

प्रति,

मा. आयुक्त
पुणे महानगरपालिका,
जि. पुणे.

Room No. : 101, 'C' Wing, Shastri Bhawan, Dr Rajender Prasad Road, New Delhi-110001, Tel : 011-23381656, 23381657, Fax : 011-23381869

सुनिल ज्ञानदेव कांबळे
आमदार
पुणे शहर विधानसभा मतदारसंघ
मा. आमदार, २७२ पुणे महानगरपालिका
५३, गुरुवार पेठ, लोणिकलन, महात्मा फुले पेठ, पुणे ४११ ०४२. दूरध्वनी : ०२०-२४२६०४८० / मोबाईल : ९८२२४११११३
ई मेल : kambleasunil18@gmail.com

दिनांक : 25/10/2021

प्रति,
मा.महानगरपालिका अति, आयुक्त (ज)
पुणे महानगरपालिका,
पुणे

विषय :-Quinquent Industries Pvt.Ltd.या कंपनीचे Draynzyme हे बायोडिग्रो ड्रेनेज चोकअप काढणारे प्रॉडक्ट वापरणे बाबत.

महोदय,

विषयांकित Quinquent Industries Pvt.Ltd. या कंपनीचे Draynzyme हे बायोडिग्रो ड्रेनेज चोकअप काढणारे प्रॉडक्ट तयार केलेले आहे.तसेच कंपनीने पुणे महानगरपालिका हद्दीमध्ये प्रभाग क्र.३० मधिल सावित्रीबाई फुले वसाहत (पु.ल.देशपांडे उद्यानाजवळ)येथे फेब्रुवारी २०२१ महीन्यात प्रायोजिक तत्वावर कंपनी मार्फत गल्ली बोळातील चेंबर्स.ड्रेनेज चोकअपची यशस्वी चाचणी करण्यात आली त्यामध्ये Draynzyme हे बॉयाडिग्रो ड्रेनेज चोकअप काढणारे प्रॉडक्ट वापरून ड्रेनेज चोकअप ची समस्या दुर होऊन दुर्गधीसुध्दा आजपर्यंत दुर झाली आहे.तरी वरील कंपनीने चांगल्या प्रकारे प्रॉडक्ट निर्माण करून ड्रेनेज समस्या दुर होणेस व नागरिकांचे आरोग्य रक्षण होणेस चांगल्या प्रकारे मदत होणार आहे.

तरी Quinquent Industries Pvt.Ltd.या कंपनीस आपल्या पुणे महानगरपालिकेत काम करण्याची संधी देण्यात यावी.

आपला

आमदार.सुनिल ज्ञानदेव कांबळे

PMC trials on Sewage water:

UNIK Lab
Environmental & Chemical Analysts

912 Sadashiv Peth, Deepvijay Apartment, Near Kalanjali Silk & Sarees, Pune - 30. | 020 24490360/61. | E-mail : info@uniklab.in

TEST REPORT

Report No.:2205/E/39 **Date:** 06/06/2022

ULR No.: TC663521000000488F

Name of the Company: Quinquent Industries Pvt.Ltd.
Warje, Pune.

Clients Ref. No.: Personal discussions with Mr. Mahesh Patvardhan.

Type of sample: Waste water (Drainage) **No. of Samples:** One

Date of receipt of sample: 31/05/2022 **Sample collected by:** Party

Analysis Start Date: 31/05/2022 **Analysis End Date;** 06/06/2022

Sr. No.	Parameters	Values			MPCB Standards	Method
		1	2	3		
1.	pH	7.36			5.5 – 9.0	IS: 3025(Part 11)-1983 (Clause 2)
2.	Total Suspended Solids	162.0			<100.0	IS :3025(Part 17)-1984
3.	COD	368.0			<250.0	APHA Ed22, 5220-C
4.	3 days BOD @27deg C	170.0			<30.0	IS: 3025(Part 44):1993 (Reaffirmed 2003)

(All values except pH are in mg/l, BLQ-Below limit of quantification,

Remarks: Sample -1 Before (Drainage Sample) 11/05/2022

Madhura Kamat
Analyst

Sudhir Bhalerao
Authorized Signatory

UL/DF/01/A- Rev03-01/09/2015

TC-4835

UNIK Lab
SINCE 1995
Environmental & Chemical Analysts

912 Sadashiv Peth, Deepvijay Apartment, Near Kalanjali Silk & Sarees, Pune - 30. | 020 24490360/61. | E-mail : info@uniklab.in

TEST REPORT

Report No.:2205/E/40 **Date:** 06/06/2022

ULR No.: TC663521000000489F

Name of the Company: Quinquent Industries Pvt.Ltd.
Warje, Pune.

Clients Ref. No.: Personal discussions with Mr. Mahesh Patvardhan.

Type of sample: Waste water Treated (Drainage) **No. of Samples:** One

Date of receipt of sample: 31/05/2022 **Sample collected by:** Party

Analysis Start Date: 31/05/2022 **Analysis End Date;** 06/06/2022

Sr. No.	Parameters	Values			MPCB Standards	Method
		1	2	3		
1.	pH	7.26			5.5 – 9.0	IS: 3025(Part 11)-1983 (Clause 2)
2.	Total Suspended Solids	18.0			<100.0	IS :3025(Part 17)-1984
3.	COD	61.0			<250.0	APHA Ed22, 5220-C
4.	3 days BOD @27deg C	16.0			<30.0	IS: 3025(Part 44):1993 (Reaffirmed 2003)

(All values except pH are in mg/l, BLQ-Below limit of quantification,

Remarks: Sample 1-After Drainzyme Treated-27/05/2022

Madhura Kamat
Analyst

Sudhir Bhalerao
Authorized Signatory

UL/DF/01/A- Rev03-01/09/2013

PMC Second trials on Sewage water:

अधिक्षक अभियंता कार्यालय
मलनि:सारण देखभाल दुरुस्ती विभाग
पुणे महानगरपालिका.
जावक क्र. 3822
दिनांक :- 9/3/2023

श्री.नितीन शिंदे
उप प्रादेशिक अधिकारी
महाराष्ट्र प्रदुषण नियंत्रण मंडळ
२ रा मजला, 'जोग सेंटर' वाकडेवाडी,
पुणे मुंबई रोड, पुणे-४११००३.

यांजकडेस.....

विषय : मे.Quinquent Industries PVT LTD या कंपनीने विकसित केलेल्या जैविक पद्धतीने ड्रेनझाईम या प्रोडक्टचा वापर करून साफसफाई करणे कामी सदर रसायना बाबतचा PH value, Total Suspended Solids value,COD value, 3day B.O.D @27°C, E coli bacteria, Hydrogen Sulphide इ.बाबींचे रिपोर्ट आपल्या विभागाकडून मिळणेबाबत.

उपरोक्त विषयाच्या अनुषंगाने आपणास सुचित करण्यात येते मे.Quinquent Industries PVT LTD या कंपनीने विकसित केलेल्या पुणे मनपाच्या कार्यक्षेतातील अस्तित्वातील मलवाहिन्या, मलकुंड यामध्ये जैविक पद्धतीने ड्रेनझाईम या प्रोडक्टचा वापर करून साफसफाई करण्यात आली आहे. सदर मलवाहिन्या, मलकुंड यांच्यामधील साफसफाई करण्या आधीचे व साफसफाई करण्यात आल्या नंतरचे पाण्याचे नमुने घेतले आहेत. त्यासंबधी घेतलेल्या पाण्याचे नमुने आपल्या विभागास पाठविण्यात आलेले आहेत.

तरी साफसफाई करणे कामी PH value, Total Suspended Solids value,COD value, 3day B.O.D @27°C, E coli bacteria, Hydrogen Sulphide इ बाबींचे रिपोर्ट आपणामार्फत देण्यात यावे ही विनंती.

मा.स.कळावे,

अधिक्षक अभियंता
मलनि:सारण देखभाल व दुरुस्ती
पुणे महानगरपालिका

सोबत: पाण्याचे नमुने.

Received on Dt.....
Clerk 13/02/2023
R. O. M. P. C. B. Pune

MAHARASHTRA POLLUTION CONTROL BOARD

SUB- REGIONAL OFFICE, PUNE-II

Jog Center Bldg.
2nd floor, Wakdewadi,
Mumbai – Pune Highway
Pune 411003

Ph. (020) 25811698

MPCB/ SROP-II/ 230308-FTS-0189 Date: 08.03.2023

To,
M/s. Superintendent Engineer,
STP -O&M Dept, Pune Municipal Corporation

Sub :- Analysis Result of JVS submitted by your good self

Ref :- Your letter dated 13.02.2023

Sir,

M/s. Quinquent Industries Pvt. Ltd., has developed bio-remedial solution for which you have submitted the letter vide no. 3622, dated 13.02.2023 along with effluent two samples.

The samples submitted by your good self has been analyzed and the copy of results are attached herewith for your ready reference.

D.A.: As above

Yours faithfully,

Nitin Shinde

(Nitin Shinde),
Sub- Regional Office, Pune- II

Copy submitted for information :-
Regional Office, MPC Board, Pune.

MAHARASHTRA POLLUTION CONTROL BOARD
REGIONAL LABORATORY, PUNE

Phone no. : 020-25811698
Visit us at : http://mpcb.gov.in
mail : sopunelab@mpcb.gov.in

"Your Service is our Duty"

Regional Laboratory, Pune, Maharashtra Pollution Control Board, Jog Center, 3rd Floor,Mumbai Pune Road, Wakdewadi,Pune- 411 003

NABL Certificate No.:	Validity
Laboratory MoEF Recognition :	Validity

Test Report No.: MPCB/RL-Pune/JVS/22-23/03/98	Date: 06/03/2023 05:28 PM

Analysis Report-Water (JVS)

Field Sample ID :	BR-0023637		
Name & Address of the Industry	Pune Municipal Corporation Local Body		
Sampling Location :	OTHERS (Before Droynzyme)		
Lab code :	MPCB/RL-Pune/JVS/22-23/2677		
Sampling Method(s) :		Sample Details (Water/Air/HW) :	Water
Sampling drawn by (Officer name):	SRO-Pune II (Shri. Nitin Shinde)	Sample Volume Received :	
Sample submitted by (Name) :	SRO-Pune II (Shri. Nitin Shinde) (SRO-Pune II)	Seal No. :	103
Date of Sample Collection.(dd/mm/yyyy) :	27/02/2023 12:15 PM	Date of Sample receipt to Laboratory (dd/mm/yyyy) :	01/03/2023 12:40 PM
Analysis start Date (dd/mm/yyyy). :	01/03/2023 03:18 PM		

Test Report

Sr.No	Parameter	Results	Unit	Method Adopted	MU(If required)
1	pH	6.4			
2	Suspended Solids (SS)	174.0	mg/l		
3	Biochemical Oxygen Demand (BOD)	327.4	mg/l		
4	Chemical Oxygen Demand (COD)	777.8	mg/l		
5	Sulphide	BDL	mg/l		

Sr.No	Parameter	Results	Unit	Method Adopted	MU(If required)
6	E. Coli (MPN technique)	NA	MPN/100 ml		

End of The Report

Abbreviations: - BDL=Below Detectable limit, N.D.=Not Detected, N.A.= Not Analyzed, * Not covered under NABL scop.

Comment (if any):
Comment for Amended Report:
Remark: - Note: This test report refers only to the sample submitted for the testing.
Results Compiled by: Dr P D Khadkikar
Results Approved by: Dr P D Khadkikar
Results Reviewed by: Dr P D Khadkikar

Dr P D Khadkikar
Scientific Officer,
I/c Regional Laboratory,
Pune,

 # This is an Electronically generated report does not require signature

Note :
1 . Results relate only to the sample/s tested, only in case of samples submitted by customer & not drawn by MPCB.
2. # indicates parameters are not in scope of NABL(ISO:17025:2017)
3. Samples will be preserved for a period 10 days from the delivery of Test Certificate.
4. Customer complaint register is available at laboratory.
5. The Contents of this Report shall not be reproduced in part or in full without written approval of laboratory.
6. MU values will be reported on request.

required)

MAHARASHTRA POLLUTION CONTROL BOARD
REGIONAL LABORATORY, PUNE

Phone no. : 020-25811698
Visit us at : http://mpcb.gov.in
mail : sopunelab@mpcb.gov.in

"Your Service is our Duty"

Regional Laboratory, Pune, Maharashtra Pollution Control Board, Jog Center, 3rd Floor,Mumbai Pune Road, Wakdewadi,Pune- 411 003

NABL Certificate No.:	Validity
Laboratory MoEF Recognition :	Validity

Test Report No.: MPCB/RL-Pune/JVS/22-23/03/99	Date: 06/03/2023 05:28 PM

Analysis Report-Water (JVS)

Field Sample ID :	BR-0023638		
Name & Address of the Industry	Pune Municipal Corporation Local Body		
Sampling Location :	OTHERS (After Droynzyme)		
Lab code :	MPCB/RL-Pune/JVS/22-23/2678		
Sampling Method(s) :		Sample Details (Water/Air/HW) :	Water
Sampling drawn by (Officer name):	SRO-Pune II (Shri. Nitin Shinde)	Sample Volume Received :	
Sample submitted by (Name) :	SRO-Pune II (Shri. Nitin Shinde) (SRO-Pune II)	Seal No. :	103
Date of Sample Collection.(dd/mm/yyyy) :	27/02/2023 12:15 PM	Date of Sample receipt to Laboratory (dd/mm/yyyy) :	01/03/2023 12:40 PM
Analysis start Date (dd/mm/yyyy). :	01/03/2023 03:18 PM		

Test Report

Sr.No	Parameter	Results	Unit	Method Adopted	MU(If required)
1	pH	4.2			
2	Suspended Solids (SS)	20.0	mg/l		
3	Biochemical Oxygen Demand (BOD)	19.8	mg/l		
4	Chemical Oxygen Demand (COD)	43.7	mg/l		
5	Sulphide	BDL	mg/l		

Sr.No	Parameter	Results	Unit	Method Adopted	MU(If required)
6	E. Coli (MPN technique)	NA	MPN/100 ml		

End of The Report

Abbreviations: - BDL=Below Detectable limit, N.D.=Not Detected, N.A.= Not Analyzed, * Not covered under NABL scop.

Comment (if any):
Comment for Amended Report:
Remark: - Note: This test report refers only to the sample submitted for the testing.
Results Compiled by: Dr P D Khadkikar
Results Approved by: Dr P D Khadkikar
Results Reviewed by: Dr P D Khadkikar

Dr P D Khadkikar
Scientific Officer,
I/c Regional Laboratory,
Pune,

 # This is an Electronically generated report does not require signature

Note :
1 . Results relate only to the sample/s tested, only in case of samples submitted by customer & not drawn by MPCB.
2. # indicates paramters are not in scope of NABL(ISO:17025:2017)
3. Samples will be preserved for a period 10 days from the delivery of Test Certificate.
4. Customer complaint register is available at laboratory.
5. The Contents of this Report shall not be reproduced in part or in full without written approval of laboratory.
6. MU values will be reported on request.

PMC Testing at Mhasoba Gate:

TC-6858

UNIK Lab
SINCE 1998
Environmental & Chemical Analysts

912 Sadashiv Peth, Deepvijay Apartment, Near Kalanjali Silk & Sarees, Pune - 30. | 020 24490360/61. | E-mail : info@uniklab.in

TEST REPORT

Report No:	2303/E/28	Issue Date	03/04/2023
ULR No	TC663523000000072F		
Name and Address of Customer	Quinquent Industries Pvt. Ltd.		
Site	Near Mhasoba Gate Sample no-1	Ref No	Personal Discussions with Mr. Mahesh Patwardhan
Sample Name	Waste Water	Sample Description	Before Drayanzyme
Sampling Done By	Client	Group and Discipline	Chemical- Pollution & Environment
Sample Receipt Date	28/03/2023	Sample Quantity	1Ltr
Start Date of Analysis	28/03/2023	End Date of Analysis	03/04/2023

Results

Sr. No.	Parameters	Results	Unit(s)	Specifications (IS 10500)	Methods
1	pH at 25°C	6.85	--	5.5 to 9.0	IS: 3025(Part 11)-1983 Clause 2
2	Total Suspended Solids	158.0	mg/L	<100	IS: 3025 (Part 17): 1984, RA
3	COD	326.4	mg/L	<250	APHA-Ed.23, 5220-C
4	3 days BOD @27deg C	140.0	mg/L	<100	IS: 3025(Part 44)-1993
5	Oil & Grease	2.0	mg/L	<10	IS: 3025(Part 39)-1991

Remark-

UNIK LAB PUNE 411030 612, Sadashiv Peth

Madhura Kamat
Analyst

Kushal Kulkarni
Authorized Signatory

----------END OF REPORT----------

Page 01 of 01

TC-4835

UNIK Lab
SINCE 1995
Environmental & Chemical Analysts

912 Sadashiv Peth, Deepvijay Apartment, Near Kalanjali Silk & Sarees, Pune - 30. | 020 24490360/61. | E-mail: info@uniklab.in

TEST REPORT

Report No:	2303/E/30	**Issue Date**	03/04/2023
ULR No	TC663523000000074F		
Name and Address of Customer	**Quinquent Industries Pvt. Ltd.**		
	Near Tanajiwadi Nala Sample No.3	**Ref No**	**Personal Discussions with Mr. Mahesh Patwardhan**
Sample Name	**Waste Water**	**Sample Description**	**After Drayanzyme**
Sampling Done By	**Client**	**Group and Discipline**	**Chemical- Pollution & Environment**
Sample Receipt Date	28/03/2023	**Sample Quantity**	**1Ltr**
Start Date of Analysis	28/03/2023	**End Date of Analysis**	03/04/2023

Results

Sr. No.	Parameters	Results	Unit(s)	Specifications (IS 10500)	Methods
1	pH at 25°C	7.12	--	5.5 to 9.0	IS: 3025(Part 11)-1983 Clause 2
2	Total Suspended Solids	36.0	mg/L	<100	IS: 3025 (Part 17): 1984, RA
3	COD	70.4	mg/L	<250	APHA-Ed.23, 5220-C
4	3 days BOD @27deg C	28.0	mg/L	<100	IS: 3025(Part 44)-1993
5	Oil & Grease	<0.5	mg/L	<10	IS: 3025(Part 39)-1991

Remark-

UNIK LAB PUNE 411030 912, Sadashiv Peth

Madhura Kamat
Analyst

Kushal Kulkarni
Authorized Signatory

----------*END OF REPORT*----------

Page 01 of 01

PMC testing at Warje Nalla:

UNIK Lab
Environmental & Chemical Analysts
912 Sadashiv Peth, Deepvijay Apartment, Near Kalanjali Silk & Sarees, Pune - 30. | 020 24490360/61. | E-mail : info@uniklab.in

TEST REPORT

Report No:	2304/E/23	Issue Date	26/04/2023
ULR No	TC663523000000101F		
Name and Address of Customer	Quinquent Industries Pvt. Ltd.		
Site	Warje Nala Sample no-1	Ref No	Personal Discussions with Mr. Mahesh Patwardhan
Sample Name	Waste Water	Sample Description	Before Drayanzyme
Sampling Done By	Client	Group and Discipline	Chemical- Pollution & Environment
Sample Receipt Date	21/04/2023	Sample Quantity	1Ltr
Start Date of Analysis	21/04/2023	End Date of Analysis	26/04/2023

Results

Sr. No.	Parameters	Results	Unit(s)	Specifications As per CPCB	Methods
1	pH at 25°C	6.65	--	6.5 to 9.0	IS: 3025(Part 11)-1983 Clause 2
2	Total Suspended Solids	142.0	mg/L	<20	IS: 3025 (Part 17): 1984, RA
3	COD	312.4	mg/L	<50	APHA-Ed.23, 5220-C
4	3 days BOD @27deg C	135.0	mg/L	<10	IS: 3025(Part 44)-1993
5	Oil & Grease	2.0	mg/L	<10	IS: 3025(Part 39)-1991

Remark-

Madhura Kamat
Analyst

Sudhir Bhalerao
Authorized Signatory

----------END OF REPORT----------

Page 01 of 02

UNIK Lab
Environmental & Chemical Analysts
912 Sadashiv Peth, Deepvijay Apartment, Near Kalanjali Silk & Sarees, Pune - 30. | 020 24490360/61. | E-mail : info@uniklab.in

TEST REPORT

Report No:	2304/E/23	Issue Date	26/04/2023
ULR No	---		
Name and Address of Customer	Quinquent Industries Pvt. Ltd.		
Site	Warje Nala Sample no-1	Ref No	Personal Discussions with Mr. Mahesh Patwardhan
Sample Name	Waste Water	Sample Description	Before Drayanzyme
Sampling Done By	Client	Group and Discipline	Chemical- Pollution & Environment
Sample Receipt Date	21/04/2023	Sample Quantity	1Ltr
Start Date of Analysis	21/04/2023	End Date of Analysis	26/04/2023

Results

Sr. No.	Parameters	Results	Unit(s)	Specifications As per CPCB	Methods
1	Total kjeldahl Nitrogen as N	14.0	mg/L	<10.0	NEERI,3.3.6D
2	Phosphate as P	6.35	mg/L	<2.0	APHA-Ed.23

Remark-

Madhura Kamat
Analyst

Sudhir Bhalerao
Authorized Signatory

----------END OF REPORT----------

TC-6636

UNIK Lab
SINCE 1995
Environmental & Chemical Analysts

912 Sadashiv Peth, Deepvijay Apartment, Near Kalanjali Silk & Sarees, Pune - 30. | 020 24490360/61. | E-mail : info@uniklab.in

TEST REPORT

Report No:	2304/E/24	**Issue Date**	26/04/2023
ULR No	TC663523000000102F		
Name and Address of Customer	**Quinquent Industries Pvt. Ltd.**		
Site	**Warje Nala Sample no-1**	**Ref No**	**Personal Discussions with Mr. Mahesh Patwardhan**
Sample Name	**Waste Water**	**Sample Description**	**After Drayanzyme G**
Sampling Done By	**Client**	**Group and Discipline**	**Chemical- Pollution & Environment**
Sample Receipt Date	21/04/2023	**Sample Quantity**	**1Ltr**
Start Date of Analysis	21/04/2023	**End Date of Analysis**	26/04/2023

Results

Sr. No.	Parameters	Results	Unit(s)	Specifications As per CPCB	Methods
1	pH at 25°C	7.0	--	6.5 to 9.0	IS: 3025(Part 11)-1983 Clause 2
2	Total Suspended Solids	26.0	mg/L	<20	IS: 3025 (Part 17): 1984, RA
3	COD	60.0	mg/L	<50	APHA-Ed.23, 5220-C
4	3 days BOD @27deg C	18.0	mg/L	<10	IS: 3025(Part 44)-1993
5	Oil & Grease	<0.5	mg/L	<10	IS: 3025(Part 39)-1991

Remark-

Madhura Kamat
Analyst

Sudhir Bhalerao
Authorized Signatory

----------*END OF REPORT*----------

UNIK Lab
Environmental & Chemical Analysts
912 Sadashiv Peth, Deepvijay Apartment, Near Kalanjali Silk & Sarees, Pune - 30. | 020 24490360/61. | E-mail : info@uniklab.in

TEST REPORT

Report No:	2304/E/24	**Issue Date**	26/04/2023
ULR No	---		
Name and Address of Customer	**Quinquent Industries Pvt. Ltd.**		
Site	**Warje Nala Sample no-1**	**Ref No**	**Personal Discussions with Mr. Mahesh Patwardhan**
Sample Name	**Waste Water**	**Sample Description**	**After Drayanzyme G**
Sampling Done By	**Client**	**Group and Discipline**	**Chemical- Pollution & Environment**
Sample Receipt Date	21/04/2023	**Sample Quantity**	1Ltr
Start Date of Analysis	21/04/2023	**End Date of Analysis**	26/04/2023

Results

Sr. No.	Parameters	Results	Unit(s)	Specifications As per CPCB	Methods
1	Total kjeldahl Nitrogen as N	8.0	mg/L	<10.0	NEERI,3.3.6D
2	Phosphate as P*	1.10	mg/L	<2.0	APHA-Ed.23

Remark-

Madhura Kamat
Analyst

Sudhir Bhalerao
Authorized Signatory

----------*END OF REPORT*----------

Page 02 of 02

PMC testing in Drains:

मुख्य अभियंता (विद्युत) कार्यालय
पुणे महानगरपालिका
जावक क्र :- 378
दिनांक :- 9/5/23

प्रति,
FHHL PVT.LTD.
(Food Hygiene & Health Laboratory)
Sr.No.126/10, Plot No.1, Hadapsar, Pune-13.

विषय :- पुणे महानगरपालिकेच्या हद्दीतील नाल्यांमधील पाण्याच्या नमुन्यांची गुणवत्ता तपासणी करून मिळणेबाबत.

वरील विषयास अनुसरून पुणे महानगरपालिका हद्दीत प्रतिदिन ८०० ते ८५० MLD मैलापाणी तयार होत असून, ४७० MLD मैलापाण्यावर ९ मैलापाणी शुद्धीकरण केंद्रातून शुद्धीकरणाची प्रकिया केली जाते. तसेच उर्वरित मैलापाणी नाल्यामध्ये प्रकिया न करता सोडले जाते. मे. महाराष्ट्र प्रदूषण नियंत्रण मंडळ, पुणे यांनी दिलेल्या आदेशनुसार नाल्यावर शुद्धीकरण प्रकिया करून नदीमध्ये सोडण्यात यावे. सदर आदेशाचे पालन करून पुणे महानगरपालिकेने मे. Quinquent Industries Pvt.Ltd. यांना जावक क्र. ३९६३ दि.२४/०३/२०२३ यांना खात्यामार्फत नाल्यावर शुद्धीकरणाची प्रकिया करणेसाठी पत्राद्वारे आदेश दिले आहेत. त्या अनुषंगाने मे. Quinquent Industries Pvt.Ltd. यांनी ब्रेनझाईम बायोडिग्री तंत्रज्ञाने पर्यावरण पूरक प्रॉडक्टचा वापर करून नाल्यावर शुद्धीकरणाची प्रकिया करण्यात आली आहे.

शुद्धीकरणाची प्रकिया केलेल्या नाल्यांमधील पाण्याच्या नमुन्यांची गुणवत्ता तपासणी आपल्या मार्फत करण्यात यावी व सदरील ब्रेनझाईम बायोडिग्री तंत्रज्ञान पर्यावरण पूरक प्रॉडक्टचा जलचर प्राण्यांसाठी उपयुक्त आहे कि नाही हे मार्गदर्शन होणेस विनंती आहे.

शुद्धीकरणाची प्रकिया केलेल्या नाल्यांमधील पाण्याच्या नमुन्यांची गुणवत्ता तपासणी पुढील प्रमाणे करणात यावी. P^H, Suspended Solids(SS), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD),Sulphide, E. Coli (MPN Techique).

सदर तपासणीसाठी लागणारे चार्जेस मे. Quinquent Industries Pvt.Ltd. (सदवर्धन :-९९२२८४६९६९) यांचे मार्फत अदा करण्यात येईल.

तरी सदरील तपासणी अहवाल लवकर देण्यात यावा.

मुख्य अभियंता (विद्युत)
मलनिःसारण विभाग
पुणे महानगरपालिका

FHHL PRIVATE LIMITED

(FOOD HYGIENE & HEALTH LABORATORY)

Testing of • Food & Processed Food Products • Water • Environmental Monitoring & Analysis • Packaging Material

Laboratory Recognized by CPCB-Ministry of Environment, Forest & Climate Change (MoEF & CC) under EPA 1986 Vide Gazette of India Notification No. Legal 42(3)/2022 (E), dated 22/07/2022. Recognition Valid up to 27/08/2023.
• ISO 14001 : 2015. • ISO 45001 : 2018.

TC-5931

TEST REPORT

Test Report No:-FHHL/2305/WW/081-1	**Report Date:- 30/05/2023**
Sample Id:- 2305/WW/081-1	Page No. :- **1 of 1**
Customer Name & Address: CHIEF ENGINEER OFFICE (ELECTRICAL) Pune Municipal Corporation.	Customer Reference Letter No. & date: - **22/05/2023**

Sample Information: -
Sample Type:- Waste Water
a] Quantity of sample received: 1 Lit x 1 No. c] Packing: - Plastic bottle
b] Sample collected/Submitted by: Customer.

Information provide by Customer: -
a] Sample Marked As :- **Before Draynzyme** b] Preservation – At 2° - 8°C

Date of Receipt in the Lab.: - **22/05/2023**
Date(s) of testing :- 22/05/2023 to 30/05/2023
Location of test performance :- In-house
Discipline:- Chemical Testing
Product Group:- Pollution & Environment
Sub Group :- Waste Water (Effluents/Sewage)

Sr. No.	Test Done	Result	Unit	Test Method
01	pH at 25°C	7.3	-	IS 3025 (part 11)
02	Total Suspended Solids	25	mg/l	IS 3025 (Part 17)
03	COD	131.04	mg/l	APHA (5220 B)
04	BOD 3 days at 27°C	45	mg/l	IS 3025 (Part 44)
05	Sulphide	<0.03	mg/l	IS 3025 (part 29)

Discipline:- Biological Testing
Group:- Pollution & Environment
Sub Group :- Sewage Water

01	Escherichia coli	Present	Per 100 ml	IS 1622

Note: Retention time of the above mentioned sample would be **15 days** after report date, subject to the remnant quantity.

Reviewed by

END OF REPORT

Sushma Thorat
Authorised Signatory
Chemical Testing

FHHL PRIVATE LIMITED

Laboratory Address : Sr. NO. 126/10, Plot No. 1, Hadapsar Industrial Estate, Hadapsar, Pune - 411 013, MH., India.
Mob. : +91-9881237321, +91-8380074695
E-mail : info@fhhl.in / environment@fhhl.in Website : www.fhhl.in

FHHL PRIVATE LIMITED

(FOOD HYGIENE & HEALTH LABORATORY)

Testing of • Food & Processed Food Products • Water • Environmental Monitoring & Analysis • Packaging Material

Laboratory Recognized by CPCB-Ministry of Environment, Forest & Climate Change (MoEF & CC) under EPA 1986 Vide Gazette of India Notification No. Legal 42(3)/2022 (E), dated 22/07/2022. Recognition Valid up to 27/08/2023.
• ISO 14001 : 2015. • ISO 45001 : 2018.

TC-5931

TEST REPORT

Test Report No:-FHHL/2305/WW/081-2 | **Report Date:- 30/05/2023**

Sample Id:- 2305/WW/081-2 | Page No. :- **1 of 1**

Customer Name & Address: CHIEF ENGINEER OFFICE (ELECTRICAL)
Pune Municipal Corporation.

Customer Reference Letter No. & date: - **22/05/2023**

Sample Information: -
Sample Type: - Waste Water
a] Quantity of sample received: 1 Lit x 1 No. c] Packing: - Plastic bottle
b] Sample collected/Submitted by: Customer.

Information provide by Customer: -
a] Sample Marked As :- **After Draynzyme** b] Preservation – At 2° - 8°C

Date of Receipt in the Lab.: - **22/05/2023**
Date(s) of testing :- 22/05/2023 to 30/05/2023

Location of test performance :- In-house

Discipline:- Chemical Testing

Product Group:- Pollution & Environment

Sub Group :- Waste Water (Effluents/Sewage)

Sr. No.	Test Done	Result	Unit	Requirements as per MPCB	Test Method
01	pH at 25°C	8.1	-	5.5 to 9.0	IS 3025 (part 11)
02	Total Suspended Solids	<1	mg/l	100, Max	IS 3025 (Part 17)
03	COD	64.48	mg/l	250, Max	APHA (5220 B)
04	BOD 3 days at 27°C	18	mg/l	100, Max	IS 3025 (Part 44)
05	Sulphide	<0.03	mg/l	2.0, Max	IS 3025 (part 29)
Discipline:- Biological Testing					
Group:- Pollution & Environment					
Sub Group :- Sewage Water					
01	Escherichia coli	Present	Per 100 ml	Not Specified	IS 1622

Remark: - Based upon results of above parameter the water sample conforms to the **MPCB** requirements.

Note: Retention time of the above mentioned sample would be **15 days** after report date, subject to the remnant quantity.

Reviewed by

END OF REPORT

Sushma Thorat
Authorised Signatory
Chemical Testing

FHHL PRIVATE LIMITED

Laboratory Address : Sr. NO. 126/10, Plot No. 1, Hadapsar Industrial Estate, Hadapsar, Pune - 411 013, MH., India
Mob. : +91-9881237321, +91-8380074695
E-mail : info@fhhl.in / environment@fhhl.in Website : www.fhhl.in

PMC work in Sewage Network :

33

महापालिका सहाय्यक आयुक्त कार्यालय, सिंहगड रोड, पुणे महानगरपालिका
कार्यालयाचा पत्ता - राजमाता जिजाऊ बहुउद्देशीय केंद्र, वडगांव बु. पुणे-४१.

कार्यालयाचा संपर्क क्र. 020 29912301 Email - sinhgadroad@punecorporation.org

जा.क्र. ३५२४
दिनांक : ३१/०८/२०२३

प्रति,
मा.अधिक्षक अभियंता
मलनि:सारण व देखभाल दुरुस्ती विभाग
पुणे महानगरपालिका

यांजकडेस...

विषय:- ड्रेनझाईमचा वापर करून ड्रेनेज लाईन इत्यादी मधील तुंबलेले सांडपाणी/मैला पाणी साफसफाई करणेचे कामाबाबत.

संदर्भ :- कार्यकारी अभियंता , मलनि:सारण देखभाल व दुरुस्ती कार्यालयाकडील जा.क्र.१०९५ दि.२६/७/२०२३ रोजीचे प्राप्त पत्र

सदर्भांकित पत्राद्वारे या विभागाकडील अस्तित्वातील मलवाहिन्यांची ड्रेनझाईमचा वापर करून जैविक पध्दतीने साफसफाई करण्याकरीता तसेच ड्रेनझाईमच्या उपयुक्ततेबाबत व प्रभावीपणे बाबत अहवाल आपल्या खात्याकडे सादर करणेबाबत कळविण्यात आलेले आहे. त्या अनुषंगाने दि.२९/७/२०२३ रोजी खालील नमूद दोन ठिकाणी १) जनता वसाहत कोंदेआळी गल्ली क्र.५ २) स.नं.१३२ निलायम ब्रिज सिंहगडरोड ड्रेनझाईमचा वापर करून जैविक पध्दतीने मलवाहिन्यांची साफसफाई करणेकरीता चाचणी घेण्यात आलेली होती. सदर जैविक पध्दतीने मलवाहिन्यांची साफसफाई करण्यात आलेली असून त्याचा अहवाल सोबत जोडत आहोत. सदरचा अहवाल हा नमूद ठिकाणी १) जनता वसाहत कोंदेआळी गल्ली क्र.५ २) स.नं.१३२ निलायम ब्रिज सिंहगडरोड दि.२९/७/२०२३ ते दि.१०/८/२०२३ दरम्यान निदर्शनास आलेल्या बाबींनुसार केलेला आहे.

सदर बाब आपले माहितीस्तव सादर.

मा.स.कळावे.

सोबत:- अहवाल

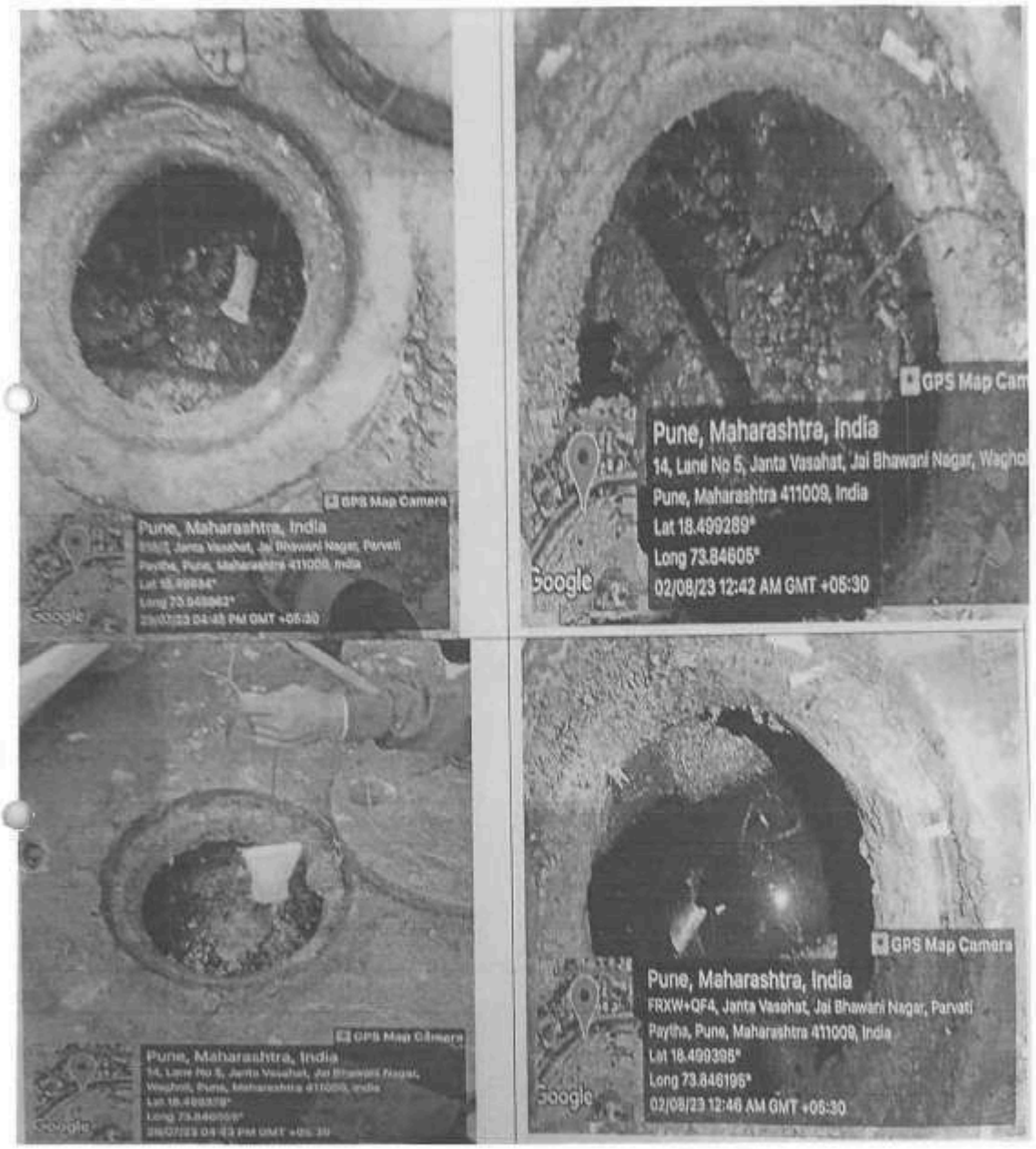
सविता खेलाट
महापालिका सहाय्यक आयुक्त
महापालिका सहाय्यक आयुक्त कार्यालय
सिंहगडरोड, पुणे महानगरपालिका

प्रत:- मा.उप आयुक्त (प.क्र.३)
पुणे महानगरपालिका

OIC

ठिकाणाचे नाव	[illegible] क्र. ५
Drainage-चा वापर करण्यात आलेल्या ड्रेनेज लाईन - ची लांबी	अंदाजे [illegible] मीटर
Drainage-चा वापर करण्याची अंदाजे तारीख	[illegible]
ड्रेनेज लाईन बाबतची पूर्वस्थिती	1. [illegible] 2. [illegible] 3. सदर ठिकाणी रस्ता अरुंद व वर्दळीचा असल्यामुळे Suction Machine [illegible] काम करणे अवघड [illegible] मिळत नव्हता

BEFORE	AFTER


GPS Map Camera
Pune, Maharashtra, India
Pune, Maharashtra, India
14, Lane No 5, Janta Vasahat, Jai Bhawani Nagar, Wagho
Pune, Maharashtra 411009, India
Lat 18.499289°
Long 73.84605°
02/08/23 12:42 AM GMT +05:30
Google
GPS Map Camera
Pune, Maharashtra, India
FRXW+QF4, Janta Vasahat, Jai Bhawani Nagar, Parvati
Paytha, Pune, Maharashtra 411009, India
Lat 18.499398°
Long 73.846196°
02/08/23 12:46 AM GMT +05:30
Google

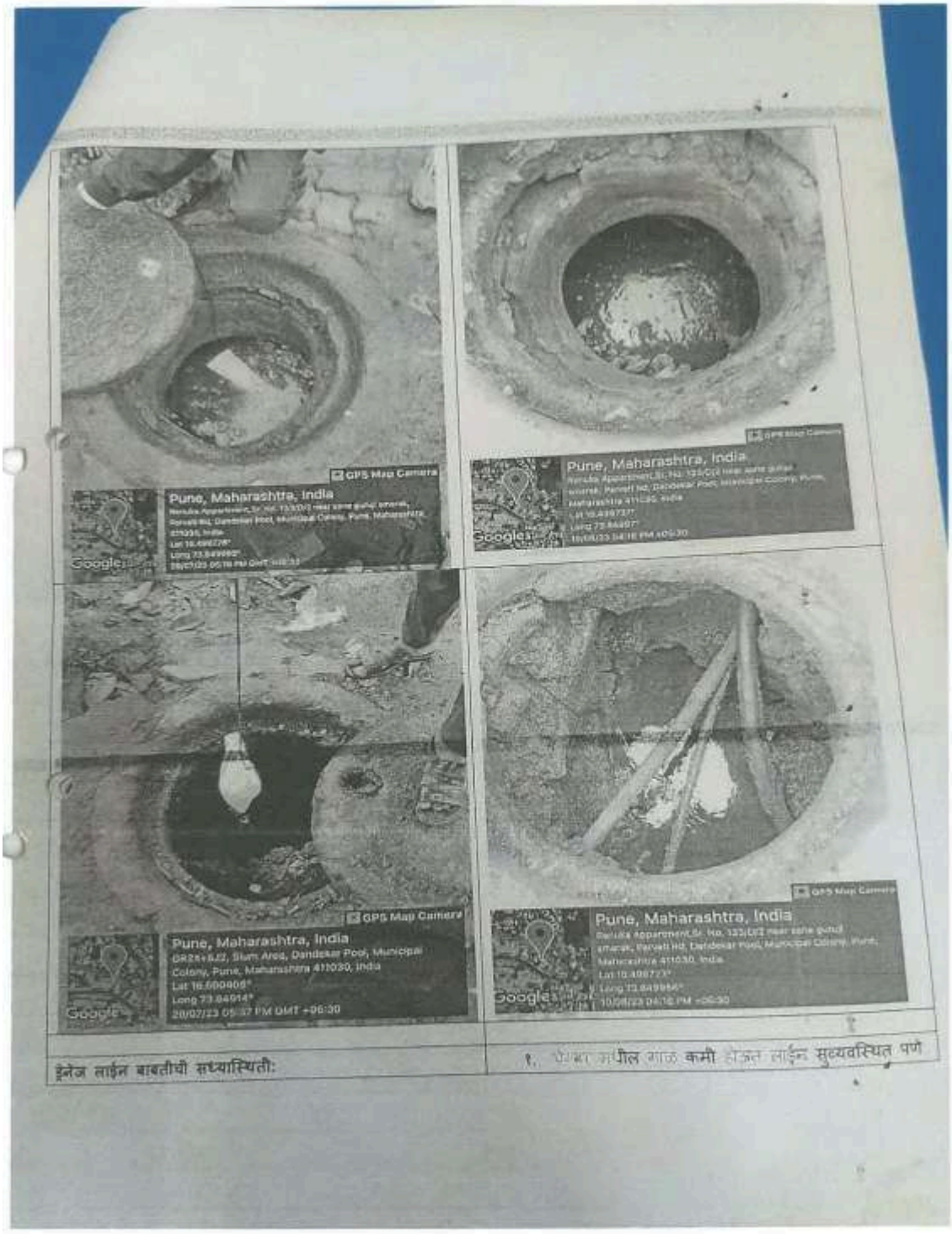
ड्रेनेज लाईन बाबतीची सध्यास्थिती:	१. चेम्बर मधील गाळ कमी होऊन लाईन सुरळीतपणे पुढे साडपाण्याचा निचरा करीत आहे. २. सदर ड्रेनेज लाईनची तुंबण्याची तक्रार इकडील कार्यालयाला त्यानंतर प्राप्त झालेली नाही (२५/७/२०२३ ते १०/८/२०२३)
शेरा:	१. सदर ठिकाणी Drenzyme प्रयोगानंतर चेम्बर मधील गाळ कमी झाल्याचे निदर्शनास आलेले आहे. २. सदर ड्रेनेज लाईन Drenzyme प्रयोगानंतर आवश्यक तसेच प्रभावीपणे (As per required bare minimum standards) साडपाण्याचा निचरा होत असल्याचे आजपावत निदर्शनास आलेले आहे. त्यामुळे गल्लीबोळामध्ये Drenzyme वापराचा प्रभावीपणा दिसून येतो.

ठिकाणाचे नाव:	स. नं. १३२ निलायम थिएटर सिंहगड रोड
Drenzyme चा वापर करण्यात आलेल्या ड्रेनेज लाईन ची लांबी:	अंदाजे २०० मीटर
Drenzyme चा वापर करण्यात आलेली तारीख:	२६/०७/२०२३
ड्रेनेज लाईन बाबतीची पूर्वस्थिती	१. सदर ड्रेनेज सिंहगड रोड मुख्य रस्त्याला आहे त्यामुळे पावसाळा व पावसाळ्याला असल्यामुळे अपुरा प्रमाणात सोसायट्यान पैणारा माल तसेच कैनल या ठिकाणचे असलेले पाणी या ड्रेनेज लाईन मध्ये साठत असते. २. अपुरा प्रमाणात माल चेम्बर मध्ये साठत असल्यामुळे ड्रेनेज लाईन तसेच चेम्बर वारंवार तुंबण्याच्या नागरिकाच्या व मा. सभासदाच्या तक्रारी नित्यनियमाने या कार्यालयाकडे प्राप्त होत होत्या. ३. चेम्बर तुंबल्याने पूर्ण सांडपाणी सिंहगड मुख्य रस्त्यावरून वाहत असायचा त्यामुळे हिंद तसेच परिसरात दुर्गंधी व अनारोग्य परिस्थिती निर्माण होत होती. ४. Suction Machine, जेट मशीनने, ड्रेनेज कोठीच्या सैवाकाट्बारे तसेच कंत्राटी कामगारांदवारे रोडने काम करूनसुद्धा हवा तसा परिणाम मिळत नव्हता.

BEFORE	AFTER
	

ठिकाणाचे नाव:	स. नं. ६२ सिंहगड रोड
Drenzyme चा वापर करण्यात आलेल्या ड्रेनेज लाईन ची लांबी:	अंदाजे २०० मीटर
Drenzyme चा वापर करण्यात आलेली तारीख:	२६/०७/२०२३
ड्रेनेज लाईन बाबतीची पूर्वस्थिती	१. सदर ड्रेनेज सिंहगड रोड मुख्य रस्त्याला आहे त्यामुळे पावसाळ्यात असल्यामुळे अपुरा प्रमाणात सोसायट्यान पैणारा माल तसेच केमिकल युक्त प्रक्रिया असलेले पाणी या ड्रेनेज लाईन मध्ये साठत असते. २. अपुरा प्रमाणात माल चेम्बर मध्ये साठत असल्यामुळे ड्रेनेज लाईन तसेच चेम्बर वारंवार तुंबण्याच्या नागरिकाच्या व मा. सभासदाच्या तक्रारी नित्यनियमाने या कार्यालयाकडे प्राप्त होत होत्या. ३. चेम्बर तुंबल्याने पूर्ण सांडपाणी सिंहगड मुख्य रस्त्यावरून वाहत असायचा त्यामुळे हिंद तसेच परिसरात दुर्गंधी व अनारोग्य परिस्थिती निर्माण होत होती. ४. Suction Machine, जेट मशीनने, ड्रेनेज कोठीच्या सैवाकट्बारे तसेच कंत्राटी कामगारांदवारे रोडने काम करूनसुद्धा हवा तसा परिणाम मिळत नव्हता.

BEFORE	AFTER
	



इनेज लाईन बाबतीची सध्यास्थिती:

१. पेव्हर ब्लॉक मॉल कमी होऊन लाईन सुव्यवस्थित पणे

ड्रेनेज लाईन बाबतीची सद्यस्थिती:	1. पेम्बर सर्पीन गाळ काढी होऊन लाईन मुखबन्दित पणे सांडपाण्याचा निचरा करीत आहे. 2. सदर ड्रेनेज लाईनची तुंबण्याची तक्रार इकडील कार्यालयास त्यानंतर प्राप्त झालेली नाही (२५/०७/२०२३ ते १०/०८/२०२३)
शेरा:	1. सदर ठिकाणी Drenzyme प्रयोगानंतर पेम्बर सर्पीन गाळ काढी झाल्याचे निदर्शनास आलेले आहे. 2. सदर ड्रेनेज लाईन ऑडिटकरी होत असल्याचे आजपावत (२५/०७/२०२३ ते १०/०८/२०२३) आढळून आले आहे. 3. सदर ड्रेनेज लाईन Drenzyme प्रयोगानंतर आवश्यक तसेच पुरेशी पाण्याचे (As per required bare minimum standards) सांडपाण्याचा निचरा होत असल्याचे आजपावत निदर्शनास आलेले आहे त्यामुळे मानसीबोळामध्ये Drenzyme वापराचा पुनरावृत्ती टिकून येतो.

मयूर गेडाम
कनिष्ठ अभियंता
महा. सहा. आयुक्त कार्यालय
सिंहगड रोड, पुणे मनपा

विजयकुमार वाघमोडे
कनिष्ठ अभियंता
महा. सहा. आयुक्त कार्यालय
सिंहगड रोड, पुणे मनपा

संदीप खलाटे
महापालिका सहा. आयुक्त
महा. सहा. आयुक्त कार्यालय
सिंहगड रोड, पुणे मनपा

महापालिका सहायक आयुक्त
येरवडा कळस धानोरी क्षेत्रिय कार्यालय
पुणे महानगरपालिका
जा.क्र. ४०६५
दि. २३/८/२३

प्रति,
मा.उप आयुक्त
परिमंडळ कार्यालय क्र १
पुणे महानगरपालिका

याजकडेस ---

विषय :- ड्रेनझाईमचा वापर करून ड्रेनेज लाईन इत्यादीमधील तुंबलेला सांडपाणी / मैलापाणी साफसफाई करणे कामाबाबत.

संदर्भ :- १. निविदा क्र PMC/DRAINAGE/2022/187.
२.कामाचे कार्यदिश कार्यकारी अभियंता मलनि:सारण देखभाल दुरुस्ती विभाग जा क्र ६९३ दि १४/६/२०२३
३.अधिक्षक अभियंता मलनि:सारण देखभाल दुरुस्ती विभाग यांचेकडील पत्र जा क्र १३९४ दि २६/७/२०२३
४.येरवडा कळस धानोरी क्षेत्रिय कार्यालयाकडील जा क्र ३८३५ दि २८/७/२०२३ चे पत्र

महोदय,

येरवडा कळस धानोरी क्षेत्रिय कार्यालय अंतर्गत येणाऱ्या धानोरी परिसरात (विठ्ठल मंदीर, धानोरी गावठाण), येरवडा परिसरात(यशवंत नगर) येथे सर्व ड्रेनेज चेंबर व मलवाहीनी जैविक पद्धतीने ड्रेनझाईम चा वापर करून साफसफाई करण्यात आलेली आहे. आपणामार्फत संदर्भ क्र ३ अन्वये उपलब्ध करून देण्यात आलेल्या निविदेमधून येरवडा कळस धानोरी क्षेत्रिय कार्यालय अंतर्गत सदरचे काम करून घेण्यात आलेले आहे विठ्ठलमंदीर, धानोरी येथील संपूर्ण लाईन गाईच्या शेणाने भरलेली होती, त्यामुळे संपूर्ण धानोरी गावठाण व आजुबाजुचा परिसर बाधित होऊन ड्रेनेज चेंबर व लाईन पूर्णपणे तुंबलेल्या होत्या. या ठिकाणी ग्रॅब मशीन, जेटींग मशीन तसेच टायगर मशीनचा वापर करूनही हा प्रश्न सुटला नव्हता. नवीन लाईन टाकण्या अगोदर एक प्रयोग म्हणून ड्रेनझाईमचा वापर करण्याचे ठरले आणि एकाच दिवसांत संपूर्ण मलवाहीनी व चेंबर संपूर्णता मोकळे झाले. तेथे रस्त्यावर पसरलेल्या शेणाच्या पाण्याची दुर्गंधीसुद्धा सदर प्रयोगाने नष्ट झाली. दि. १४/८/२०२३ रोजी धानोरी परिसरात मलवाहीनी साफसफाई करिता ड्रेनझाईमचा वापर करण्यात आला. अद्याप पर्वत सदर परिसरातुन परत कोणतीही प्रकार प्राप्त झालेली नाही ही वस्तुस्थिती आहे.

तसेच दि २५/०८/२०२३ रोजी येरवडा परिसरातील यशवंत नगर या ठिकाणी ड्रेनझाईमचा वापर परत एकदा करण्यात आला त्यावेळी सुद्धा वरीलप्रमाणे संपूर्ण चेंबर व मलवाहीन्यांची साफसफाई अत्यंत उत्कृष्टपणे झालेली आहे.

वरील दोन्ही प्रकारचे रिझल्ट पहाता ड्रेनझाईम हे जैविक प्रोडक्ट मलवाहीन्या व चेंबर साफसफाई करिता अत्यंत उपयुक्त व फायदेशीर आहे.आपले विभागाकडील संदर्भ क्र ३ च्या अनुषंगाने येरवडा कळस धानोरी क्षेत्रिय कार्यालयाकडील माहिती खालीलप्रमाणे सादर करीत आहोत.

अ.क्र	ड्रेनझाईमचा वापर/उपयुक्तता संबधित प्रश्न	शेरा

	घरीसाठ कामे करण्यापुर्वीचे व काम पुर्ण झालेनंतरचे फोटोग्राफ सादर करावेत.	आणा,त्याबाबतचे फोटोग्राफ सोबत जोडले आहे .
२	या निविदाचे किती परिमाण/ काम करण्यात आलेले आहे.	प्राथमिकच्या वेळी १० कॅप्सुलचा वापर केला गेला.
३	ड्रेनझाईमचा वापर करण्याने सांडपाणी / मैलापाणी सफाईच्या कामामध्ये चोक झालेल्या मलवाहिन्या व सुरळ्या आली आहे किंवा कसे? व ड्रेनझाईम वापराच्या परिणामकारकता, प्रभावीपणा व उपयुक्तता याबाबत आपले निरीक्षण / निष्कर्ष काय आहे ?	चोक झालेल्या मलवाहिन्या व चेंबर पूर्णपणे साफ झाल्याचे व मलवाहीन्या व्यवस्थित कार्यरत झाल्याचे निदर्शनास आले आहे. तेथील दुर्गंधी नाहीशी झालेली आहे.तथापि या पद्धतीचा वापर करणेत आला तेथील मलवाहिन्या मधील जमा झालेला गाळ सुद्धा काढण्याचे संबधित एजन्सीकडून करून घेणे व त्याबाबतची सुमारे ६ महिने असावा व तशी अट सुधीन निविदेत समाविष्ट करावी अशी विनंती असुन ,त्यास या कार्यालयाची शिफारस आहे .
४	ड्रेनझाईमचा वापर पारंपारिक यांत्रिक पद्धतीने सफाईच्या तुलनेत आर्थिक बाबाबतचा निर्णय आपल्या व कार्यक्षमतेच्या दृष्टीने फायदेशीर आहे किंवा कसे? याबाबत यांत्रिक स्तरावर घेणे योग्य राहील.	वरील परिसरातील रिझल्ट पहाता ज्या ठिकाणी यांत्रिक पद्धतीने साफसफाई करणे शक्य होत नाही,तसेच ज्या ठिकाणी यांत्रिक पद्धतीनेही साफसफाई होत नाही अश्या ठिकाणी ड्रेनझाईमचा वापर फायदेशीर ठरेल ,तथापि आर्थिकदृष्ट्या फायदेशीर आहे अगर कसे याबाबतचा निर्णय वरिष्ठ पातळीवर घेणे योग्य होइल .

तरी वरीलप्रमाणे ड्रेनझाईम हे जैविक प्रोडक्ट वापरून करावयाच्या चेंबर व मलवाहिनी साफसफाई बाबतचा अहवाल आपले अवलोकनार्थ सादर करीत आहोत.

मा.स.कळावे.

13/3/20
अरुण जगदाळे
कनिष्ठ अभियंता
वेरवडा कळस धानोरी क्षेत्रिय कार्यालय
पुणे महानगरपालिका

Sayaleeh
सायली सुर्यवंशी
शाखा अभियंता
वेरवडा कळस धानोरी क्षेत्रिय कार्यालय
पुणे महानगरपालिका

मनोहर शिंदे
उप अभियंता
वेरवडा कळस धानोरी क्षेत्रिय कार्यालय
पुणे महानगरपालिका

13/3/20
इंद्रायणी करचे
महापालिका सहाय्यक आयुक्त
वेरवडा कळस धानोरी क्षेत्रिय कार्यालय
पुणे महानगरपालिका

प्रत:- मा. अधिक्षक अभियंता,
मलनिःसारण देखभाल व दुरुस्ती वि.

PMC work at Ramnadi:

SKYLAB
ANALYTICAL LABORATORY

- ENVIRONMENTAL TESTING
- FOOD & MICROBIOLOGICAL TESTING
- TEXTILE TESTING
- METALS, CHEMICAL TESTING
- TURNKEY, ENVIRONMENT CONSULTANCY
- SHE AUDIT & TRAINING

TEST REPORT

NAME & ADDRESS OF CUSTOMER:
To,
Commissioner,
Pune Municipal Corporation,
Pune, Maharashtra 411004

REPORT NO : SLK/23-24/PR/PMC/PSW/02-01
REPORT DATE :12/02/2024
CUSTOMER REF : उका/पर्या-२८९
REF DATE :21/06/2022

SAMPLE TYPE: **SURFACE WATER ANALYSIS**

SAMPLING PLAN& METHOD NO.: IS 3025 Part 1
SAMPLE RECEIPT DATE :06/02/2024
ANALYSIS START DATE :06/02/2024
SAMPLE COLLECTED BY:SKYLAB

Location Name	Sampling Date	Parameter				
		pH	TSS	COD (mg/L)	BOD (mg/L)	O & G (mg/L)
Ram Nadi Near Ram Nagar (Before Treatment)	05/02/2024	6.94	16	105	31.3	<5
Ram Nadi Near Ram Nagar (Before Treatment)	05/02/2024	6.79	56	118	35.6	5.4
Ram Nadi Near Ram Nagar (Before Treatment)	05/02/2024	6.85	68	106	31.6	5.6
Ram Nadi, Near Ram Nagar (After Treatment)	06/02/2024	7.12	<5	94	22.68	<5

For SKYLAB ANALYTICAL LABORATORY

Technical Manager
Authorized Signatory

END OF REPORT

1. This report reflects findings only for the above sample tested/monitored and only for time and place of monitoring/testing.
2. This report is confidential & cannot be re-produced in part or full without permission of SKYLAB Analytical Laboratory.
3. Any attempt of forgery or misleading use of this report by any person/organization etc will attract suitable legal action against them by Skylab Analytical laboratory.

Certified by ISO 9001:2015 & ISO 45001:2018
Recognized by Ministry of Environment, Forest & Climate Change (MoEFCC), Govt. of India, valid from 08.12.2023
202,CFC-3, Asmeeta Texpa, Addl. Kalyan-Bhiwandi Industrial Area, MIDC Village Kon, Tal.Bhiwandi, Dist.Thane
Mob. No- 9820386785/9867577309-312/8422929165
Email- mails@skylabenviro.com Website- www.skylabenviro.com

SKYLAB
ANALYTICAL LABORATORY

- ENVIRONMENTAL TESTING
- FOOD & MICROBIOLOGICAL TESTING
- TEXTILE TESTING
- METALS, CHEMICAL TESTING
- TURNKEY, ENVIRONMENT CONSULTANCY
- SHE AUDIT & TRAINING

TEST REPORT

NAME & ADDRESS OF CUSTOMER:
To,
Commissioner,
Pune Municipal Corporation,
Pune, Maharashtra 411004

REPORT NO : SLK/23-24/PR/PMC/PSW/02-02
REPORT DATE :13/02/2024
CUSTOMER REF : उका/पर्या-२८९
REF DATE :21/06/2022

SAMPLE TYPE: **SURFACE WATER ANALYSIS**

SAMPLING PLAN& METHOD NO.: IS 3025 Part 1
SAMPLE RECEIPT DATE :07/02/2024
ANALYSIS START DATE :07/02/2024
SAMPLE COLLECTED BY:SKYLAB

Location Name	Sampling Date	Parameter				
		pH	TSS	COD (mg/L)	BOD (mg/L)	O & G (mg/L)
Ram Nadi, Near Ram Nagar (After Treatment)	07/02/2024	7.01	22	76	18.46	<5

For SKYLAB ANALYTICAL LABORATORY

Technical Manager
Authorized Signatory

END OF REPORT

1. This report reflects findings only for the above sample tested/monitored and only for time and place of monitoring/testing.
2. This report is confidential & cannot be re-produced in part or full without permission of SKYLAB Analytical Laboratory.
3. Any attempt of forgery or misleading use of this report by any person/organization etc will attract suitable legal action against them by Skylab Analytical Laboratory.

Scientific studies and safety assessments, if available, addressing aspects such as biomagnification potential, impacts on microbial ecology, and outcomes of chronic toxicity evaluations.

Toxicity Evaluations:

Charotar University of Science and Technology:

CHARUSAT

CHAROTAR UNIVERSITY OF SCIENCE & TECHNOLOGY

Formed under Gujarat State Act No. : 8 of 2009

Accredited Grade A by NAAC

Dec. 20, 2022

TO WHOM IT MAY CONCERN

This is to certify that the sample (Enzyme Based Treatment Pack, (Dranzyme)) which was provided to us to know its toxicity in water. We performed intermediate term toxicity assay using Guppy fishes (*Poecilia reticulata*) as per attached protocol following Standard Methods of Examination of Water Waste Water (23rd Vol. APHA). There was no death of even a single fish in two weeks' time. Sample does not have any toxicity and is safe.

Datta Madamwar
Scientific Advisor
Dec 20, 2022

Datta Madamwar, Ph.D
Scientific Advisor
P.D. Patel Institute of Applied Sciences
Charotar University of Science and Technology
CHARUSAT Campus, Changa-388421.
Dist. Anand, Gujarat, India

CHARUSAT : Campus - Changa, Off Nadiad - Petlad Highway, Gujarat - 388 421, INDIA.

CSIR - Indian Institute of Toxicology Research:

Study on Acute Fish Embryo:

FINAL REPORT

STUDY TITLE

ACUTE FISH EMBRYO TOXICITY TEST OF DRAYNZYME

TESTITEM: DRAYNZYME

STUDY NO.: CSIR-IITR/GLP/395

STUDY COMPLETED ON:
16/04/2025

SPONSOR

PUNE MUNICIPAL CORPORATION
SHIVAJI NAGAR, PUNE 411005

TEST FACILITY

TOXICITY TESTING: GLP TEST FACILITY
CSIR-INDIAN INSTITUTE OF TOXICOLOGY RESEARCH
CRK CAMPUS, GHERU, SAROJINI NAGAR INDUSTRIAL AREA
KANPUR ROAD, LUCKNOW-226008
UTTAR PRADESH, INDIA

TABLE OF CONTENTS

STATEMENT OF CONFIDENTIALITY

The report contains confidential and proprietary information Pune Municipal Corporation Shivaji Nagar, Pune 411005. The contents of this report will not be disclosed to anyone without an expressed or a written approval of competent authority of Pune Municipal Corporation Shivaji Nagar.

STATEMENT OF GLP COMPLIANCE

This study was performed in compliance with the OECD Principles of Good Laboratory Practice for the testing of chemicals as specified by International [C (97) 186/Final] Legislation. This study was conducted in accordance with the Standard Operating Procedures of Toxicity Testing: GLP Test Facility, CSIR-Indian Institute of Toxicology Research and the mutually agreed study plan which was signed by the Study Director on 21/03/2025 after the email consent from sponsor on 24/03/2025.

DECLARATION

The Study Director hereby declares that the work was performed under her supervision and in accordance with the described procedures. It is assured that the reported results faithfully represent the raw data obtained during the experimental work. No circumstances have been left unreported.

Study Director
Date: 16/04/2025

Test Facility Management
Date: 16/04/2025

Deputy Test Facility Management
Vishaktata Parikshan : GLP Anuroop Suvidha
Toxicity Testing: GLP Compliant Facility CSIR-IITR Lucknow, India

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

QUALITY ASSURANCE STATEMENT

Study No.: CSIR-IITR/GLP/395, "Acute Fish Embryo Toxicity Test of Draynzyme" has been inspected in accordance with the OECD Principles of Good Laboratory Practice for the testing of chemicals as specified by International [C (97) 186/Final] Legislation.

This study was inspected and findings reported to the Management and Study Director on the dates shown below:

INSPECTION DATE/S	PHASE	REPORTING DATE/S
	Initiation Phase	
24/02/2025	Draft Study Plan Review	24/02/2025
21/03/2025	Final Study Plan review	21/03/2025
	In-Life Phase	
	Limit Test	
24/03/2025	Breeding Record	24/03/2025
25/03/2025	Embryo collection and dosing	25/03/2025
26/03/2025	24-hour observation and exposure	26/03/2025
27/03/2025	48-hour observation and exposure	27/03/2025
28/03/2025	72-hour observation and exposure	28/03/2025
	Reporting Phase	
03/04/2025	Draft Report Review	03/04/2025
16/04/2025	Final Report Review	16/04/2025

Inspections were performed according to the Standard Operating Procedures of the Test Facility's Quality Assurance Unit. The report was inspected as per the approved study plan and pertinent raw data is accurately reflected in the report.

Date: 16/04/2025

(Vandh Dogra)

Quality Assurance Unit
Toxicity Testing: GLP Test Facility
CSIR-Indian Institute of Toxicology Research
CRK Campus, Gheru, Sarojini Nagar Industrial
Area Kanpur Road, Lucknow-226008, India

LIST OF COMMONLY USED ABBREVIATIONS AND SYMBOLS

CSIR	Council of Scientific and Industrial Research
CoA	Certificate of Analysis
GLP	Good Laboratory Practices
IITR	Indian Institute of Toxicology Research
LC_{50}	Lethal Concentration
NAD	No Abnormalities Detected
NABL	National Accreditation Board for Testing and Calibration Laboratory
OECD	Organization for Economic Cooperation and Development
TIIS	Test Item Information Sheet
°C	Degree Celsius
h	Hour
l	Litre
mg	Milligram
mL	Millilitre

1. STUDY DETAILS

Study Title	:	Acute Fish Embryo Toxicity Test of Draynzyme
Test Item	:	Draynzyme
Study Number	:	CSIR-IITR/GLP/395
Sponsor	:	Pune Municipal Corporation Shivaji nagar, Pune 411005 Test conducted for Pune Municipal Corporation (In compliance to NGT matter of OA 323 of 2024)
Sponsor's Representative	:	Mangesh Dighe Environment Officer Pune Municipal Corporation E Mail: indradhanushya@punecorporation.org
Test Facility	:	Toxicity Testing: GLP Test Facility CSIR-Indian Institute of Toxicology Research CRK Campus, Gheru, Sarojini Nagar Industrial Area Kanpur Road, Lucknow-226008,India
Study Schedule		
Study Start Date	:	21/03/2025
Limit Test		
Experiment Start Date		24/03/2025
Date of Breeding	:	24/03/2025
Embryo Collection	:	25/03/2025
Test item exposure	:	25/03/2025
24 Hour Observation	:	26/03/2025
48 Hour Observation	:	27/03/2025
72 Hour Observation	:	28/03/2025
96 Hour Observation	:	29/03/2025
Analytical Measurement	:	
Experiment Completion Date	:	29/03/2025
Study Completion Date	:	16/04/2025

2. STUDY PERSONNEL

The following personnel participated in the conduct of the study.

Name	Function
Ms Priya Maurya	Study Director
Ms Vijaya Shree	Study Personnel
Mr Sudhanshu Mishra	Study Personnel

3. SUMMARY

Acute fish embryo toxicity was performed as per the OECD Guideline for the testing of Chemicals, Number 236 with the test item, Dryanzyme using zebrafish (*Danio rerio*) embryos. The test item was formulated in reconstituted water as per the OECD Guideline.

Prior to the test item exposure, healthy adult male and female fishes (breeding pairs) in 2:1 ratio were kept in egg collection chamber with separator. On the next morning, once the lights were turned on, separator was removed and the fishes were allowed to spawn for 30 minutes. After spawning, the embryos were collected carefully by decanting the water within one hour of fertilization. The embryos were then washed with E3 medium three-four times to prevent the fungal infection and further incubated in the Reconstituted water at 27°C until effluent exposure. Fertilized Embryos were selected under Stereomicroscope at 16 – 64 cell stage (1.5 hours post fertilization; hpf) for the subsequent experimental exposures.

Dryanzyme was found to be non-toxic at a limit test concentration of 100 mg/L in previous acute studies conducted in *Daphnia magna* in the test facility. Hence, embryos were exposed to 100 mg/L Dryanzyme concentrations in limit test along with control and 4 mg/L of 3, 4-dichloroaniline as positive control groups. Homogenous suspension of the test item was prepared by continuous stirring in reconstituted water for overnight using magnetic stirrer. From this, 2 mL of test solution was filled in 24-well plate prior to the embryo exposure. Fertilized embryos (approximately twice the number required) were exposed into the respective concentrations in petri dishes. The exposure was carried out semi-static by renewing the test media with freshly prepared test item for every 24 hours. 20 embryos per concentration were used along with 4 embryos as internal plate control with reconstituted water alone. The embryos were placed individually in 24-well plates, randomly positioned and incubated at 27°C in incubator for 96 hours. The embryos were then observed at 24-hour intervals under the microscope for the appearance of any developmental defects and mortality.

Appearance of apical end points such as coagulation, lack of somite formation, lack of heartbeat and non-detachment of tail were noted and presence of any one of the end points in embryo was considered to be lethal. At the end of 96 hour, embryos exposed to 100 mg/L of Dryanzyme along with control group resulted in 0% mortality whereas 4 mg/L concentration of 3,4-dichloroaniline as a positive control exhibited 80% embryo mortality.

4. OBJECTIVE

The purpose of this study was to assess the fish embryo acute toxicity test of Dryanzyme on embryonic stages of fish, followed by an observation period of 96 hours under semi-static conditions.

5. STUDY COMPLIANCE

The study was performed in accordance with the following:

1. OECD Principles of Good Laboratory Practice for the testing of chemicals as specified by International [C (97) 186/Final] Legislation.
2. OECD Test Guideline No. 236–Fish Embryo Acute Toxicity Test
3. The mutually agreed study plan and the Standard Operating Procedures of the test facility (CSIR-IITR/ECO/027, Revision 2).
4. IAEC Approval (IITR/IAEC/36/25) Dated: 20/03/2025.

6. AMENDEMENT AND DEVIATION

This study has no amendment and deviation.

6. MATERIALS AND METHODS

6.1 Materials

6.1.1 Test Item Information

(As furnished by the Sponsor)

Test Item	:	DWE/2005A-1KG ILD Draynzyme
Common Name	:	Draynzyme
Chemical Name	:	Not Provided
Accession Number	:	Not Provided
Batch / Lot Details	:	01/01/24
Date of Manufacture	:	January, 2024
Date of Expiry	:	January, 2026
Physical Appearance	:	Mustard yellow gel encapsulated inside polyester sack
Recommended storage	:	Store away from heat
Purity	:	Not Provided
Solubility	:	Slowly dissolve into flowing water over a long period of time
Intended Usage	:	Treatment of contamination created by raw sewage exposure
Manufactured By	:	Dhara Biotech, Nr, Gaushala, Sarsa – Vasad chokdi, Bhalej Road, Sarsa, Anand, Gujrat - 388365
Supplied By	:	Quin Quent Industries Pvt Ltd S No. 131/2A Rajyog Colony Warje Pune

		- 411052

6.1.2 Identity of the Test Item

The Test Item information Sheet (TIIS), Certificate of Analysis (CoA) and Material Safety Data Sheet (MSDS) has been provided by the sponsor. The responsibility for the correct identity and purity of the test item rests with the sponsor. The authenticity of the test item was not being conducted at the test facility.

6.1.3 Test System & Test Conditions

Species	:	*Danio rerio* embryos
Justification for the selection of species	:	Recommended by the regulatory guideline (OECD 236) for aquatic toxicity assessment.
Source	:	Embryos collected from In-house fish stock, Ecotoxicology Lab, CRK Campus, CSIR-Indian Institute of Toxicology Research, Lucknow.
Test Room Details	:	Room No: 46 & 48,
Test Type	:	Semi-Static
Test Vessel	:	24 well plates
Number of Replicates	:	**Limit Test:** 20 embryos placed individually for control, 100 mg/Land 4 mg/L positive control.
Light	:	16-hour light and 8-hour darkness
Test Medium	:	Reconstituted water
pH Range	:	**Limit Test** Control: 6.89 – 7.04 Treatment: 6.93 – 7.06
Temperature (°C)	:	**Limit Test** Control: 26.52 – 26.66 Treatment: 26.55 – 26.68
Dissolved Oxygen (%)	:	**Limit Test** Control: 82.8 – 90.9 Treatment: 82.6 – 90.7
Hardness (mg/L)	:	**Limit Test** (at the start of the test): 152
Test Duration	:	96 hours

6.2 Methods

6.2.1 Embryo Collection

Wild type zebrafish from in-house stock were maintained at 25-27ºCtemperature, pH 6.50-8.50 and 16:08 h light-dark cycle. Prior to the test item exposure, healthy adult male and female fishes (breeding pairs) in 2:1 ratio were kept in egg collection chamber with separator. On the next morning, once the lights were turned on, separator was removed and the fishes were allowed to spawn for 30 minutes. After spawning, the embryos were collected carefully by decanting the water within one hour of fertilization. The embryos were then washed with reconstituted water and incubated at 27°C until test item exposure. Fertilized embryos were then selected under Stereomicroscope at 16 – 64 cell stage (1.5 hours post fertilization; hpf) for the subsequent experimental exposures.

6.2.2 Vehicle

Reconstituted water will be used to prepare the desired test concentration

6.2.3 Dose Formulation

Limit test was carried out at a concentration of 100 mg/L of Dryanzyme. Stock concentration of 0.1 mg/mL Dryanzyme was prepared by dissolving 100 mg in 1000 mL of reconstituted water. Homogenous suspension of the test item was prepared by continuous stirring in reconstituted water for overnight using magnetic stirrer. From this, 2 mL of test solution was filled in each 24-well plate prior to the embryo exposure. Control group consists of reconstituted water without Dryanzyme. The exposure was carried out semi-static by renewing the test media with freshly prepared test item for every 24 hours.

3, 4-dichloroaniline was used as appositive control at 4 mg/L concentration. Stock concentration of 0.004 mg/mL 3,4-DCA was prepared by dissolving 4 mg in 1000 mL of reconstituted water. From this, 2 mL of test solution was filled in each 24-well plate prior to the embryo exposure. Exposure to 4 mg/L of 3, 4-dichloroaniline was static. The embryos were remained in the test solution continuously for a period of 96-hours.

6.2.4 Limit Test

Dryanzyme was found to be non-toxic at a limit test concentration of 100 mg/L in previous acute studies conducted in *Daphnia magna* in the test facility. Hence, the embryos were exposed to 100 mg/L Dryanzyme concentration along with a control and positive control (4 mg/L of 3, 4 - dichloroaniline) groups. The test solutions were prepared in a volume of 1000 mL. Stock solution with 100 mg/L concentration was prepared by dissolving 100 mg of Dryanzyme in 1000 mL of test media.

Fertilized embryos (approximately twice the number required) were exposed into the respective concentrations in petri dishes. 20 embryos per concentration were used along with 4 embryos as internal plate control with reconstituted water alone. The embryos were placed individually in 24-well plates, randomly positioned and incubated at 27°C in incubator for 96 hours. The embryos were then observed at 24-hour intervals under the microscope for the appearance of any developmental defects and mortality.

6.2.5 Treatment

During limit test, 20 embryos per concentration were used along with 4 embryos as internal plate control. The embryos were placed individually in 24-well plates, randomly positioned and incubated at 27°C in incubator for 96 hours.

6.2.6 Physico-chemical Parameters

The test medium was analyzed for pH, temperature, and dissolved oxygen during the initial and final phase of 0–24-hour, 24–48-hour, 48-72 hour and 72–96-hour intervals in control and treated groups (Table 1-3).

7. OBSERVATIONS

The embryos were then observed at 24-hour intervals under the stereo microscope for a period of 96 hours for the appearance of any apical end points as the manifestation of developmental defects and embryo lethality. Appearance of apical end points such as coagulation, lack of somites, lack of heart beat, and non-detachment of tail were noted and presence of any one of the end points in embryo was considered to be lethal.

8. RESULTS

8.1 Embryo Mortality

Embryos exposed to 100 mg/L Dryanzyme concentration resulted in 0% mortality along with control during the limit test. Embryos exposed to 4 mg/L concentration of positive control (3, 4 –dichloroaniline) resulted in 80% lethality at the end of 96- hour exposure. Table 4 shows the details of apical end points observed and percent embryo mortality after Dryanzyme exposure.

8.2 Hatching Rate

In limit Test, 100% hatching rate was noted in control, internal plate and embryos exposed to 100 mg/L Dryanzyme concentration (Table 5).

9. VALIDITY CRITERIA

The study is considered to be valid since the following criterion was met:

1. The overall fertilization rate of all embryos collected was 88.83% (should be ≥70% as per the guideline)
2. The water temperature was maintained between 26.52 – 26.66°C in all test chambers during the limit test (should be between 25-27°C as per the guideline).
3. Overall survival of control embryos in Limit Test was 100% (should be ≥90% as per the guideline).
4. Hatching rate in control embryos was 100% at the end of 96 hour in Limit Test (should be ≥80% at the end of 96-hour exposure).
5. The dissolved oxygen concentration in control and highest test concentrations was greater than 90.9% of air saturation value (should be ≥ 80% of air saturation value).

10. STATISTICAL ANALYSIS

No statistical analysis was carried out as study was concluded as a limit test.

11. DATA COMPILATION

Data are summarized in tabular form for each treatment and control groups. The number of embryos used and mortality details at each observation are reported.

12. CONCLUSION

Based on the test results, the LC_{50} of Dryanzyme for *Danio rerio* embryos observed for a period of 96 hours was found to be greater than 100 mg/L.

13. ARCHIVING

The following has been archived at the test facility for 9 years after completion of the study: study plan, all raw data, draft and final reports. A representative sample of test Item has been sent from the Test Item Control Office to the Archives in the test facility. The sample shall be stored for a period of 9 years from the date of this final report. Sponsor's approval would be sought before discarding of any archived study materials.

14. REPORT DISTRIBUTION

The study report will be distributed as follows:

Test Facility : One signed final report in original (Copy No.1/2) and an electronic copy in the PDF format.

Sponsor : One signed final report in original (Copy No. 2/2)

TABLE 1: PHYSICO-CHEMICAL PARAMETERS– LIMIT TEST

Parameter	Conc. (mg/L)	0-24 Hour		24-48 Hour		48-72 Hour		72-96 Hour		MEAN			
		New 0h	Old 24h	New 24h	Old 48h	New 48h	Old 72h	New 72h	Old 96h	New		Old	
										Mean	SD	Mean	SD
pH	CONTROL	6.91	7.04	6.89	7.01	6.92	7.02	6.91	7.04	6.91	0.01	7.03	0.02
	100	6.95	7.06	6.93	7.05	6.97	7.06	6.95	7.05	6.95	0.02	7.06	0.01
	3,4 - DCA (Positive Control)	6.42								6.42			

TABLE 2: PHYSICO-CHEMICAL PARAMETERS– LIMIT TEST

Parameter	Conc. (mg/L)	0-24 Hour		24-48 Hour		48-72 Hour		72-96 Hour		MEAN			
		New 0h	Old 24h	New 24h	Old 48h	New 48h	Old 72h	New 72h	Old 96h	New		Old	
										Mean	SD	Mean	SD
Temp. (°C)	CONTROL	26.66	26.52	26.62	26.59	26.61	26.62	26.63	26.61	26.63	0.02	26.59	0.05
	100	26.68	26.55	26.65	26.58	26.62	26.61	26.65	26.62	26.65	0.02	26.59	0.03
	3,4 - DCA (Positive Control)	26.69								26.69			

TABLE 3: PHYSICO-CHEMICAL PARAMETERS– LIMIT TEST

Parameter	Conc. (mg/L)	0-24 Hour		24-48 Hour		48-72 Hour		72-96 Hour		MEAN			
		New 0h	Old 24h	New 24h	Old 48h	New 48h	Old 72h	New 72h	Old 96h	New		Old	
										Mean	SD	Mean	SD
Dissolved Oxygen	CONTROL	89.4	84.6	90.2	85.1	89.9	83.6	90.9	82.8	90.1	0.6	84.0	1.0
	100	89.3	84.9	90.3	84.8	89.7	83.1	90.7	82.6	90.0	0.6	83.9	1.2
	3,4 - DCA (Positive Control)	86.5								86.5			

NOTE: PC-Positive Control; **CONC**. - Concentration; **DCA** - 3, 4-dichloroaniline

TABLE 4: MORTALITY DATA – LIMIT TEST

End points Observed	Group	Concentration (mg/L)	Embryos/ Conc.	Number of Lethal Embryos				Cumulative Mortality (%)
				24h	48h	72h	96h	
Coagulation Lack of Somites Non-detachment of tail Lack of heart beat	G1	Control	20	0/20	0/20	0/20	0/20	0
		Internal Plate Control	4	0/4	0/4	0/4	0/4	0
	G2	100	20	0/20	0/20	0/20	0/20	0
		Internal Plate Control	4	0/4	0/4	0/4	0/4	0
	G3	4 mg/L of 3,4– DCA (Positive Control)	20	0/20	4/20	5/20	7/20	80
		Internal Plate Control	4	0/4	0/4	0/4	0/4	0

Note: IPC-Internal Plate Control; **PC**-Positive Control; **3, 4-DCA**- 3, 4-dichloroaniline.

TABLE 5: EMBRYO HATCHING RATE DATA – LIMIT TEST

Group	Concentration (mg/L)	Embryos Exposed	Embryos Hatched at 48 hours	% Hatching Rate
G1	Control	20	16	80
	Internal Plate Control	4	2	50
G2	100	20	16	80
	Internal Plate Control	4	3	75
G3	4 mg/L of 3, 4– DCA	20	06	30
	Internal Plate Control	4	3	75

Note: 3, 4-DCA- 3, 4-dichloroaniline

ANNEXURE–I

STUDY PLAN

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

STUDY PLAN

STUDY No.: CSIR-IITR/GLP/395

ACUTE FISH EMBRYO TOXICITY TEST OF DRAYNZYME

TEST ITEM: DRAYNZYME

SPONSOR
PUNE MUNICIPAL CORPORATION
SHIVAJI NAGAR, PUNE 411005

TEST FACILITY

TOXICITY TESTING: GLP TEST FACILITY
CSIR-INDIAN INSTITUTE OF TOXICOLOGY RESEARCH
CRK CAMPUS, GHERU, SAROJINI NAGAR INDUSTRIAL AREA
KANPUR ROAD, LUCKNOW-226008
UTTAR PRADESH, INDIA

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

TABLE OF CONTENTS

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

1. **STUDY DETAILS**

Study Title	:	Acute Fish Embryo Toxicity Test of Draynzyme
Test Item	:	DWE/2005A-1KG ILD Draynzyme
Study Number	:	CSIR-IITR/GLP/395
Sponsor	:	Pune Municipal Corporation Shivaji nagar, Pune 411005 Test conducted for Pune Municipal Corporation (In compliance to NGT matter of OA 323 of 2024)
Sponsor's Representative	:	Mangesh Dighe Environment Officer, Pune Municipal Corporation E Mail: indradhanushya@punecorporation.org
Test Facility	:	Toxicity Testing: GLP Test Facility CSIR-Indian Institute of Toxicology Research CRK Campus, Gheru, Sarojini Nagar Industrial Area Kanpur Road, Lucknow-226008, India
Study Director	:	Ms Priya Maurya Ecotoxicology Laboratory Vishaktata Parikshan: GLP Test Facility CSIR-Indian Institute of Toxicology Research CRK Campus, Gheru, Sarojini Nagar Industrial Area Kanpur Road, Lucknow-226008, India Contact Details: +91-522-2476051(Extn:210) +91-9554664279 E Mail: priyamaurya528@gmail.com
Study Personnel	:	Ms Vijaya Shree Mr Sudhanshu Mishra
Study Schedule **Study Start Date** **Limit Test** **Experiment Start Date** Date of Breeding Embryo Collection Test item exposure 24 Hour Observation 48 Hour Observation 72 Hour Observation 96 Hour Observation	: : : : : : : : :	 21/03/2025 24/03/2025 24/03/2025 25/03/2025 25/03/2025 26/03/2025 27/03/2025 28/03/2025 29/03/2025

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

Experiment Completion Date	:	29/03/2025
Study Completion Date	:	DD/04/2025

2. **QUALITY ASSURANCE**
The Quality Assurance Unit of the Test Facility will inspect the study, the raw data, the draft and final reports. Findings of all inspections will be reported to the Management and to the Study Director. The details of phase inspected, Inspection dates and reporting dates will be entered as QA-statement in the study report.

The Quality Assurance Unit has reviewed the study plan and will receive a copy thereof.

3. **STUDY COMPLIANCE**
The study will be performed in accordance with the following:
- OECD Principles of Good Laboratory Practice for the testing of chemicals as specified by International [C (97) 186/Final] Legislation
- OECD Test Guideline No. 236 –Fish Embryo Acute Toxicity Test.
- The mutually agreed study plan and the Standard Operating Procedures of the test facility (CSIR-IITR/ECO/027; Rev 2).
- OECD Guidance Document No: 23 – Guidance Document on Aqueous-Phase Aquatic Toxicity Testing of Difficult Test Chemicals, 08 February 2019.
- IAEC Approval (IITR/IAEC/36/25) Dated: 20/03/2025.

4. **AMENDMENT PROCEDURES**
This study plan may be amended or subjected to alterations. In each case, any amendment to the approved study plan and the reasons for such amendments will be documented and realized only after written / e mail consent from the study Sponsor and review by the Quality Assurance Unit and Test Facility Management. If immediate action is necessary, verbal agreement from the Sponsor will be confirmed as soon as possible by study plan amendment followed by written consent from the sponsor. Minor changes (unplanned) of the study plan which do not influence the procedures or the outcome of the study may be subject to the

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

discretion of the Study Director, but will be mentioned in the report as deviations.

5. **SAFETY PRECAUTIONS**

Gloves, face mask and goggles (if required) will be used in addition to protective body garments and shoes to ensure adequate personal health and safety. In case of eye contact, the eye will be washed thoroughly with water and medical treatment will be sought. In case of skin contact, it will be washed with soap and water with subsequent medical aid.

6. **OBJECTIVE**

The purpose of the study is to assess the acute embryo toxicity of test item, Draynzyme to zebra fish (*Danio rerio*) embryos. The sensitivity and reliability of the experimental technique employed will be assessed with 3,4-dichloroaniline at 4 mg/L concentration as per OECD test guideline 236.

7. **MATERIALS AND METHODS**

7.1 Materials

7.1.1 Test Item Information

(As furnished by the sponsor)

Test Item	:	DWE/2005A-1KG ILD Draynzyme
Common Name	:	Draynzyme
Chemical Name	:	Not Provided
Accession Number	:	Not Provided
Batch / Lot Details	:	01/01/24
Date of Manufacture	:	January, 2024
Date of Expiry	:	January, 2026
Physical Appearance	:	Mustard yellow gel encapsulated inside polyester sack
Recommended storage	:	Store away from heat
Purity	:	Not Provided
Solubility	:	Slowly dissolve into flowing water over a long period of time
Intended Usage	:	Treatment of contamination created by raw sewage exposure
Manufactured By	:	Dhara Biotech, Nr, Gaushala, Sarsa – Vasad chokdi, Bhalej Road, Sarsa, Anand, Gujrat - 388365

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

Supplied By	:	Quin Quent Industries Pvt Ltd S No. 131/2A Rajyog Colony Warje Pune - 411052

7.1.2 Identity of the Test Item

The Test Item information Sheet (TIIS), Certificate of Analysis (CoA) and Material Safety Data Sheet (MSDS) has been provided by the sponsor. The responsibility for the correct identity and purity of the test item rests with the sponsor. The authenticity of the test item was not being conducted at the test facility.

7.1.3 Test System & Test Conditions

Species	:	*Danio rerio* embryos
Justification for the selection of species	:	Recommended by the regulatory guideline (OECD 236) for the aquatic toxicity assessment.
Source	:	Embryos collected from In-house fish stock, Ecotoxicology Lab, CRK Campus, CSIR-Indian Institute of Toxicology Research, Lucknow
Test Room Details	:	Room No: 46 & 48
Test Type	:	Semi-Static
Test Vessel	:	24 well plates
Number of Replicates	:	**Limit Test:** 20 embryos placed individually for Control, 100 mg/L Draynzyme, and 4 mg/L positive control.
Light	:	16-hour light and 8-hour darkness
Test Medium	:	Reconstituted water
Test Medium pH Range	:	6.50 – 8.50
Temperature	:	25 – 27°C
Dissolved Oxygen	:	≥80% air saturation value
Hardness	:	100 – 300 mg/l
Test Duration	:	96 hours

7.2 Methods

7.2.1. Embryo Collection

Adult healthy male and female zebrafishes in 2:1 ratio will be maintained in breeding chamber for embryo collection before the onset of darkness on the day prior to the test item exposure. The

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

breeding pairs will be maintained at a temperature of 25-27°C with 16-hour light: 8-hour dark photoperiod.

Spawning and fertilization will take place within 30 minutes after the onset of light in the following morning (i.e., on the day of test item exposure). The embryos will be collected, washed with reconstituted water, and used for test item exposure.

7.2.2. Vehicle

Reconstituted water will be used to prepare the desired test concentration.

7.2.3. Test Item Preparation

The stock solution will be prepared in reconstituted water shortly before the start of the test from which the test groups will be prepared. Since the test item is not soluble in water, stock solution is prepared by adding the required quantity of test item in the reconstituted water and stirred continuously for 24 hours as per the OECD guidance document 23. The aqueous phase of the test solution will be used for daphnia exposure.

7.2.4. Limit Test

Drayzyme was found to be non-toxic at a limit test concentration of 100 mg/L in previous acute studies conducted on *daphnia magna*. Hence, fish embryo acute toxicity test will be carried out as a limit test by exposing the embryos to 100 mg/L concentration of test item. 20 embryos each will be used for control, 100 mg/L Drayzyme, and 4 mg/L 3,4-dichloroaniline as positive control in 24-well plate. In each group, 4 embryos will be exposed to reconstituted water and serve as internal plate control.

The exposure will be initiated within 1.5 hour after the fertilization by exposing twice the number of embryos required per treatment group in glass petri dishes. Then the embryos will be visualized under the microscope, separated from unfertilized eggs, and transferred to 24-well plates. The test will be carried out as semi-static exposure by refilling the well plates with 2 ml of freshly prepared test solutions and incubated in the incubator for a period of 96-hours at every 24-hour intervals. If no mortality is observed during the 96-hour exposure period, the study will be concluded as a limit test.

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

8. **OBSERVATIONS**

pH, temperature, dissolved oxygen & hardness of system water will be recorded on day of breeding for embryo collection. Dissolved oxygen, temperature and pH values will be measured at the start of the test during the test medium renewal, in fresh and old media, in the control, teat item, and positive control treated groups. Hardness of dilution water will be measured at the start of the test

Embryo mortality will be recorded on every 24-hour intervals by observing apical endpoints like coagulation of embryo, lack of somite formation, non-detachment of tail and lack of heartbeat. These apical end points are the indicators of embryo lethality. Any positive outcome in one of these observations means that the embryo is dead. Heartbeat will be visible after 48-hour and absence of heartbeat will be recorded after 48, 72, and 96 hours.

Hatching rate will be recorded from 48-hour onwards and reported. Although hatching is not an endpoint used for the calculation of the LC_{50}, hatching ensures exposure of the embryo without a potential barrier function of the chorion, and as such may help data interpretation.

9. **VALIDITY CRITERIA**

(i) The overall fertilisation rate of all eggs collected should be ≥ 70%.
(ii) The water temperature should be maintained between 25-27°C in test chambers at any time during the test.
(iii) Overall survival of embryos in the control group should be ≥90% at the end of 96-hour exposure.
(iv) Hatching rate in the control group should be ≥80% at the end of 96-hour exposure.
(v) Dissolved oxygen in the control and highest test concentration should be ≥80% of air saturation value.

10. **STATISTICAL ANALYSIS**

No statistical analysis will be performed since the study will be conducted as a limit test.

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

11. **DATA COMPILATION**

Data will be summarized in a tabular form, the number of embryos used, percentage of lethality at each concentration during 96-hour exposure. Test media pH, temperature, dissolved oxygen, hardness will also be tabulated.

12. **FINAL REPORT**

The final report will be prepared in compliance to the principles of GLP and normally include, but not limited to the following:

- A descriptive title.
- The name and address of the Sponsor and the test facility along with the details of study schedule.
- The names of all personnel involved in the study.
- A compliance statement signed by the Study Director that all applicable GLP regulations were followed in the conduct of the study.
- Quality Assurance (QA) statement: that states that the report accurately reflects the raw data obtained during the performance of the study and including the dates of QA activities and the dates reported to study director and management.
- The Test Item and its code, composition and other appropriate characteristics and vehicle with identification by name.
- Complete description of the test system including species, source, number, test conditions, photoperiod, and acclimation.
- Statistical analysis of the results (if applicable).
- Method of preparation of stock and test solutions.
- Graph of the concentration mortality curve at the end of the test.
- LC_{50} values, with 95% confidence limits.

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

- Highest concentration causing no mortality, lowest concentration causing 100% mortality.
- Physico-chemical parameters details.
- A description of the results; discussion and conclusion.
- A description of all study plan deviations, if any.
- A description of all circumstances that may have affected the quality or integrity of the study.
- The storage locations of all raw data, specimens, reports, Test Item reference sample and the archiving period.

13. ARCHIVING

The following will be archived at the test facility for at least 9 years (3 cycles of GLP) after completion of the study: study plan, all raw data, draft and final reports, a representative sample of Test Item (approximately one gram), etc. Before discarding of any archived study materials, the Sponsor will be contacted for the disposal.

14. STUDY PLAN DISTRIBUTION

The final study plan (original copies) will be distributed as follows:
Test Facility: One signed study plan in original (Copy No. 1/2)
Sponsor : One signed study plan in original (Copy No. 2/2)
Document Control: One controlled copy
Quality Assurance Unit: One controlled copy
Study Personnel: One controlled copy

15. REPORT DISTRIBUTION

The study report will be distributed as follows:

Test Facility : One signed final report in original (Copy No. 1/2) and an electronic copy in PDF format.
Sponsor : One signed final report in original (Copy No. 2/2).

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

16. AGREEMENT

This study plan for Study No.: CSIR-IITR/GLP/395, "Fish Embryo Acute Fish embryo Toxicity Test of Draynzyme" has been mutually agreed:

for **TEST FACILITY**	for **STUDY SPONSOR**
1. _____	1. _____
STUDY DIRECTOR	SPONSOR REPRESENTATIVE
Date: 21/02/2025	Date: Email Consent received on: 24/02/2025

2. _____
QUALITY ASSURANCE UNIT
Date: 21/02/2025

3. _____
TEST FACILITY MANAGEMENT
Date: 21/03/2025

Study No. CSIR-IITR/GLP/395
Copy No. 1/2

ANNEXURE – II

CERTIFICATE OF ANALYSIS

Green Leaf

Test Report
Water Sample Analysis Report

Client's Name & Address: **M/s. Dhara Bio-tech** Near SarasChokdi, Near Gaushala, Kunjrav Road, Sarsa, Anand 388365, Gujarat. **Contact Person:** Mr. Vasantlal Patel	**Report No:** GLEPL/240124/01 **Issue Date:** 01/02/2024

Lab ID Code	:	GLEPL/240124/WL_1			
Sample Description	:	Product Sample	Purpose	:	As per Client requirement
Date of Sampling	:	Submitted by Client (Dhara Bio-tech)	Sample collected/Submitted by	:	Submitted by Client (Dhara Bio-tech)
Date of Sample Received	:	24/01/2024	Test Parameters	:	As per Client requirement
Date of starting Analysis	:	25/01/2024	Quantity/No. of sample	:	1No. Batch No. 01/01/24
Date of completion Analysis	:	31/01/2024	Packed/Seal	:	Sealed (13/01/24)

Result Table

Sr. No.	Test Parameters	Test Method	Unit	Results
1	Drayzyme for Bacteria Count	ISO 16649-3	MPN/100 mL	Absent

Remark: In accordance to G.S.R 613(E)-This product does not contain any Bacteria, thus does not require 'Appraisal by genetic Engineering Appraisal Committee).

Chemist

R.V. Dare

Authorized Signatory
Rekha Dare

Notes: (1) The results pertain to tested items only.
(2) This report shall not be reproduced, except in full, without written approval of the laboratory.
(3) Authenticity of this Report could be validated with office copy at Greenleaf Evirotech Pvt. Ltd.
(4) Perishable samples will be destroyed after testing, others after 7 days from the date of issue of the report, unless otherwise agreed with the customer or as required by the applicable regulations.

CIN: U74140GJ2010PTC059798

Greenleaf EnvirotechPvt. Ltd., Nr. RangoliFlats,Radhanpur Road, Mehsana – 384002. Gujarat, India.
Tel : +91-9725519974, E-mail: info@glepl.com, Web: www.glepl.com
Branch Office: 304, Kankavati Complex, Singanpor-Causway Road, Katargam, Surat– 395004

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

ANNEXURE –III

TEST SYSTEM CHARACTERIZATION CERTIFICATE

IGIB
INSTITUTE OF GENOMICS & INTEGRATIVE BIOLOGY
Genomes Knowledge Partner

जीनोमिकी और समवेत जीव विज्ञान संस्थान
(वैज्ञानिक तथा औद्योगिक अनुसंधान परिषद)
विश्वविद्यालय परिसर माल रोड, दिल्ली ११०००७

Institute of Genomics & Integrative Biology
(COUNCIL OF SCIENTIFIC & INDUSTRIAL RESEARCH)
DELHI UNIVERSITY CAMPUS
MALL ROAD, DELHI-110007, INDIA

20 July 2018

To Whom It May Concern

This is to certify that the Assam Wild Type strain (ASWT) zebrafish embryos (approx. 500 numbers) from the CSIR-Institute of Genomics and Integrative Biology (CSIR IGIB) were provided to the CSIR-Indian Institute of Toxicology Research, Lucknow on June 14, 2018.

The embryos should be raised and maintained under controlled environmental conditions for experimental and breeding purposes. The ASWT zebrafish should be used for research application only. They should not be used for commercial purpose unless prior permission is obtained from CSIR IGIB.

Sincerely,

Sridhar Sivasubbu, Ph.D.,
Principal Scientist, CSIR-Institute of Genomics & Integrative Biology,
Associate Professor, Academy of Scientific and Innovative Research,
Sukdev Vihar, Mathura Road,
Delhi -100025,
India.
sridhar@igib.in
s.sivasubbu@igib.res.in

ANNEXURE –IV
GLP CERTIFICATE

सत्यमेव जयते

National Good Laboratory Practice (GLP) Compliance Monitoring Authority (NGCMA)
Department of Science and Technology
GOVERNMENT OF INDIA

Certificate of GLP Compliance

This is to certify that

Toxicity Testing: GLP Test Facility, CSIR-Indian Institute of Toxicology Research
CRK Campus, Gheru, Sarojini Nagar Industrial Area
Lucknow-226008, Uttar Pradesh (India)

is a GLP certified test facility in compliance with the NGCMA's Document No. GLP-101 "Terms & Conditions of NGCMA for obtaining and maintaining GLP certification by a test facility" and OECD Principles of GLP.

The test facility conducts the below-mentioned tests/ studies:

- **Toxicity Studies**
- **Mutagenicity Studies**
- **Environmental Toxicity Studies on Aquatic and Terrestrial Organisms**
- **Analytical and Clinical Chemistry Testing**

The specific area(s) of expertise, test item(s) and test system(s) are listed in the annexure overleaf.

Validity: June 5, 2023 – June 4, 2026

Certificate No. :GLP/C-213/2023
Issue Date :07-12-2023

(Dr. Ekta Kapoor)
Head, NGCMA

Study on Freshwater Alga Growth Inhibition:

FINAL REPORT

STUDY TITLE

FRESHWATER ALGA GROWTH INHIBITION TEST OF DRAYNZYME

TEST ITEM: DRAYNZYME

STUDY NO.: CSIR-IITR/GLP/396

STUDY COMPLETED ON:
16/04/2025

SPONSOR
PUNE MUNICIPAL CORPORATION
SHIVAJI NAGAR, PUNE 411005

TEST FACILITY
TOXICITY TESTING: GLP TEST FACILITY
CSIR-INDIAN INSTITUTE OF TOXICOLOGY RESEARCH
CRK CAMPUS, GHERU, SAROJINI NAGAR INDUSTRIAL AREA
KANPUR ROAD, LUCKNOW-226008
INDIA

TABLE OF CONTENTS

STATEMENT OF CONFIDENTIALITY

The report contains confidential and proprietary information to Pune Municipal Corporation Shivaji Nagar, Pune 411005. The contents of this report will not be disclosed to anyone without an expressed or approval of competent authority of Pune Municipal Corporation Shivaji Nagar.

STATEMENT OF COMPLIANCE

The study was performed using the OECD Principles of Good Laboratory Practice for the testing of chemicals as specified by International [C (97) 186/Final] Legislation as a guidance document. This study was conducted in accordance with the Standard Operating Procedures of Toxicity Testing: GLP Test Facility, CSIR-Indian Institute of Toxicology Research and the mutually agreed study plan, which was signed by the Study Director on 05/03/2025, for which email approval was received from the sponsor on 27/02/2025.

DECLARATION

The Study Director hereby declares that the work was performed under her supervision and in accordance with the described procedures. It is assured that the reported results represent the raw data obtained during the experimental work. No circumstances have been left unreported.

Study Director
Date: 16/04/2025

Test Facility Management
Date: 16/04/2025

Deputy Test Facility Management
Vishaktata Parikshan : GLP Anuroop Suvidha
Toxicity Testing: GLP Compliant Facility CSIR-IITR Lucknow, India

QUALITY ASSURANCE STATEMENT

Study No.: CSIR-IITR/GLP/396, "Freshwater Alga Growth Inhibition Test of Draynzyme" has been inspected in accordance with the OECD Principles of Good Laboratory Practice for the testing of chemicals as specified by International [C (97) 186/Final] Legislation.

This study was inspected, and findings reported to the Management and Study Director on the dates shown below:

INSPECTION DATE	PHASE	REPORTING DATE
	Initiation Phase	
19/02/2025	Draft Study Plan review	19/02/2025
05/03/2025	Final Study Plan review	05/03/2025
	In-Life Phase	
06/03/2025	Pre-culture cell count of Limit Test	06/03/2025
10/03/2025	0-hour pre-culture cell count of Limit Test and Test Item Exposure	10/03/2025
11/03/2025	24-hour cell count of Limit Test	11/03/2025
12/03/2025	48-hour cell count of Limit Test	12/03/2025
13/03/2025	72-hour cell count of Limit Test	13/03/2025
	Reporting Phase	
03/04/2025	Draft Report Review	04/04/2025
16/04/2025	Final Report Review	16/04/2025

Inspections were performed according to the Standard Operating Procedures of the Test Facility's Quality Assurance Unit. The report was inspected as per the approved study plan and pertinent raw data is accurately reflected in the report.

Date: 16/04/2025

(Navdeep Dwivedi)
Quality Assurance Unit
Toxicity Testing: GLP Test Facility
CSIR-Indian Institute of Toxicology Research
CRK Campus, Gheru, Sarojini Nagar Industrial Area Kanpur Road, Lucknow-226008, India

LIST OF COMMONLY USED ABBREVIATIONS AND SYMBOLS

°C	Degree Celsius
CoA	Certificate of Analysis
CSIR	Council of Scientific and Industrial Research
EC_{50}	Effective Concentration
E_rC_{50}	Average Specific Growth Rate
E_yC_{50}	Algal Growth Yield
G	gram
GLP	Good Laboratory Practices
IITR	Indian Institute of Toxicology Research
LOEC	Lowest Observed Effect Concentration
mg/L	Milligram/liter
mL	Millilitre
NAD	No Abnormalities Detected
NOEC	No Observed Effect Concentration
OECD	Organization for Economic Cooperation and Development
%	Percentage
SD	Standard Deviation

1. STUDY DETAILS

Study Title	:	Freshwater Alga Growth Inhibition Test of Draynzyme
Test Item	:	DWE/2005A-1KG ILD Draynzyme
Study Number	:	CSIR-IITR/GLP/396
Sponsor	:	Pune Municipal Corporation Shivaji nagar, Pune 411005 Test conducted for Pune Municipal Corporation (In compliance to NGT matter of OA 323 of 2024)
Sponsor's Representative	:	Mangesh Dighe Environment Officer Pune Municipal Corporation E Mail: indradhanushya@punecorporation.org
Test Facility	:	Toxicity Testing: GLP Test Facility CSIR-Indian Institute of Toxicology Research CRK Campus, Gheru, Sarojini Nagar Industrial Area Kanpur Road, Lucknow-226008, India
Study Schedule **Study Start Date**	:	05/03/2025
Limit Test **Experiment Start Date**		06/03/2025 – 10/03/2025
Pre-culture	:	10/03/2025
Test Item Exposure	:	11/03/2025
24 Hour Observation	:	12/03/2025
48 Hour Observation	:	13/03/2025
72 Hour Observation	:	
Experiment Completion Date	:	13/03/2025
Study Completion Date	:	16/04/2025

2. STUDY PERSONNEL

The following personnel participated in the conduct of the study.

Name	Function
Ms Vijaya Shree	Study Director
Ms Priya Maurya	Study Personnel
Mr Sudhanshu Mishra	Study Personnel

3. SUMMARY

DWE/2005A-1KG ILD Draynzyme was found to be non-toxic at a limit test concentration of 100 mg/L in previous acute study conducted on daphnia in the test facility. Hence, Freshwater Alga Growth Inhibition Test of DWE/2005A-1KG ILD Draynzyme will be carried out as a limit test at 100 mg/L concentration of test item. Prior to the test item exposure, pre-culture was maintained by inoculating $5x10^3$ cells/ml in a conical flask containing 100 ml of algal growth medium. The volume of algal growth medium was 100 ml in each control and treatment flask. Six replicates for both control and test concentrations were maintained for DWE/2005A-1KG ILD Draynzyme exposure. After the exposure on day 0, algal cells were observed microscopically and the cell count performed at 24, 48 and 72 h using Hemocytometer in all replicates of the control and treatment groups. Microscopic observation revealed that the cells were normal, and no abnormal appearance of algal cells was observed on exposure to the DWE/2005A-1KG ILD Draynzyme.

Average Specific Growth Rate:

The percent inhibition on the average growth rate at the end of 72 h was 0.09% for 100 mg/L concentration of DWE/2005A-1KG ILD Draynzyme.

From the average specific growth rate recorded, the concentration that inhibited 50% growth (E_rC_{50}) of the freshwater green algae, *Pseudokirchneriella subcapitata* was found to be greater than 100 mg/L of DWE/2005A-1KG ILD Draynzyme.

The no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) are not applicable since the study was conducted as a limit test.

Yield (biomass):

The percent inhibition on the yield at the end of 72 hour was 0.48% for 100 mg/L concentration of DWE/2005A-1KG ILD Draynzyme.

From the yield recorded, the concentration that inhibited 50% yield (E_yC_{50}) of the freshwater green algae, *Pseudokirchneriella subcapitata* was calculated as greater than 100 mg/L of DWE/2005A-1KG ILD Draynzyme.

The no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) are not applicable since the study was conducted as a limit test.

4. OBJECTIVE

The purpose of the study was to determine the effect of test item, DWE/2005A-1KG ILD Draynzyme on the exponentially growing culture of unicellular green algae, *Pseudokirchneriella subcapitata* with an observation period of 72 hours. The effect of the test item on the growth of the algal culture compared with control was determined and the concentration which inhibited growth rate by 50% (E_rC_{50}) and the concentration that inhibited biomass by 50% (E_yC_{50}) were calculated.

5. STUDY COMPLIANCE

- The study will be performed in accordance with the following:
- OECD Principles of Good Laboratory Practice for the testing of chemicals as specified by International [C (97) 186/Final] Legislation
- OECD Test Guideline No. 201 – "Freshwater Alga and Cyanobacteria Growth Inhibition Test", adopted on 23 March 2006.
- The mutually agreed study plan and the Standard Operating Procedures of the test facility (CSIR-IITR/ECO/024; Revision 02).

6. AMENDMENT AND DEVIATION

This study has no deviation and amendment.

7. MATERIALS AND METHODS

7.1 Materials

7.1.1 Test Item Information

(As furnished by the Sponsor)

Test Item	:	DWE/2005A-1KG ILD Draynzyme
Common Name	:	Draynzyme
Chemical Name	:	Not Provided
Accession Number	:	Not Provided
Batch / Lot Details	:	01/01/24
Date of Manufacture	:	January, 2024
Date of Expiry	:	January, 2026
Physical Appearance	:	Mustard yellow gel encapsulated inside polyester sack
Recommended storage	:	Store away from heat
Purity	:	Not Provided
Solubility	:	Slowly dissolve into flowing water over a long period of time
Intended Usage	:	Treatment of contamination created by raw sewage exposure
Manufactured By	:	Dhara Biotech, Nr, Gaushala, Sarsa – Vasad chokdi, Bhalej Road, Sarsa, Anand, Gujrat - 388365

Supplied By	:	Quin Quent Industries Pvt Ltd S No. 131/2A Rajyog Colony Warje Pune - 411052

7.1.2 Identity of the Test Item

The Test Item information Sheet (TIIS), Certificate of Analysis (CoA) and Material Safety Data Sheet (MSDS) has been provided by the sponsor. The responsibility for the correct identity and purity of the test item rests with the sponsor. The authenticity of the test item was not being conducted at the test facility.

7.1.3 Test System & Test Conditions

Species	:	*Pseudokirchneriella subcapitata* (Unicellular green alga)
Growth Condition	:	Exponentially growing cultures
Justification for the selection of species	:	Recommended by the regulatory guideline (OECD 201) for the toxicity assessment.
Source	:	Algae culture, Ecotoxicology Lab, CRK Campus, CSIR-Indian Institute of Toxicology Research, Lucknow
Test Room Details	:	Room No: 47 & 48
Test Method	:	Static Test
Test Vessel	:	250 ml conical flask
Test Volume	:	100 ml
Algae Inoculation	:	**Limit Test:** $5x10^3$ cells/ml
Number of Replicates	:	**Limit Test** 6 replicates for each test concentration and control.
Light (Lux)	: :	Continuous cool white light **Pre-culture** 5730 – 7890 Lux **Limit Test** 5730 -7840 Lux
Temperature (°C)	: :	**Pre-culture** 22.0 – 22.2 **Limit Test** 21.8 – 22.2 °C
Test Medium	:	OECD recommended algal growth medium
pH Range	: :	**Pre-culture** 8.31 **Limit Test** Start of the test (0 h) Control: 8.24 Treatment: 8.30

		End of the test (72 h) Control: 8.21 – 8.25 Treatment: 8.27 – 8.33
Test Duration	:	72 hours

7.2 Methods

7.2.1 Pre-Culture

Inoculum culture in the test medium was prepared prior to start of the test to allow exponential growth of algae until the exposure initiation. Pre-culture was maintained under the same conditions as the test culture. The increase in biomass in the algal culture was measured to ensure the growth is normal under the culture conditions.

7.2.2 Vehicle

The test item is not soluble in distilled water and solvents, then the required quantity of the test item was added in the OECD medium and stirred continuously for 24 hours as per the OECD guidance document 23. The aqueous phase of the test solution will be used for algae exposure.

7.2.3 Dose Formulation

Limit test was performed at 100 mg/L test concentration for the test item, DWE/2005A-1KG ILD Draynzyme. A stock concentration of 0.1 mg/mL was prepared by dissolving 100 mg DWE/2005A-1KG ILD Draynzyme in 1000 mL of OECD algal growth medium. From this, 600 mL was used for algae exposure in six replicates (100 mL each replicate) of 250 mL conical flasks. Remaining volume used for pH. Control group consist of algal growth medium without the test item.

7.2.4 Test Procedure

Equal volume of algal growth medium was maintained in all control and treatment groups. Required test concentrations were prepared from stock solution using algal growth medium. On inoculation, control and treatment flasks were kept in the shaker incubator with light intensity range between 4440 – 8880 lux under continuous illumination with shaking speed at 100 rpm. The light intensity was recorded daily once during the study duration (0 - 72 h). Cell density was estimated on day 0 (pre-culture used for inoculation) and then at 24, 48 and 72 h. Algal cells were counted using a hemocytometer under the microscope by taking 10 µl from respective treatment flasks using micropipette.

8. OBSERVATIONS

Microscopic observations were performed in both control and treatment flasks once every 24 h to verify any abnormal morphological appearance of the algae due to the test item exposure in treatment groups during the entire study duration.

pH values were measured at the beginning in bulk preparation of control and test concentrations whereas the pH was measured in all replicates of control and treatment groups at the end of the study. Light intensity was recorded daily during the conduct of the test.

8.1 Percent inhibiton of growth rate

The percent growth rate inhibition for each treatment replicate was calculated using the formula:

$$\%Ir = \frac{\mu_C - \mu_T}{\mu_C} \times 100$$

Where,
%Ir is percent inhibition in average specific growth rate
μ_C is mean value for average specific growth rate (μ) in the control group
μ_T is the average specific growth rate for the treatment replicate.

8.2 Average specific growth rate

Average specific growth rates were recorded in test solutions and the concentration with specified x% growth rate inhibition (e.g., 50%) was determined and expressed as E_rC_x (e.g., E_rC_{50}).

The average specific growth rate for a specific period was calculated on the basis of the logarithmic increase of biomass during the test period expressed as per day using the following formula:

$$\mu_{i-j} = \frac{\ln X_j - \ln X_i}{t_j - t_i}$$

Where,
μ_{i-j} is the average specific growth rate from time i to j
X_i is the biomass at time i
X_j is the biomass at time j

8.3 Yield

Yield was calculated as the biomass at the end of the test minus the starting biomass for each single vessel of control and treatment groups. For each test concentration and control, the mean value for yield along

with variance estimates was calculated. The percent inhibition in yield (%Iy) is calculated using the formula:

$$\%Iy = \frac{(Y_c - Y_t)}{Y_c} \times 100$$

Where,
%Iy is percent inhibition of yield
Y_c is mean value for yield in the control group
Y_t is mean value for yield in the treatment replicate

From the yield recorded in a series of test concentrations, the concentration that causes a specified x% inhibition of yield (e.g.50%) was calculated and expressed as E_yC_x (e.g. E_yC_{50})

9. STATISTICAL ANALYSIS

No statistical analysis was carried out for the determination of E_rC_{50} and E_yC_{50} limit test. Student t-test and Levene's test for equality of variances was conducted to find any significant difference in growth rate between control and 100 mg/L DWE/2005A-1KG ILD Draynzyme treated group.

10. DATA COMPILATION

Data was summarized in a tabular form with the number of cells and other measurement variables in each test and control vessels at each observation. Algal cell count, pH and light intensity data, percent inhibition for average specific growth rate and yield, EC_{50}, E_rC_{50}, E_yC_{50}, NOEC, LOEC based on average specific growth rate and yield were tabulated.

11. RESULTS OF TEST ITEM

11.1 Cell Count

The algal biomass in each flask was determined daily during the test period at 24, 48 and 72 h after the test item exposure during the limit test. Algal cell was counted using a haemocytometer and measurements were made by pipetting small volume (10 µl) of test solution from each replicate. Individual cell counts at 24, 48 & 72 hour during the limit test for test item was shown in tabulated form (Tables 1-3).

11.2 Microscopic Observations

Algal cells in the control and treatment replicates were appeared normal in terms of morphology.

11.3 pH and Light Intensity

pH values were measured at the beginning and end of the test in all treatment and control groups during the limit test. The values were found within the range as mentioned in the OECD test guideline (Table 4). Light intensity was recorded daily during the limit test (Tables 5).

11.4 Average Specific Growth Rate

During the limit test, the percent inhibition on the average growth rate at the end of 72 h was 0.09% for 100 mg/L DWE/2005A-1KG ILD Draynzyme (Table 6).

From the average specific growth rate recorded, the concentration that inhibited 50% growth (E_rC_{50}) and yield (E_yC_{50}) of the freshwater green algae, *Pseudokirchneriella subcapitata* was calculated as greater than 100 mg/L of DWE/2005A-1KG ILD Draynzyme (Table 8). No statistically significant ($p>0.05$) difference in the growth rate was noted between the control and DWE/2005A-1KG ILD Draynzyme treated group.

The no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) are not calculated since the study was conducted as a limit test.

11.5 Yield

During the limit test, the percent inhibition on the yield at the end of 72 hour was 0.48%, for 100 mg/L DWE/2005A-1KG ILD Draynzyme (Table 7).

From the yield recorded, the concentration that inhibited 50% yield (E_yC_{50}) of the freshwater green algae, *Pseudokirchneriella subcapitata* was calculated as greater than 100 mg/L of DWE/2005A-1KG ILD Draynzyme (Table 8). No statistically significant ($p>0.05$) difference in the yield was noted between the control and DWE/2005A-1KG ILD Draynzyme treated group.

The no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) are not applicable since the study was conducted as a limit test.

12. VALIDITY CRITERIA

The study is considered valid as the following criterion was met:

1. The biomass in the control culture increased exponentially by a factor of 182 (more than 16 as per the guideline) within the 72-hour test period. This corresponds to a specific growth rate of 1.73/day (Table 9).

2. The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures was 8.46% (not exceeding 35% as per the guideline (Table 10).

3. The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures was 0.10% (not exceeding 7% as per the guideline (Table 11).

13. CONCLUSION

The effective concentration of DWE/2005A-1KG ILD Draynzyme that inhibits algal growth rate (E_rC_{50}) observed for a period of 72 h in freshwater green algae, *Pseudokirchneriella subcapitata* after DWE/2005A-1KG ILD Draynzyme exposure was found to be greater than 100 mg/L.

The effective concentration of DWE/2005A-1KG ILD Draynzyme that inhibits algal yield (E_yC_{50}) observed for a period of 72 h in freshwater green algae, *Pseudokirchneriella subcapitata* after DWE/2005A-1KG ILD Draynzyme exposure was found to be greater than 100 mg/L.

LOEC and NOEC was not calculated since the test was conducted at the limit test concentration of 100 mg/L.

14. ARCHIVING

The following have been archived at the test facility for 9 years after completion of the study: all raw data, study plan, draft, and final reports. A representative sample of test Item has been sent from the Test Item Control Office to the Archives in the test facility. The sample shall be stored for a period of 9 years from the date of this final report. Before discarding of any archived study materials, consent of the Sponsor will be sought for the disposal.

15. REPORT DISTRIBUTION

The study report will be distributed as follows:

Test Facility : One signed final report in original (Copy No. 1/2) and an electronic copy in the PDF format.

Sponsor : One signed final report in original (Copy No. 2/2).

TABLE 1: CELL COUNT – 24 HOUR

Initial cell count: $5x10^3$ cells/ml

Test Conc. (mg/L) & Replicate		Cell Count					Sum	Mean (Cells/ml)	Average No. of Cells/ml
		S1	S2	S3	S4	S5			
Control	R1	2	4	3	4	3	16	32000	26000
	R2	2	2	4	3	1	12	24000	
	R3	0	4	3	2	3	12	24000	
	R4	3	4	2	1	4	14	28000	
	R5	4	2	3	2	1	12	24000	
	R6	3	3	2	1	3	12	24000	
100	R1	1	3	1	4	2	11	22000	21667
	R2	2	1	3	3	4	13	26000	
	R3	3	0	4	3	2	12	24000	
	R4	2	1	2	4	1	10	20000	
	R5	3	2	1	0	2	8	16000	
	R6	4	2	1	3	1	11	22000	

NOTE: Conc: Concentration; S1-S5 – Squares in Haemocytometer.

TABLE 2: CELL COUNT – 48 HOUR

Initial cell count: $5x10^3$ cells/ml

Test Conc. (mg/L) & Replicate		Cell Count					Sum	Mean (Cells/ml)	Average No. of Cells/ml
		S1	S2	S3	S4	S5			
Control	R1	17	21	15	13	15	81	162000	164333
	R2	14	23	12	15	18	82	164000	
	R3	13	17	19	15	20	84	168000	
	R4	15	17	14	19	18	83	166000	
	R5	14	20	17	16	13	80	160000	
	R6	20	15	19	17	12	83	166000	
100	R1	19	11	14	12	16	72	144000	158000
	R2	19	18	16	15	17	85	170000	
	R3	13	17	15	14	18	77	154000	
	R4	19	18	15	11	17	80	160000	
	R5	15	11	17	12	20	75	150000	
	R6	19	13	18	20	15	85	170000	

NOTE: Conc: Concentration; S1-S5 – Squares in Haemocytometer.

TABLE 3: CELL COUNT – 72 HOUR

Initial cell count: $5x10^3$ cells/ml

Test Conc. (mg/L) & Replicate		Cell Count					Sum	Mean (Cells/ml)	Average No. of Cells/ml
		S1	S2	S3	S4	S5			
Control	R1	94	90	88	95	88	455	910000	907667
	R2	87	94	86	92	93	452	904000	
	R3	96	90	91	89	87	453	906000	
	R4	85	89	92	96	90	452	904000	
	R5	88	98	88	94	85	453	906000	
	R6	93	88	89	98	90	458	916000	
100	R1	92	95	88	92	85	452	904000	903333
	R2	93	91	95	89	84	452	904000	
	R3	96	91	87	91	88	453	906000	
	R4	92	94	88	90	87	451	902000	
	R5	94	88	93	91	86	452	904000	
	R6	88	90	95	96	81	450	900000	

NOTE: Conc: Concentration; S1-S5 – Squares in Haemocytometer

TABLE 4: pH MEASUREMENT

pH at the start (0 h) of the experiment in control and treatment groups

Group	Concentration (mg/L)	pH Value
G1	Control	8.24
G2	100	8.30

pH at the end (72 h) of the experiment in control and treatment replicates

Group	Concentration (mg/L)	Replicates						pH Range
		R1	R2	R3	R4	R5	R6	
G1	Control	8.21	8.25	8.23	8.24	8.21	8.23	8.21 - 8.25
G2	100	8.28	8.27	8.30	8.33	8.31	8.29	8.27 – 8.33

TABLE 5: LIGHT INTENSITY (LUX)

Pre-Culture						
Hour	C1	C2	C3	C4	Center	Mean
0	5760	7070	5740	6960	7810	6668
24	5750	7290	5650	6940	7790	6684
48	5760	7160	5750	6950	7830	6690
72	5750	7280	5750	6930	7880	6718
96	5730	7250	5760	6890	7890	6704

Limit Test						
Hour	C1	C2	C3	C4	Center	Mean
0	5740	7050	5790	6950	7820	6670
24	5760	7110	5770	6940	7790	6674
48	5750	7140	5790	6960	7810	6690
72	5730	7120	5760	6950	7840	6680

TABLE 6: PERCENT INHIBITION – GROWTH RATE

Group	Concentration (mg/L)	Inhibition (%)
G1	Control	-
G2	100	0.09

TABLE 7: PERCENT INHIBITION – YIELD

Group	Concentration (mg/L)	Inhibition (%)
G1	Control	-
G2	100	0.48

TABLE 8: STATISTICAL ANALYSIS

Average Growth Rate	
E_rC_{50}	>100 mg/L
NOEC	Not Applicable
LOEC	Not Applicable

Yield	
E_yC_{50}	>100 mg/L
NOEC	Not Applicable
LOEC	Not Applicable

TABLE 9: AVERAGE EXPONENTIAL GROWTH RATE/DAY IN CONTROL

Average Exponential Growth Rate/Day			
Day 0-1	**Day 1-2**	**Day 2-3**	**Mean**
1.64	1.85	1.71	1.73

TABLE 10: PERCENT COEFFICIENT OF VARIATION (CV) FOR SECTION-BY-SECTION SPECIFIC GROWTH RATE IN CONTROL

Group	Replicates	% CV
Control	R1	6.77
	R2	10.27
	R3	11.15
	R4	2.50
	R5	9.48
	R6	10.62
	Mean	**8.46**

TABLE 11: PERCENT COEFFICIENT OF VARIATION (CV) FOR AVERAGE SPECIFIC GROWTH RATE IN CONTROL

Group	Replicates	Growth Rate			Mean
		0-24 h	24-48 h	48-72 h	
Control	R1	1.86	1.62	1.73	1.73
	R2	1.57	1.92	1.71	1.73
	R3	1.57	1.95	1.69	1.73
	R4	1.72	1.78	1.69	1.73
	R5	1.57	1.90	1.73	1.73
	R6	1.57	1.93	1.71	1.74
	Mean				**1.73**
	SD				**0.00**
	% CV				**0.10**

TABLE 12: Reference study results
Study No.: CSIR-IITR/GLP/276
Test Item: Potassium Dichromate

Average Growth Rate	
E_rC_{50}	0.410 mg/L
NOEC	0.003 mg/L
LOEC	0.009 mg/L

Yield	
E_yC_{50}	0.078 mg/L
NOEC	0.003 mg/L
LOEC	0.009 mg/L

ANNEXURE – I

STUDY PLAN

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

STUDY PLAN

STUDY No.: CSIR-IITR/GLP/396

FRESHWATER ALGA GROWTH INHIBITION TEST OF DRAYNZYME

TEST ITEM: DRAYNZYME

SPONSOR
PUNE MUNICIPAL CORPORATION
SHIVAJI NAGAR, PUNE 411005

TEST FACILITY
VISHAKTATA PARIKSHAN: GLP ANUROOP SUVIDHA
CSIR-INDIAN INSTITUTE OF TOXICOLOGY RESEARCH
CRK CAMPUS, GHERU, SAROJINI NAGAR INDUSTRIAL AREA
KANPUR ROAD, LUCKNOW-226008
INDIA

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

TABLE OF CONTENTS

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

1. **STUDY DETAILS**

Study Title	:	Freshwater Alga Growth Inhibition Test of Drayrzyme
Test Item	:	DWE/2005A-1KG ILD Drayrzyme
Study Number	:	CSIR-IITR/GLP/396
Sponsor	:	Pune Municipal Corporation Shivaji nagar, Pune 411005 Test conducted for Pune Municipal Corporation (In compliance to NGT matter of OA 323 of 2024)
Sponsor's Representative	:	Mangesh Dighe Environment Officer, Pune Municipal Corporation E Mail: indradhanushya@punecorporation.org
Test Facility	:	Vishaktata Parikshan: GLP Anuroop Suvidha CSIR-Indian Institute of Toxicology Research CRK Campus, Gheru, Sarojini Nagar Industrial Area Kanpur Road, Lucknow-226008, India
Study Director	:	Ms Vijaya Shree Ecotoxicology Laboratory Vishaktata Parikshan: GLP Anuroop Suvidha CSIR-Indian Institute of Toxicology Research CRK Campus, Gheru, Sarojini Nagar Industrial Area Kanpur Road, Lucknow-226008, India Contact Details: +91-522-2476051, +91-7052335668 E Mail: vijayashree0208@gmail.com
Study Personnel	:	Ms Priya Maurya Mr Sudhanshu Mishra

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

Study Schedule		
Study Start Date	:	05/03/2025
Limit Test		
Experiment Start Date	:	06/03/2025
Pre-culture	:	06/03/2025 – 10/03/2025
Test Item Exposure	:	10/03/2025
24 Hour Observation	:	11/03/2025
48 Hour Observation	:	12/03/2025
72 Hour Observation	:	13/03/2025
Experiment Completion Date	:	13/03/2025
Study Completion Date	:	DD/04/2025

2. **QUALITY ASSURANCE**
The Quality Assurance Unit of the Test Facility will inspect the study, the raw data, the draft and final reports. Findings of all inspections will be reported to the Management and to the Study Director. The details of phase inspected, inspection dates and reporting dates will be entered as QA-statement in the study report.

The Quality Assurance Unit has reviewed the study plan and will receive a copy thereof.

3. **STUDY COMPLIANCE**
The study will be performed in accordance with the following:

- OECD Principles of Good Laboratory Practice for the testing of chemicals as specified by International [C (97) 186/Final] Legislation
- OECD Test Guideline No. 201 – "Freshwater Alga and Cyanobacteria Growth Inhibition Test", adopted on 23 March 2006.
- The mutually agreed study plan and the Standard Operating Procedures of the test facility (CSIR-IITR/ECO/024; Revision 02).

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

4. **AMENDMENT PROCEDURES**

This study plan may be amended or subjected to alterations. In each case, any amendment to the approved study plan and the reasons for such amendments will be documented and realized only after written / telephonic / E-mail consent from the study Sponsor and review by the Quality Assurance Unit and Test Facility Management. If immediate action is necessary, verbal agreement from the Sponsor will be confirmed as soon as possible by study plan amendment. Minor changes (unplanned) of the study plan which do not influence the procedures or the outcome of the study may be subject to the discretion of the Study Director, but will be mentioned in the report as deviations.

5. **SAFETY PRECAUTIONS**

Gloves, face-mask, and goggles (if required) will be used in addition to protective body garments and shoes to ensure adequate personal health and safety. In case of eye contact, the eye will be washed thoroughly with water and medical treatment will be sought. In case of skin contact, it will be washed with soap and water with subsequent medical aid.

6. **OBJECTIVE**

The purpose of the study is to determine the effects of test item, DWE/2005A-1KG ILD Draynzyme on exponentially growing culture of unicellular green algae, *Pseudokirchneriella subcapitata* with an observation period of 72 hours. The effect of test item on the growth of the algal culture compared with control will be determined.

7. **MATERIALS AND METHODS**

7.1 **Materials**

7.1.1 **Test Item Information**

(As furnished by the Sponsor)

Test Item	:	DWE/2005A-1KG ILD Draynzyme
Common Name	:	Draynzyme
Chemical Name	:	Not Provided
Accession Number	:	Not Provided
Batch / Lot Details	:	01/01/24
Date of Manufacture	:	January, 2024
Date of Expiry	:	January, 2026
Physical Appearance	:	Mustard yellow gel encapsulated inside polyester sack
Recommended storage	:	Store away from heat
Purity	:	Not Provided

Vishaktata Parikshan: GLP Anurcop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

Solubility	:	Slowly dissolve into flowing water over a long period of time
Intended Usage	:	Treatment of contamination created by raw sewage exposure
Manufactured By	:	Dhara Biotech, Nr. Gaushala, Sarsa – Vasad chokdi, Bhalej Road, Sarsa, Anand, Gujrat - 388365
Supplied By	:	Quin Quent Industries Pvt Ltd S No. 131/2A Rajyog Colony Warje Pune - 411052

7.1.2 **Identity of the Test Item**

The Test Item information Sheet (TIIS), Certificate of Analysis (CoA) and Material Safety Data Sheet (MSDS) has been provided by the sponsor. The responsibility for the correct identity and purity of the test item rests with the sponsor. The authenticity of the test item was not being conducted at the test facility.

7.1.3 **Test System & Test Conditions**

Species	:	*Pseudokirchneriella subcapitata* (Unicellular green alga)
Growth Condition	:	Exponentially growing cultures
Justification for the selection of species	:	Recommended by the regulatory guideline (OECD) for aquatic toxicity assessment.
Source	:	Algae culture, Ecotoxicology Lab, CRK Campus, CSIR-Indian Institute of Toxicology Research (IITR).
Test Room Details	:	Room No: 47 & 48
Test Method	:	Static Test
Test Vessel	:	250 ml conical flask
Test Volume	:	100 ml
Number of Replicates	:	**Limit Test** 6 replicates for each test concentration and control.
Light	:	Continuous cool white light (4440-9880 lux)
Test Medium	:	OECD Medium
Test Medium pH Range (in control)	:	8.1 (should not vary by more than 1.5 units i.e., 8.1 – 9.6)
Test Duration	:	72-hours

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

7.2 **Methods**

7.2.3 **Pre-culture**

An inoculum culture in the test medium will be prepared 96 hours prior to start of the test to allow exponential growth of culture. The pre-culture will be maintained under the same conditions as the test culture. The increase in biomass in the inoculum culture will be measured to ensure the growth is normal under the culture conditions.

7.2.4 **Vehicle**

OECD medium prepared in distilled water will be used to prepare the stock solution for the study and the same will be documented in the raw data and report.

7.2.5 **Test Procedure**

All test solutions will contain the same volume of OECD medium and initial biomass of test alga. Required test concentrations will be prepared from stock solution using OECD medium. On inoculation, control and treatment flasks will be kept in the shaker incubator with light intensity ranges between 4440 – 8880 lux under continuous illumination.

The flasks will be randomly repositioned daily in the cooling incubator and gently shaken at 100 rpm daily during the entire test duration. The light intensity will be recorded daily once during the study duration (72-hours). Cell density will be estimated on day 0 (pre-culture used for inoculation) and then approximately at 24-, 48- and 72-hours using haemocytometer.

7.2.6 **Test and Control Groups**

Six replicates for each treatment and control groups will be maintained during the limit test.

7.2.7 **Limit Test**

DWE/2005A-1KG ILD Draynzyme was found to be non-toxic at a limit test concentration of 100 mg/L in previous acute study conducted on daphnia in the test facility. Hence, freshwater algae growth inhibition test will be carried out as a limit test by exposing the algal cells to 100 mg/L concentration of test item.

7.2.8 **Dose Formulation**

The stock solution will be prepared in OECD medium prior to the exposure from which the test groups will be prepared. Since the test item is not soluble in water and solvents, stock solution is

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

prepared by adding the required quantity of test item in the OECD medium and stirred continuously for 24 hours as per the OECD guidance document 23. The aqueous phase of the test solution will be used for algae exposure.

The test solution will be divided in six replicates as 100 mL per replicate along with six replicates of control algal media without the test item.

8. **OBSERVATIONS**

Microscopic observations will be performed to verify the normal appearance of inoculums culture and any abnormal morphological appearance of the algae due to the test item exposure will be noted during the study duration.

pH values will be measured at the beginning in bulk preparation of control and test concentrations whereas the pH content will be measured in all replicates of control and treatment groups at the end of the study. Light intensity and temperature will be recorded daily during the study duration.

9. **VALIDITY CRITERIA**

1. The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period. This corresponds to a specific growth rate of 0.92/day.

2. The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) the control cultures must not exceed 35%.

3. The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7%.

10. **PERCENT INHIBITON OF GROWTH RATE**

The percent growth rate inhibition for each treatment replicate is calculated using the formula:

$$\%Ir = \frac{\mu_C - \mu_T}{\mu_C} \times 100$$

%Ir is percent inhibition in average specific growth rate
μ_C is mean value for average specific growth rate (μ) in the control group
μ_T is the average specific growth rate for the treatment replicate.

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

11. **AVERAGE SPECIFIC GROWTH RATE**

Average specific growth rates will be recorded in test solutions and the concentration with specified x% growth rate inhibition (e.g. 50%) will be determined and expressed as E_rC_x (e.g. E_rC_{50}).

The average specific growth rate for a specific period will be calculated based on the logarithmic increase of biomass during the test period expressed as per day

$$\mu_{i,j} = \frac{\ln X_j - \ln X_i}{t_j - t_i}$$

$\mu_{i,j}$ is the average specific growth rate from time i to j
X_i is the biomass at time i
X_j is the biomass at time j

12. **YIELD**

Yield is calculated as the biomass at the end of the test minus the starting biomass for each single vessel of control and treatments. For each test concentration and control, the mean value for yield along with variance estimates will be calculated. The percent inhibition in yield (%Iy) is calculated using the formula:

$$\%Iy = \frac{(Y_c - Y_t)}{Y_c} \times 100$$

%Iy is percent inhibition of yield
Y_c is mean value for yield in the control group
Y_t is mean value for yield in the treatment replicate

From the yield recorded in a series of test concentrations, the concentration that cause a specified x% inhibition of yield (e.g.50%) will be calculated and expressed as E_yC_x (e.g. E_yC_{50})

13. **STATISTICAL ANALYSIS**

Since the study is carried out as a limit test, no statistical analysis will be performed.

14. **DATA COMPILATION**

Data will be summarized in a tabular form with the number of cells and other measurement variables in each test and control vessels at each observation. Algal cell count, pH and light intensity data,

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

percent inhibition for average specific growth rate and yield will be recorded.

15. DATA AND FINAL REPORT

The data will be summarized in tabular form with the number of cells and other measurement variables in each test and control vessels at each observation period. The final report will be prepared in compliance to the principles of GLP and normally include, but not limited to the following:

- A descriptive title.
- The name and address of the Sponsor and the Test Facility along with the details of study schedule.
- The names of all personnel involved in the study.
- A compliance statement signed by the Study Director that all applicable GLP regulations were followed in the conduct of the study.
- Quality Assurance (QA) statement; that states that the report accurately reflects the raw data obtained during the performance of the study and including the dates of QA activities and the dates reported to study director and management.
- The Test Item details and its code, composition and other appropriate physical and chemical characteristics, certificate of analysis (COA) etc.
- Complete description of the test system including species, source, number, test conditions, photoperiod, and acclimation.
- Method of preparation of stock and test solutions. Details of test concentrations and replicates (e.g. number of replicates, number of test concentrations and geometric progression used)
- Biomass for each flask at each measuring point and method for measuring biomass.
- Growth curves (plot of biomass versus time)

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

- Calculated response variables for each treatment replicate, with mean values and coefficient of variation for replicates.
- Tabulation of data, Graphical representation of concentration – effect relationship at the end of the test.
- Estimates of toxicity for response variables like EC_{50} values, NOEC and LOEC, with 95% confidence limits (if applicable).
- If ANOVA has been used, the size of the effect which can be detected (e.g. the least significant difference, if applicable).
- Discussion of the results, including any influence on the outcome of the test resulting from deviations from the OECD guideline.
- A description of all study plan deviations, if any.
- A description of all circumstances that may have affected the quality or integrity of the study.
- The storage locations of all raw data, specimens, reports, test item reference sample and the archiving period.
- GLP certificate

16. ARCHIVING

The following will be archived at the test facility for at least 9 years (3 cycles of GLP) after completion of the study: study plan, all raw data, draft and final reports, a representative sample of Test Item (approximately one gram), etc. Before discarding of any archived study materials, the Sponsor will be contacted for the disposal.

17. STUDY PLAN DISTRIBUTION

The final study plan (original copies) will be distributed as follows:
Test Facility : One signed study plan in original (Copy No. 1/2)
Sponsor : One signed study plan in original (Copy No. 2/2)
Document Control: One controlled copy
Quality Assurance Unit: One controlled copy
Study Personnel: One controlled copy

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

18. **REPORT DISTRIBUTION**

The study report will be distributed as follows:

Test Facility : One signed final report in original (Copy No. 1/2) and an electronic copy in the PDF format.

Sponsor : One signed final report in original (Copy No. 2/2).

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

19. AGREEMENT

This study plan for Study No.: CSIR-IITR/GLP/396, "Freshwater Alga Growth Inhibition Test of Draynzyme" has been mutually agreed:

for **TEST FACILITY**	for **STUDY SPONSOR**
1. _____	1. _____
STUDY DIRECTOR	SPONSOR REPRESENTATIVE
Date: 05/03/2025	Date:
	Email Consent Received on: 24/02/2025

2. _____
QUALITY ASSURANCE UNIT
Date: 05/03/2025

3. _____
TEST FACILITY MANAGEMENT
Date: 05/03/2025

ANNEXURE – II
CERTIFICATE OF ANALYSIS

Green Leaf

Test Report
Water Sample Analysis Report

Client's Name & Address: M/s. Dhara Bio-tech Near SarasCholdi, Near Gaushala, Kunjrav Road, Sarsa, Anand 388365, Gujarat. **Contact Person:** Mr. Vasantlal Patel	**Report No:** GLEPL/240124/01 **Issue Date:** 01/02/2024

Lab ID Code	:	GLEPL/240124/WL$_1$			
Sample Description	:	Product Sample	Purpose	:	As per Client requirement
Date of Sampling	:	Submitted by Client (Dhara Bio-tech)	Sample collected/Submitted by	:	Submitted by Client (Dhara Bio-tech)
Date of Sample Received	:	24/01/2024	Test Parameters	:	As per Client requirement
Date of starting Analysis	:	25/01/2024	Quantity/No. of sample	:	1No. Batch No. 01/01/24
Date of completion Analysis	:	31/01/2024	Packed/Seal	:	Sealed (13/01/24)

Result Table

Sr. No.	Test Parameters	Test Method	Unit	Results
1	Drayzyme for Bacteria Count	ISO 16649-3	MPN/100 mL	Absent

Remark: In accordance to G.S.R 613(E)-This product does not contain any Bacteria, thus does not require 'Appraisal by genetic Engineering Appraisal Committee).

Chemist

Authorized Signatory
Rekha Dare

Notes: (1) The results pertain to tested items only.
(2) This report shall not be reproduced, except in full, without written approval of the laboratory.
(3) Authenticity of this Report could be validated with office copy at Greenleaf Envirotech Pvt. Ltd.
(4) Perishable samples will be destroyed after testing, others after 7 days from the date of issue of the report, unless otherwise agreed with the customer or as required by the applicable regulations.

CIN: U74140GJ2010PTC059798
Greenleaf EnvirotechPvt. Ltd., Nr. RangoliFlats,Radhanpur Road, Mehsana – 384002. Gujarat, India.
Tel : +91-9725519974, E-mail: info@glepl.com, Web: www.glepl.com
Branch Office: 304, Kankavati Complex, Singanpor-Couzway Road, Katargam, Surat--395004

ANNEXURE – III

TEST SYSTEM CHARACTERIZATION CERTIFICATE

ATCC®

CERTIFICATE OF ANALYSIS

ATCC® Number:	22662™
Lot Number:	70051530
Organism:	*Pseudokirchneriella subcapitata*
Concentration:	2.3 x 10^7 cells/mL [vials contain 0.5 mL]
	Tested on 16MAY2022
Product Format:	Protist cells suspended in an appropriate cryoprotectant
Expiration Date:	Not applicable
Storage Conditions:	-80°C or colder for frozen cultures; Note: Do not store frozen vials in freezers with a defrost cycle, as this will expose the vials to increased temperatures. Store in vapor phase liquid nitrogen (approximately -196°C) for long term storage (more than one week).

Test / Method	Specification	Result
Viability (Visual observation method)	An appropriate culture vessel containing the recommended growth medium is inoculated with sample material and incubated according to the instructions on the item's product information sheet. Growth is confirmed through microscopic observation.	Pass
Cell morphology (Visual observation method)	Sample material is examined by light microscopy to confirm that cell morphology conforms to the appropriate reference description.	Pass
Purity (Visual observation method)	Sample material is microscopically observed and determined to be free from obvious contamination by undesirable fungi, bacteria, and other protists. (Note that monoxenic or xenic strains are expected to have other organisms present. These strains are observed for the presence of undesirable contaminants only.) Axenic cultures are tested for bacterial and fungal contamination using a variety of non-selective growth media. Cultures grown in host cell lines are tested for mycoplasma contaminants using a PCR-based assay.	Pass

Jo Salisbury
Digitally signed by Jo Salisbury
Date: 2022.07.07 09:24:31 -04'00'
Quality Assurance Specialist, Quality Assurance

ATCC hereby represents and warrants that the material provided under this certificate is pure and has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and correct to the best of the company's knowledge and belief. This certificate does not extend to the growth and/or passage of any living organism or cell line beyond what is supplied within the container received from ATCC.

This product is intended to be used for laboratory research use only. It is not intended for use in humans, animals, or for diagnostics. Appropriate Biosafety Level (BSL) practices should always be used with this material. Refer to the Product Information Sheet for instructions on the correct use of this product.

ATCC products may not be resold, modified for resale, used to provide commercial services, or to manufacture commercial products without prior written agreement from ATCC.

ATCC
10801 University Boulevard
Manassas, VA 20110-2209 USA
www.atcc.org

800-638-6597 or 703-365-2700
Fax: 703-365-2750
E-mail: tech@atcc.org
or contact your local distributor

ANNEXURE – IV

GLP CERTIFICATE

National Good Laboratory Practice (GLP) Compliance Monitoring Authority (NGCMA)
Department of Science and Technology
GOVERNMENT OF INDIA

Certificate of GLP Compliance

This is to certify that

Toxicity Testing: GLP Test Facility, CSIR-Indian Institute of Toxicology Research
CRK Campus, Gheru, Sarojini Nagar Industrial Area
Lucknow-226008, Uttar Pradesh (India)

is a GLP certified test facility in compliance with the NGCMA's Document No. GLP-101 "Terms & Conditions of NGCMA for obtaining and maintaining GLP certification by a test facility" and OECD Principles of GLP.

The test facility conducts the below-mentioned tests/ studies:

- **Toxicity Studies**
- **Mutagenicity Studies**
- **Environmental Toxicity Studies on Aquatic and Terrestrial Organisms**
- **Analytical and Clinical Chemistry Testing**

The specific area(s) of expertise, test item(s) and test system(s) are listed in the annexure overleaf.

Validity: June 5, 2023 – June 4, 2026

Certificate No. :GLP/C-213/2023
Issue Date :07-12-2023

(Dr. Ekta Kapoor)
Head, NGCMA

Study on Daphinia sp. Acute immobilization test:

FINAL REPORT

STUDY TITLE

DAPHNIA SP. ACUTE IMMOBILIZATION TEST OF DRAYNZYME

TESTITEM: DRAYNZYME

STUDY NO.: CSIR-IITR/GLP/398

STUDY COMPLETED ON:
25/02/2025

SPONSOR

PUNE MUNICIPAL CORPORATION
SHIVAJI NAGAR, PUNE 411005

TEST FACILITY

VISHAKTATA PARIKSHAN: GLP ANUROOP SUVIDHA
CSIR-INDIAN INSTITUTE OF TOXICOLOGY RESEARCH
CRK CAMPUS, GHERU, SAROJINI NAGAR INDUSTRIAL AREA
KANPUR ROAD, LUCKNOW-226008
UTTAR PRADESH, INDIA

TABLE OF CONTENTS

STATEMENT OF CONFIDENTIALITY

The report contains confidential and proprietary information to Pune Municipal Corporation Shivaji Nagar, Pune 411005.The contents of this report will not be disclosed to anyone without an expressed or approval of competent authority of Pune Municipal Corporation Shivaji Nagar.

STATEMENT OF GLP COMPLIANCE

This study was performed in compliance with the OECD Principles of Good Laboratory Practice for the testing of chemicals as specified by International [C (97) 186/Final] Legislation. This study was conducted in accordance with the Standard Operating Procedures of Vishaktata Parikshan: GLP Anuroop Suvidha, CSIR-Indian Institute of Toxicology Research and the mutually agreed study plan which was signed by the Study Director on 05/02/2025 for which email approval was received from the sponsor on 04/02/2025.

DECLARATION

The Study Director hereby declares that the work was performed under her supervision and in accordance with the described procedures. It is assured that the reported results represent the raw data obtained during the experimental work. No circumstances have been left unreported.

Study Director
Date: 25/02/2025

Test Facility Management
Date: 25/02/2025

Deputy Test Facility Management
Vishaktata Parikshan : GLP Anuroop Suvidha
Toxicity Testing: GLP Compliant Facility CSIR-IITR Lucknow India

QUALITY ASSURANCE STATEMENT

Study No.: CSIR-IITR/GLP/398, "Daphnia sp. Acute Immobilization Test of Draynzyme" has been inspected in accordance with the OECD Principles of Good Laboratory Practice for the testing of chemicals as specified by International [C (97) 186/Final] Legislation.

This study was inspected and findings reported to the Management and Study Director on the dates shown below:

INSPECTION DATE	PHASE	REPORTING DATE
	Initiation Phase	
01/01/2025	Draft Study Plan review	01/01/2025
06/02/2025	Final Study Plan review	06/02/2025
	In-Life Phase	
07/02/2025	Exposure of daphnids during range finding study	07/02/2025
08/02/2025	24-hour observation	08/02/2025
11/02/2025	Exposure of daphnids during limit test	11/02/2025
12/02/2025	24hour observation of daphnids	12/02/2025
13/02/2025	48 hour observation	13/02/2025
	Reporting Phase	
19/02/2025	Draft Report Review	19/02/2025
25/02/2025	Final Report Review	25/02/2025

Inspections were performed according to the Standard Operating Procedures of the Test Facility's Quality Assurance Unit. The report was inspected as per the approved study plan and pertinent raw data is accurately reflected in the report.

Date: 25/02/2025 (Nupur)

Quality Assurance Unit
Vishaktata Parikshan: GLP Anuroop Suvidha
CSIR-Indian Institute of Toxicology Research
CRK Campus, Gheru, Sarojini Nagar Industrial Area Kanpur Road, Lucknow-226008, India.

LIST OF COMMONLY USED ABBREVIATIONS AND SYMBOLS

CSIR	Council of Scientific and Industrial Research
CoA	Certificate of Analysis
°C	Degree Celsius
GLP	Good Laboratory Practices
h	Hour
IITR	Indian Institute of Toxicology Research
l	Litre
mg	Milligram
mL	Millilitre
OECD	Organization for Economic Cooperation and Development
TIIS	Test Item Information Sheet

1. STUDY DETAILS

Study Title	:	Daphnia sp. Acute Immobilization Test of Dryanzyme
Test Item	:	DWE/2005A-1KG ILD Draynzyme
Study Number	:	CSIR-IITR/GLP/398
Sponsor	:	Pune Municipal Corporation Shivaji nagar, Pune 411005 Test conducted for Pune Municipal Corporation (In compliance to NGT matter of OA 323 of 2024)
Sponsor's Representative	:	Mangesh Dighe Environment Officer, Pune Municipal Corporation E Mail: indradhanushya@punecorporation.org
Test Facility	:	Vishaktata Parikshan: GLP Anuroop Suvidha CSIR-Indian Institute of Toxicology Research CRK Campus, Gheru, Sarojini Nagar Industrial Area, Kanpur Road, Lucknow-226008, India
Study Schedule		
Study Start Date	:	05/02/2025
Range Finding Study Experiment Start Date	:	07/02/2025
Test Item Exposure	:	07/02/2025
24-hour Observation	:	08/02/2025
48-hour Observation	:	09/02/2025
Experimental Completion Date	:	09/02/2025
Main Study/Limit Test Experiment Start Date	:	11/02/2025
Test Item Exposure	:	11/02/2025
24-hour Observation	:	12/02/2025
48-hour Observation	:	13/02/2025
Experimental Completion Date	:	13/02/2025
Study Completion Date	:	25/02/2025

2. STUDY PERSONNEL

The following personnel participated in the conduct of the study.

Name	Function
Ms Priya Maurya	Study Director
Ms Vijaya Shree	Study Personnel
Mr Sudhanshu Mishra	Study Personnel

3. SUMMARY

Acute immobilization study in *Daphnia magna* was conducted in accordance with OECD guideline 202 for the test item Dryanzyme. Less than 24-hour old daphnids (neonates) were collected from the brooders for test item exposure. Daphnids were observed for immobilization at 24 and 48-hour after the test item exposure.

A range finding experiment was conducted with test concentrations of 0.01, 0.1, 1, 10 and 100 mg/L of Dryanzyme. Stock concentration of 0.1 mg/mL Dryanzyme was prepared by dissolving 100 mg in 1000 mL of M4 media. The contents were stirrer for 24 hour using magnetic stirrer to get the aqueous filtrate as per the OECD guidance document 23. A volume of 100 mL test solution for each group was prepared by diluting 10 µL, 100 µL, 1 mL, 10 mL and 100 mL of Dryanzyme stock solution in 99.99 mL, 99.9 mL, 99 mL and 90 mL of M4 media for 0.01, 0.1, 1.0, 10.0 and 100 mg/L of Dryanzyme concentrations respectively. Control group consists of M4 media without Dryanzyme. Two replicates with 50 mL of test medium were maintained for treatment and control groups with 5 daphnids per replicate. After the exposure on day 0, daphnids were observed for immobility and toxicity signs at 24 and 48-hour.

No immobilization was observed in control and daphnids exposed to 0.01, 0.1,1, 10 and 100 mg/L of Dryanzyme concentrations at 24 and 48-hour post-exposure. Thus, the percent immobilization at the end of 48 hour was recorded to be 0% immobility in control and all test concentrations of Dryanzyme. pH, temperature, hardness, and dissolved oxygen were found to be within the range during the study duration.

Based on the results of range finding study, limit test was carried out at a concentration of 100 mg/L of Dryanzyme. Stock concentration of 0.1 mg/mL Dryanzyme was prepared by dissolving 100 mg in 1000 mL of M4 media. The contents were stirrer for 24 hour using magnetic stirrer to get the aqueous filtrate as per the OECD guidance document 23. Four replicates with 50 mL of test medium were maintained for treatment and control groups with 5 daphnids per replicate.

No immobilization was observed in control and daphnids exposed to 100 mg/L Dryanzyme concentration throughout the study duration. Thus, the cumulative immobility at the end of 48-hour duration was found to be 0% in control and 0% immobility at 100 mg/L Dryanzyme concentration. pH, temperature, hardness, and dissolved oxygen were found to be within the range during the study duration.

Based on the test results, the EC_{50} of the test item, Dryanzyme for *Daphnia magna* observed for a period of 48-hour was found to be greater than 100 mg/L.

4. OBJECTIVE

The purpose of this study was to assess the acute toxicity of Dryanzyme to young daphnids aged less than 24 h.

5. STUDY COMPLIANCE

The study was performed in accordance with the following:
1. OECD Principles of Good Laboratory Practice for the testing of chemicals as specified by International [C (97) 186/Final] Legislation.
2. OECD Test Guideline No. 202–Daphnia Sp. Acute Immobilization Test.
3. The mutually agreed study plan and the Standard Operating Procedures of the test facility (CSIR-IITR/ECO/018, Revision 3)

6. STUDY AMENDMENT AND DEVIATION

This study has no amendment and no deviation.

7. MATERIALS AND METHODS

7.1 Materials

7.1.1 Test Item Information

(As furnished by the Sponsor)

Test Item	:	DWE/2005A-1KG ILD Draynzyme
Common Name	:	Draynzyme
Chemical Name	:	Not Provided
Accession Number	:	Not Provided
Batch / Lot Details	:	01/01/24
Date of Manufacture	:	January, 2024
Date of Expiry	:	January, 2026
Physical Appearance	:	Mustard yellow gel encapsulated inside polyester sack
Recommended storage	:	Store away from heat
Purity	:	Not Provided
Solubility	:	Slowly dissolve into flowing water over a long period of time
Intended Usage	:	Treatment of contamination created by raw sewage exposure
Manufactured By	:	Dhara Biotech, Nr, Gaushala, Sarsa – Vasad chokdi, Bhalej Road, Sarsa,

		Anand, Gujrat - 388365
Supplied By	:	Quin Quent Industries Pvt Ltd S No. 131/2A Rajyog Colony Warje Pune - 411052

7.1.2 Identity of the Test Item

The Test Item information Sheet (TIIS) has been provided by the sponsor. Certificate of Analysis (CoA) and Material Safety Data Sheet (MSDS) has not been provided by the sponsor. The responsibility for the correct identity and purity of the test item rests with the sponsor. The authenticity of the test item was not being conducted at the test facility.

7.1.3 Test System & Test Conditions

Species	:	*Daphnia magna*
Age	:	Less than 24-hour old neonates
Source	:	Daphnia culture, Ecotoxicology Lab, CRK Campus, CSIR-Indian Institute of Toxicology Research (IITR).
Justification for the selection of species	:	Recommended by the regulatory guideline (OECD 202) for aquatic toxicity assessment
Test Room Details	:	Room No:46
Test Method	:	Static
Test Vessel	:	100 glass beakers
Volume of Test Solution	:	50
No. of Replicates	:	**Range Finding Study:** 2 replicates. **Limit Test:** 4 replicates
Number of Daphnids / replicate	:	5 daphnids / replicate
Light	:	16-hour light and 8-hour darkness
Culture Medium	:	M4 Media
Temperature (°C)	:	**Range Finding Study** Control: 19.2 – 20.3°C Treatment: 19.2 – 20.3°C **Limit Test** Control: 19.4 – 20.2°C Treatment: 19.4 – 20.3°C
pH	:	**Range Finding Study** Control: 7.57– 7.93 Treatment: 7.61 – 8.07 **Limit Test**

		Control: 7.62 – 7.98 Treatment: 7.92 – 8.09
Dissolved Oxygen (DO)	:	**Range Finding Study** Control: 6.21 – 6.81 mg/L Treatment: 6.18 – 6.82 mg/L **Limit Test** Control: 6.25 – 6.89 mg/L Treatment: 6.24 – 6.94 mg/L
Hardness (M4 Medium)	:	**Range Finding Study** 161 mg/L **Limit Test** 154 mg/L
Test Duration	:	48-hour

7.2 Methods

7.2.1 Collection of less than 24-hour old Daphnids

Sufficient numbers of less than 24-hour old daphnids (neonates) were collected from the individual brooders of more than first brood progeny for test item exposure.

7.2.2 Vehicle

Since the test item was soluble in water, M4 media was used to prepare the intended test concentrations for daphnids exposure.

7.2.3 Dose Formulation

A range finding experiment was conducted with test concentrations of 0.01, 0.1, 1, 10 and 100 mg/L of Dryanzyme. Stock concentration of 0.1 mg/mL Dryanzyme was prepared by dissolving 100 mg in 1000 mL of M4 media. The contents were stirrer for 24 hour using magnetic stirrer to get the aqueous filtrate as per the OECD guidance document 23. A volume of 100 mL test solution for each group was prepared by diluting 10 µL, 100 µL, 1 mL, 10 mL and 100 mL of Dryanzyme stock solution in 99.99 mL, 99.9 mL, 99 mL and 90 mL of M4 media for 0.01, 0.1, 1.0, 10.0 and 100 mg/L of Dryanzyme concentrations respectively. Control group consists of M4 media without Dryanzyme.

Based on the results of range finding study, limit test was carried out at a concentration of 100 mg/L of Dryanzyme. Stock concentration of 0.1 mg/mL Dryanzyme was prepared by dissolving 100 mg in 1000 mL of M4 media. The contents were stirrer for 24 hour using magnetic stirrer to get the aqueous filtrate as per the OECD guidance document 23. Four replicates with 50 mL of

test medium were maintained for treatment and control groups with 5 daphnids per replicate.

7.2.4 Treatment

Test vessels were filled with 50 mL of the test solution at different concentrations. Less than 24 hour old daphnids were then released into the respective test vessels. In range finding experiment, 60 daphnids were divided into six groups of 5 daphnids each, one group in each test replicate. Control group daphnids were exposed to 50 mL of M4 medium without the test item. During limit test, 40 daphnids were divided into two groups of 5 daphnids each, one group in each test replicate. Control group daphnids were exposed to 50 mL of M4 medium without the test item.

7.2.5 Physico-chemical Parameters

The test medium was analyzed for pH, temperature and dissolved oxygen at the beginning and end of the study in all the test groups, during range finding and Limit test. Hardness was analyzed once at the start of the experiment in control group during range finding and limit test.

8. OBSERVATIONS

Each test vessel was checked for immobilized daphnids and any abnormal behavior at 24 and 48 hours after exposure to Dryanzyme. Daphnids that were not able to swim within 15 seconds after gentle agitation of the test vessel were considered to be immobilized (even if they can still move their antennae).

9. RESULTS

9.1 Physico-chemical Parameters

pH, temperature, and dissolved oxygen were measured at the beginning and end of the study. Hardness was determined once at the start of the test. All these parameters were found to be within the range during the range finding study and limit test (Table 1 and Table 3).

9.2 Immobilization

During the range finding experiment, no immobilization was observed in control and daphnids exposed to 0.01, 0.1, 1.0, 10.0 and 100 mg/L of Dryanzyme concentrations at the end of 24-hour and 48-hour of post-exposure. Thus, the percent immobilization at the end of 48 hour was recorded to be 0% immobility in control, 0.01, 0.1, 1.0, 10.0 and 100 mg/L of Dryanzyme (Table 2).

During the Limit test, no immobilization was observed in control and daphnids exposed to 100 mg/L Dryanzyme concentrations at the end of 24 and 48-hour. Thus, the cumulative immobility at the end of 48-hour duration was found to be

0% in control, and 0% immobility was found to be 100 mg/L Dryanzyme concentrations (Table 4).

10. VALIDITY CRITERIA

The study is valid since the following criterion was met:

1. In control, no immobilization was observed throughout the experiment period.
2. The dissolved oxygen concentration at the end of the test was ≥ 3 mg/L in control and test replicate vessels.

11. STATISTICAL ANALYSIS

No statistical analysis was carried out as the main study was concluded with limit test.

12. DATA COMPILATION

Data are summarized in tabular form for each treatment and control groups. The number of daphnids used and immobilization at each observation are reported along with the record of physico-chemical parameters.

13. CONCLUSION

Based on the test results, the EC_{50} of the test item, Dryanzyme for *Daphnia magna* observed for a period of 48-hour was found to be greater than 100 mg/L.

14. ARCHIVING

The following has been archived at the test facility for 9 years after completion of the study: study plan, all raw data, draft, and final reports. A representative sample of test Item has been sent from the Test Item Control Office to the Archives in the test facility. The sample shall be stored for a period of 9 years from the date of this final report. Sponsor's approval would be sought before discarding of any archived study materials.

15. REPORT DISTRIBUTION

The study report will be distributed as follows:

Test Facility : One signed final report in original (Copy No.1/2) and an electronic copy in the PDF format.

Sponsor : One signed final report in original (Copy No. 2/2).

TABLE 1: PHYSICO-CHEMICAL PARAMETERS– RANGE FINDING STUDY

Concentration(mg/L)	Control		0.01		0.1	
	0 h	48 h	0 h	48 h	0 h	48 h
pH	7.58	7.93	7.62	7.95	7.89	7.97
Temperature (°C)	19.2	20.2	19.3	20.2	19.3	20.1
Dissolved Oxygen (mg/L)	6.80	6.22	6.78	6.20	6.79	6.20
Hardness* (mg/L)	161	-	-	-	-	-
Concentration (mg/L)	1		10		100	
	0h	48 h	0 h	48 h	0 h	48 h
pH	7.91	7.98	7.96	8.02	7.96	8.07
Temperature (°C)	19.4	20.3	19.3	20.2	19.3	20.1
Dissolved Oxygen (mg/L)	6.80	6.22	6.78	6.19	6.82	6.22

Note:* - Analysed at the start of the experiment; h – hour

TABLE 2: IMMOBILIZATION DATA – RANGE FINDING STUDY

Conc. (mg/L)	No. of Daphnids/ Replicate	24 h		Percent Immobility	48 h		Percent Immobility	Cumulative Percent Immobility
		Replicates			Replicates			
		1	2		1	2		
Control	5	0	0	0	0	0	0	0
0.01	5	0	0	0	0	0	0	0
0.1	5	0	0	0	0	0	0	0
1	5	0	0	0	0	0	0	0
10	5	0	0	0	0	0	0	0
100	5	0	0	0	0	0	0	0

Note: Conc.: Concentration; h - hour

TABLE 3: PHYSICO-CHEMICAL PARAMETERS–LIMIT TEST

Concentration(mg/L)	Control		100	
	0 h	48 h	0 h	48 h
pH	7.63	7.98	7.93	8.08
Temperature (°C)	19.5	20.2	19.5	20.2
Dissolved Oxygen (mg/L)	6.89	6.27	6.93	6.25
Hardness* (mg/L)	154	-	-	-

Note:*- Analysed at the start of the experiment; h – hour

TABLE 4: IMMOBILIZATION DATA – LIMIT TEST

Conc. (mg/L)	No. of Daphnids / Replicate	24 h					48 h					Cumulative Percent Immobility
		Replicates				Percent Immobility	Replicates				Percent Immobility	
		1	2	3	4		1	2	3	4		
Control	5	0	0	0	0	0	0	0	0	0	0	0
100	5	0	0	0	0	0	0	0	0	0	0	0

Note: Conc.: Concentration; h – hour

ANNEXURE – I

RESULTS OF REFERENCE STUDY

Study No.: CSIR/IITR/GLP/382

SUMMARY

Acute immobilization study in *Daphnia magna* was conducted in accordance with OECD guideline 202 for the test item potassium dichromate. Less than 24-hour old daphnids (neonates) were collected from the brooders of individual culture for test item exposure. Daphnids were observed for immobilization at 24 and 48 h after the test item exposure.

A preliminary range finding experiment was conducted and the concentrations selected were 0.001, 0.01, 0.1, 1, and 5mg/L respectively. Stock concentration of 0.04 mg/mL potassium dichromate was prepared by dissolving 10 mg in 250 mL of M4 media. A volume of 100 mL of test solution for each group was prepared by diluting 2.5 µL, 25 µL, 250 µL, 2.5 mL, and 12.5 mL of stock solution in 99.998, 99.98, 99.75, 97.5, and 87.5 mL of M4 media for 0.001, 0.01, 0.1, 1, and 5 mg/L concentrations respectively. Control group consists of M4 media without potassium dichromate. Two replicates with 50 mL of test medium were maintained for each treatment and control groups with 5 daphnids per replicate. After the exposure on day 0, daphnids were observed for immobility and toxicity signs at 24 and 48 h.

Physico-chemical parameters like pH. Dissolved oxygen, hardness and temperature were measured at the start and end of study and observed within the range as per the OECD guideline 202. No immobilization was observed in control, 0.001, 0.01, 0.1 mg/L treatment groups whereas daphnids exposed to 1 and 5 mg/L resulted in 70% and 100%immobility at 24-hour observation. Similar trend was noticed at 48 h observation except an additional immobilization of 20% was observed in 1 mg/L treatment group. Thus, the cumulative percent immobility at the end of 48 hour was recorded to be 0%in control, 0.001, 0.01, 0.1 mg/L treatment groups followed by 90% and 100% immobility in 1 and 5 mg/L treatment groups respectively.

Based on the results of range finding study, main study was conducted at 0.10, 0.15, 0.23, 0.34, 0.51, 0.76, 1.14 and 1.71 mg/L test concentrations with a Separation factor of 1.5.Stock concentration of 0.04 mg/mL potassium dichromate was prepared by dissolving 10 mg in 250 mL of M4 media. A volume of 200 mL of test solution for each group was prepared by diluting 600 µL, 900 µL, 1.38 mL, 2.04 mL, 3.06 mL, 4.56 mL, 6.84 mL, and 10.26

mL of test item in 239.4, 239.1, 238.62, 237.96, 236.94, 235.44, 233.16 and 229.74 mL of M4 media for 0.10, 0.15, 0.23, 0.34, 0.51, 0.76, 1.14 and 1.71 mg/L test concentrations respectively. Control group consists of M4 media without potassium dichromate. Four replicates with 50 mL of test medium were maintained for each treatment and control groups with 5 daphnids per replicate. After the exposure on day 0, daphnids were observed for immobility and toxicity signs at 24 and 48 h.

Physico-chemical parameters like pH, dissolved oxygen, hardness, and temperature were measured at the start and end of study and observed within the range as per the OECD guideline 202. No immobilization was observed in control, 0.10, 0.15, 0.23, 0.34, and 0.51 mg/L treatment groups whereas daphnids exposed to 0.76, 1.14, and 1.71 mg/L resulted in 5%, 30%, and 100% immobility at 24-hour observation. Similar trend was noticed at 48 h observation except an additional immobilization of 35% and 70% was observed in 0.76 and 1.14 mg/L treatment groups. Thus, the cumulative percent immobility at the end of 48 hour was recorded to be 0%in control, 0.10, 0.15, 0.23, 0.34, and 0.51 mg/L treatment groups followed by 40%, 100% and 100% immobility in 0.76, 1.14, and 1.71 mg/L treatment groups respectively.

Based on the test results, 24 h EC_{50} of potassium dichromate for *Daphnia magna* was found to be1.182 mg/L with 1.047mg/Land 1.351mg/L as lower and upper confidence limits respectively.

48 h EC_{50} of potassium dichromate for *Daphnia magna* was found to be 0.789 mg/L with 0.714 mg/L and 0.878 mg/L as lower and upper confidence limits respectively.

LOEC and NOEC of potassium dichromate for *Daphnia magna* was found to be 0.76 mg/L and 0.51 mg/L concentrations.

ANNEXURE – II

STUDY PLAN

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

STUDY PLAN

STUDY No.: CSIR-IITR/GLP/398

DAPHNIA SP. ACUTE IMMOBILIZATION TEST OF DRAYNZYME

TEST ITEM: DRAYNZYME

SPONSOR

PUNE MUNICIPAL CORPORATION
SHIVAJI NAGAR, PUNE 411005

TEST FACILITY

VISHAKTATA PARIKSHAN: GLP ANUROOP SUVIDHA
CSIR-INDIAN INSTITUTE OF TOXICOLOGY RESEARCH
CRK CAMPUS, GHERU, SAROJINI NAGAR INDUSTRIAL AREA
KANPUR ROAD, LUCKNOW-226008
UTTAR PRADESH, INDIA

Study No. CSIR-IITR/GLP/398
Copy No. 1/2

Page 1/13

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

TABLE OF CONTENTS

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

1. **STUDY DETAILS**

Study Title	:	Daphnia sp. Acute Immobilization Test of Drayrzyme
Test Item	:	DWE/2005A-1KG ILD Drayrzyme
Study Number	:	CSIR-IITR/GLP/398
Sponsor	:	Pune Municipal Corporation Shivaji nagar, Pune 411005
Sponsor's Representative	:	Mangesh Dighe Environment Officer, Pune Municipal Corporation E Mail: indradhanushya@punecorporation.org
Test Facility	:	Vishaktata Parikshan: GLP Anuroop Suvidha CSIR-Indian Institute of Toxicology Research CRK Campus, Gheru, Sarojini Nagar Industrial Area Kanpur Road, Lucknow-226008, India
Study Director	:	Ms Priya Maurya Ecotoxicology Laboratory Vishaktata Parikshan: GLP Anuroop Suvidha CSIR-Indian Institute of Toxicology Research CRK Campus, Gheru, Sarojini Nagar Industrial Area Kanpur Road, Lucknow-226008, India Contact Details: +91-522-2476051(Extn:210) +91-9554664279 E Mail: priyamaurya528@gmail.com
Study Personnel	:	Ms Vijaya Shree Mr Sudhanshu Mishra
Study Schedule		
Study Start Date	:	05/02/2025
Range Finding Study Experiment Start Date	:	07/02/2025
Test Item Exposure	:	07/02/2025
24-hour Observation	:	08/02/2025
48-hour Observation	:	09/02/2025
Experimental Completion Date	:	09/02/2025
Main Study/Limit Test		

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

Experiment Start Date	:	11/02/2025
Test Item Exposure	:	11/02/2025
24-hour Observation	:	12/02/2025
48-hour Observation	:	13/02/2025
Experimental Completion Date	:	13/02/2025
Study Completion Date	:	DD/02/2025

2. **QUALITY ASSURANCE**

The Quality Assurance Unit of the Test Facility will inspect the study, the raw data, the draft and final reports. Findings of all inspections will be reported to the Management and to the Study Director. The details of phase inspected, Inspection dates and reporting dates will be entered as QA-statement in the study report.

The Quality Assurance Unit has reviewed the study plan and will receive a copy thereof.

3. **STUDY COMPLIANCE**

The study will be performed in accordance with the following:

- OECD Principles of Good Laboratory Practice for the testing of chemicals as specified by International [C (97) 186/Final] Legislation
- OECD Test Guideline No. 202 – "*Daphnia* sp. Acute Immobilization Test", adopted on 13th April 2004.
- The mutually agreed study plan and the Standard Operating Procedures of the test facility (CSIR-IITR/ECO/018; Revision 03).

4. **AMENDMENT PROCEDURES**

This study plan may be amended or subjected to alterations. In each case, any amendment to the approved study plan and the reasons for such amendments will be documented and realized only after written / telephonic / E-mail consent from the study Sponsor and review by the Quality Assurance Unit and Test Facility Management. If immediate action is necessary, verbal agreement from the Sponsor will be confirmed as soon as possible by study

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

plan amendment. Minor changes (unplanned) of the study plan which do not influence the procedures, or the outcome of the study may be subject to the discretion of the Study Director but will be mentioned in the report as deviations.

5. **SAFETY PRECAUTIONS**

Gloves, facemask, and goggles (if required) will be used in addition to protective body garments and shoes to ensure adequate personal health and safety. In case of eye contact, the eye will be washed thoroughly with water and medical treatment will be sought. In case of skin contact, it will be washed with soap and water with subsequent medical aid.

6. **OBJECTIVE**

The purpose of the study is to assess the acute immobilization of test item, DWE/2005A-1KG ILD Draynzyme exposed to young daphnids aged less than '24' hours at the start of the test, followed by an observation period of '48' hours under static condition and immobilization is recorded at 24 and 48 hours.

7. **MATERIALS AND METHODS**

7.1 **Materials**

7.1.1 **Test Item Information**

(As furnished by the Sponsor)

Test Item	:	DWE/2005A-1KG ILD Draynzyme
Common Name	:	Draynzyme
Chemical Name	:	Not Provided
Accession Number	:	Not Provided
Batch / Lot Details	:	01/01/24
Date of Manufacture	:	January, 2024
Date of Expiry	:	January, 2026
Physical Appearance	:	Mustard yellow gel encapsulated inside polyester sack
Recommended storage	:	Store away from heat
Purity	:	Not Provided
Solubility	:	Slowly dissolve into flowing water over a long period of time
Intended Usage	:	Treatment of contamination created by raw sewage exposure

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

Manufactured By	:	Dhara Biotech, Nr. Gaushala, Sarsa – Vasad chokdi, Bhalej Road, Sarsa, Anand, Gujrat - 388365
Supplied By	:	Quin Quent Industries Pvt Ltd S No. 131/2A Rajyog Colony Warje Pune - 411052

7.1.2 **Identity of the Test Item**

The Test Item information Sheet (TIIS), Certificate of Analysis (CoA) and Material Safety Data Sheet (MSDS) has been provided by the sponsor. The responsibility for the correct identity and purity of the test item rests with the sponsor. The authenticity of the test item was not being conducted at the test facility.

7.1.3 **Test System & Test Conditions**

Species	:	*Daphnia magna*
Age	:	Less than 24-hour old neonates.
Justification for the selection of species	:	Recommended by the regulatory guideline (OECD) for the toxicity assessment.
Source	:	Daphnia culture, Ecotoxicology Lab, CRK Campus, CSIR-Indian Institute of Toxicology Research (IITR).
Test Room Details	:	Room No: 46
Test Method	:	Static
Test Vessel	:	100 ml glass beakers
Test Volume	:	50 ml
Number of Replicates	:	**Range Finding Study:** 2 replicates for each test concentration and control. **Main Study:** 4 replicates for each test concentration and control.
Light	:	16 hours light and 8 hours darkness.
Feeding	:	During the test, daphnids will not be fed.
Test Medium	:	M4 Media
Hardness of Medium	:	140-250 mg/l

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

Test Medium pH Range	:	6 to 9 (should not vary by more than 1.5 units)
Dissolved Oxygen	:	≥3 mg/L
Test Duration	:	48 hours

7.2 Methods

7.2.1 < 24-hour old neonate daphnids collection
Sufficient numbers of <24-hour old neonate daphnids will be collected from the individual brooders (maintained as individual cultures) of more than first brood progeny in the M4 media prior to the test item exposure.

7.2.2 Vehicle
M4 medium prepared in distilled water will be used to prepare the stock solution for the study and the same will be documented in the raw data and report.

7.2.3 Test Item Preparation
The stock solution will be prepared in M4 medium immediately prior to the exposure from which the test groups will be prepared. Since the test item is not soluble in water, stock solution is prepared by adding the required quantity of test item in the M4 medium and stirred continuously for 24 hours as per the OECD guidance document 23. The aqueous phase of the test solution will be used for daphnia exposure.

7.2.4 Test and Control Groups
For range finding study, two replicates will be maintained for each treatment and control having five daphnids for each replicate.

For main study, four replicates will be maintained for each treatment and control having five daphnids for each replicate.

7.2.5 Range Finding Study
A preliminary range finding study will be conducted with a series of widely spaced concentrations like 0.01, 0.1, 1.0, 10.0, and 100 mg/L. The stock solution will be prepared with M4 medium and required volume will then be added to each test group for the required test concentrations.

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

For each replicate, 50 ml of the test solution will be placed in the respective test vessel of 100 ml capacity. 05 daphnids will be placed in each test vessel using a wide mouth pipette carefully. The vessels will then be kept under the test conditions for 48 hours.

7.2.6 **Limit Test**

If the highest concentration of the test item (i.e., 100 mg/L) shows 0 percent immobilization at the end of 48-hour exposure period in a range finding study, then the study is restricted to a limit test.

Limit test will be conducted in four replicates at 100 mg/L test concentration with five daphnids per replicate besides the control group with M4 media only.

7.2.7 **Main Study**

If immobility is noted during the range finding study, the main study will be conducted with minimum five test concentrations with a separation factor preferably not exceeding 2.2. The doses selected for main study will be provided in study report and recorded in the raw data sheet.

During the main study, four replicates will be maintained for each test concentration and control groups where each replicate will contain 05 daphnids. For each replicate, 50 ml of test solution will be placed in the respective test vessel. 05 daphnids will be placed in each test vessel using a wide mouth pipette carefully. The vessels will then be kept under the test conditions for 48 hours.

8. **OBSERVATIONS**

During the range finding and main study/limit test, dissolved oxygen, pH, and temperature of the test medium will be recorded on 0 hour and 48 hours of the treatment and control groups. Hardness will be measured at the start of the range finding and main study/limit test in control group only.

Daphnids that are not able to swim for a few seconds, after gentle agitation of the vessel will be considered as immobilized (even if they can still move their antennae). In addition to immobility, any abnormal behavior or appearance will be recorded at 24 and 48 hours.

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

9. **VALIDITY CRITERIA**
   1. Daphnids in control should not show more than 10 percent immobilization.
   2. The dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels.

10. **STATISTICAL ANALYSIS**
The mortality/concentration data will be used to calculate the Median Effective Concentration (EC_{50}) and its confidence limits. Finney's Probit Analysis will be applied to calculate the LC_{50} with 95% confidence limits and graph showing concentration/effect curve will also be plotted. No statistical analysis will be performed for the limit test and range finding experiment.

11. **DATA COMPILATION**
Data will be summarized in a tabular form, the number of daphnids used, and number of dead and live daphnids per treatment at start and during the experiment (24 h and 48 h). Dissolved oxygen, pH and temperature at the start and end of the experiment will also be tabulated.

12. **DATA AND FINAL REPORT**
The data will be summarized in tabular form, showing for each test group the number of daphnids at the start and end of the test, death of individual daphnids at different concentration levels and description of toxic effects. The final report will be prepared in compliance to the principles of GLP and normally include, but not limited to the following.

- A descriptive title.
- The name and address of the Sponsor and the test facility along with the details of study schedule.
- The names of all personnel involved in the study.
- A compliance statement signed by the Study Director that all applicable GLP regulations were followed in the conduct of the study.

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

- Quality Assurance (QA) statement; that states that the report accurately reflects the raw data obtained during the performance of the study and including the dates of QA activities and the dates reported to study director and management.
- The Test Item and its code, composition and other appropriate characteristics and vehicle with identification by name.
- Complete description of the test system including species, source, number, test conditions, photoperiod, and acclimation.
- Statisilcal analysis of the results (if applicable).
- Method of preparation of stock and test solutions.
- Graph of the concentration mortality curve at the end of the test.
- EC_{50} values, with 95% confidence limits at each of the recommended observation times (24 hour and 48 hour), if possible.
- A description of the results; discussion and conclusion.
- A description of all study plan deviations, if any.
- A description of all circumstances that may have affected the quality or integrity of the study.
- The storage locations of all raw data, specimens, reports, test Item reference sample and the archiving period.

13. **ARCHIVING**

The following will be archived at the test facility for at least 9 years (3 cycles of GLP) after completion of the study: study plan, all raw data, draft and final reports, a representative sample of Test Item (approximately one gram), etc. Before discarding of any archived study materials, the Sponsor will be contacted for the disposal.

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

14. **STUDY PLAN DISTRIBUTION**

The final study plan (original copies) will be distributed as follows:
Test Facility : One signed study plan in original (Copy No. 1/2)
Sponsor : One signed study plan in original (Copy No. 2/2)
Document Control: One controlled copy
Quality Assurance Unit: One controlled copy
Study Personnel: One controlled copy

15. **REPORT DISTRIBUTION**

The study report will be distributed as follows:
Test Facility : One signed final report in original (Copy No. 1/2) and an electronic copy in the PDF format.
Sponsor : One signed final report in original (Copy No. 2/2).

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

16. **AGREEMENT**

This study plan for Study No.: CSIR-IITR/GLP/398, "Daphnia sp. Acute Immobilization Test of Drayzyme" has been mutually agreed:

for **TEST FACILITY**	for **STUDY SPONSOR**
1. _____	1. _____
STUDY DIRECTOR	SPONSOR REPRESENTATIVE
Date: 05/02/2025	Date: Email Consent received on: 04/02/2025

2. _____
QUALITY ASSURANCE UNIT
Date: 05/02/2025

3. _____
TEST FACILITY MANAGEMENT
Date: 05/02/2025

ANNEXURE – III
CERTIFICATE OF ANALYSIS

Green Leaf

Test Report
Water Sample Analysis Report

Client's Name & Address: **M/s. Dhara Bio-tech** Near SarasChokdi, Near Gaushala, Kunjrav Road, Sarsa, Anand 388365, Gujarat. **Contact Person:** Mr. Vasantlal Patel	**Report No:** GLEPL/240124/01 **Issue Date:** 01/02/2024

Lab ID Code	:	GLEPL/240124/WL;			
Sample Description	:	Product Sample	Purpose	:	As per Client requirement
Date of Sampling	:	Submitted by Client (Dhara Bio-tech)	Sample collected/Submitted by	:	Submitted by Client (Dhara Bio-tech)
Date of Sample Received	:	24/01/2024	Test Parameters	:	As per Client requirement
Date of starting Analysis	:	25/01/2024	Quantity/No. of sample	:	1No. Batch No. 01/01/24
Date of completion Analysis	:	31/01/2024	Packed/Seal	:	Sealed (13/01/24)

Result Table

Sr. No.	Test Parameters	Test Method	Unit	Results
1	Drayzyme for Bacteria Count	ISO 16649-3	MPN/100 mL	Absent

Remark: In accordance to G.S.R 613(E)-This product does not contain any Bacteria, thus does not require 'Appraisal by genetic Engineering Appraisal Committee).

Chemist

Authorized Signatory
Rekha Dare

Notes: (1) The results pertain to tested items only.
(2) This report shall not be reproduced, except in full, without written approval of the laboratory.
(3) Authenticity of this Report could be validated with office copy at Greenleaf Evirotech Pvt. Ltd.
(4) Perishable samples will be destroyed after testing, others after 7 days from the date of issue of the report, unless otherwise agreed with the customer or as required by the applicable regulations.

CIN: U74140GJ2010PTC059798
Greenleaf EnvirotechPvt. Ltd., Nr. RangoliFlats,Radhanpur Road, Mehsana – 384002. Gujarat, India.
Tel : +91-9725519974, E-mail: info@glepl.com, Web: www.glepl.com
Branch Office: 304, Kankavati Complex, Singanpor-Cauzway Road, Katargam, Surat– 395004

ANNEXURE – IV
TEST SYSTEM CHARACTERIZATION CERTIFICATE

Mobile: 09415871277
E-mail: serajuddin2164@rediffmail.com

DEPARTMENT OF ZOOLOGY
LUCKNOW UNIVERSITY, LUCKNOW-226007

DR. M. SERAJUDDIN *PROFESSOR*

To whom it may concerned

The specimens of *Daphnia* submitted by **Dr. Anbumani Sadasivan**, Scientist, Ecotoxicology Lab (GLP Test Facility) of CSIR-IITR are verified as *Daphnia magna* (originally supplied by Carolina Biologicals, USA). The specimens are identified based on the following taxonomic key characters which are mentioned below:

Daphniidae:

General - body enclosed in a carapace

Genus ***Daphnia***: Possess a prominent shell spine (i.e. "tail")

- Antennules attached to ventral side of head and not covered by fornices. Antennules are not inserted at the anterior and of the ventral edge of the head
- The rostrum is present and the dorsal and ventral margins are spinous with no pubercaece. A dorsal carina is present and there are no cervical spines present.
- The carapace extends anteriourly along the mid dorsal line as a broad strip between the halves of the head.
- Shield and the margin of the post abdomen is sinuate.
- The overall size is 4.5 to 5 mm in length

Ref.: Peanak, R.W. 1989. Freshwater Invertebrates of United States. 3rd Edition, Willey and Sons: 386-388pp.

Dated:)2 May, 2017

(Dr. M. Serajuddin)

Dr. M. Serajuddin
Professor
Department of Zoology
Lucknow University

ANNEXURE-V
GLP CERTIFICATE

National Good Laboratory Practice (GLP) Compliance Monitoring Authority (NGCMA)
Department of Science and Technology
GOVERNMENT OF INDIA

Certificate of GLP Compliance

This is to certify that

Toxicity Testing: GLP Test Facility, CSIR-Indian Institute of Toxicology Research
CRK Campus, Gheru, Sarojini Nagar Industrial Area
Lucknow-226008, Uttar Pradesh (India)

is a GLP certified test facility in compliance with the NGCMA's Document No. GLP-101 "Terms & Conditions of NGCMA for obtaining and maintaining GLP certification by a test facility" and OECD Principles of GLP.

The test facility conducts the below-mentioned tests/ studies:

- **Toxicity Studies**
- **Mutagenicity Studies**
- **Environmental Toxicity Studies on Aquatic and Terrestrial Organisms**
- **Analytical and Clinical Chemistry Testing**

The specific area(s) of expertise, test item(s) and test system(s) are listed in the annexure overleaf.

Validity: June 5, 2023 – June 4, 2026

Certificate No. :GLP/C-213/2023
Issue Date :07-12-2023

(Dr. Ekta Kapoor)
Head, NGCMA

Study on Earthworm, acute toxicity test:

VishaktataParikshan: GLP AnuroopSuvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

FINAL REPORT

STUDY TITLE

EARTHWORM, ACUTE TOXICITY TEST OF DRAYNZYME

TESTITEM: DRAYNZYME

STUDY NO.: CSIR-IITR/GLP/397

STUDY COMPLETED ON:
15/04/2025

SPONSOR
PUNE MUNICIPAL CORPORATION
SHIVAJI NAGAR, PUNE 411005

TEST FACILITY
TOXICITY TESTING: GLP TEST FACILITY
CSIR-INDIAN INSTITUTE OF TOXICOLOGY RESEARCH
CRK CAMPUS, GHERU, SAROJINI NAGAR INDUSTRIAL AREA
KANPUR ROAD, LUCKNOW-226008
INDIA

TABLE OF CONTENTS

STATEMENT OF CONFIDENTIALITY

The report contains confidential and proprietary information to Pune Municipal Corporation Shivaji Nagar, Pune 411005.The contents of this report will not be disclosed to anyone without an expressed or approval ofcompetent authority of Pune Municipal Corporation Shivaji Nagar.

STATEMENT OF GLP COMPLIANCE

This study was performed in compliance with the OECD Principles of Good Laboratory Practice for the testing of chemicals as specified by International [C (97) 186/Final] Legislation. This study was conducted in accordance with the Standard Operating Procedures of Toxicity Testing: GLP Test Facility, CSIR-Indian Institute of Toxicology Research and the mutually agreed study plan which was signed by the Study Director on 05/02/2025 after the email consent from sponsor on 04/02/2025.

DECLARATION

The Study Director hereby declares that the work was performed under her supervision and in accordance with the described procedures. It is assured that the reported results faithfully represent the raw data obtained during the experimental work. No circumstances have been left unreported.

Study Director
Date: 15/04/2025

Test Facility Management
Date: 15/04/2025

Deputy Test Facility Management
Vishaktata Parikshan : GLP Anuroop Suvidha
Toxicity Testing: GLP Compliant Facility CSIR-IITR Lucknow, India

VishaktataParikshan: GLP AnuroopSuvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

QUALITY ASSURANCE STATEMENT

Study No.: CSIR-IITR/GLP/397, "Earthworm, Acute Toxicity Test of Draynzyme" has been inspected in accordance with the OECD Principles of Good Laboratory Practice for the testing of chemicals as specified by International [C (97) 186/Final] Legislation.

This study was inspected and findings reported to the Management and Study Director on the dates shown below:

INSPECTION DATE	PHASE	REPORTING DATE
	Initiation Phase	
01/01/2025	Draft Study Plan Review	01/01/2025
06/02/2025	Final Study Plan Review	06/02/2025
	In-Life Phase	
06/02/2025	Acclimatization of Earthworms for Range Finding Study	06/02/2025
07/02/2025	Earthworm Exposure to Test Item during Range Finding Study	07/02/2025
14/02/2025	Day 7 Observation of Range Finding Study	14/02/2025
21/02/2025	Day 14 Observation of Range Finding Study	21/02/2025
24/02/2025	Acclimatization of Earthworms for Limit Test	24/02/2025
25/02/2025	Earthworm Exposure to Test Item during Limit Test	25/02/2025
04/03/2025	Day 7 observation of Limit Test	04/03/2025
11/03/2025	Day 14 observation of Limit Test	11/03/2025
	Reporting Phase	
27/03/2025	Draft Report Review	01/04/2025
15/04/2025	Final Report Review	15/04/2025

Inspections were performed according to the Standard Operating Procedures of the Test Facility's Quality Assurance Unit. The report was inspected as per the approved study plan and pertinent raw data is accurately reflected in the report

Date: 15/04/2025

(Vandita Dogra)
Quality Assurance Unit
Toxicity Testing: GLP Test Facility
CSIR-Indian Institute of Toxicology Research
CRK Campus Gheru, Sarojini Nagar Industrial
Area Kanpur Road, Lucknow-226008, India

LIST OF COMMONLY USED ABBREVIATIONS AND SYMBOL

CSIR	Council of Scientific and Industrial Research
°C	Degree Celsius
g	Gram
GLP	Good Laboratory Practices
IITR	Indian Institute of Toxicology Research
kg	Kilogram
LC_{50}	Median Lethal Concentration
mg	Milligram
ml	Milliliter
NAD	No Abnormalities Detected
OECD	Organization for Economic Cooperation and Development
SD	Standard Deviation
TIIS	Test Item Information Sheet

1. STUDY DETAILS

Study Title	:	Earthworm, Acute Toxicity Test of Draynzyme
Test Item	:	DWE/2005A-1KG ILD Draynzyme
Study Number	:	CSIR-IITR/GLP/397
Sponsor	:	Pune Municipal Corporation Shivaji nagar, Pune 411005 Test conducted for Pune Municipal Corporation (In compliance to NGT matter of OA 323 of 2024)
Sponsor's Representative	:	Mangesh Dighe Environment Officer Pune Municipal Corporation E Mail: indradhanushya@punecorporation.org
Test Facility	:	Toxicity Testing: GLP Test Facility CSIR-Indian Institute of Toxicology Research CRK Campus, Gheru, Sarojini Nagar Industrial Area Kanpur Road,Lucknow-226008, India
Study Schedule		
Study Start Date	:	05/03/2025
Limit Test Experiment Start Date		
Pre-culture	:	06/03/2025 – 10/03/2025
Test Item Exposure	:	10/03/2025
24 Hour Observation	:	11/03/2025
48 Hour Observation	:	12/03/2025
72 Hour Observation	:	13/03/2025
Experiment Completion Date	:	13/03/2025
Study Completion Date	:	15/04/2025

2. STUDY PERSONNEL

The following personnel participated in the conduct of the study.

Name	Function
Ms Vijaya Shree	Study Director
Ms Priya Maurya	Study Personnel
Mr Sudhanshu Mishra	Study Personnel

3. SUMMARY

Acute toxicity study in Earthworm (*Eisenia fetida*) was performed as per the OECD Guideline for the testing of Chemicals, Number 207 with the test item DWE/2005A-1KG ILD Draynzyme. The earthworms were acclimatized one day prior to the exposure in the artificial soil. All earthworms were normal during the acclimatization period.

A preliminary range finding experiment was conducted with test concentrations of 1.0, 10.0, 100.0 and 1000.0 mg/Kg (dry weight of artificial soil) along with a concurrent control group. Four replicates were maintained for treatment and control group with 10 earthworms per replicate. The test item was formulated in distilled water. Daily light intensity measurements were found to be within 400-800 lux respectively. Prior to the release of earthworms into the respective replicate test vessel, body weight of earthworms was measured. The test vessels were covered loosely with the lid to avoid earthworm escape and prevent the drying of artificial soil on surface. The test vessels with earthworms were incubated for a period of 14 days with continuous illumination for mortality assessment. Mortality was assessed on day 7 and 14 by emptying the test vessel into a tray and counted the number of worms in each replicate of the respective treatment group. Earthworms in all the treatment groups including control was found to be live with no abnormalities during the 14-day exposure period. No treatment related mortality was observed in 1.0, 10.0, 100.0 and 1000.0 mg/Kg (dry weight of artificial soil) DWE/2005A-1KG ILD Draynzyme treatment groups throughout the study duration in four replicates.

Based on the results of range finding study, the main study was carried out as a limit test at 1000 mg/Kg (dry weight of artificial soil) concentration along with a concurrent control group. Four replicates were maintained for treatment and control group with 10 earthworms per replicate. The exposure and maintenance protocol were same as mentioned above in the range finding study. Mortality was assessed on day 7 and 14 by emptying the test vessel into a tray and counted the number of worms in each replicate of the respective treatment group. Earthworms in the limit test concentration (1000 mg/kg) group including control was found to be live with no abnormalities during the 14-day exposure period. No treatment related mortality was observed in1000 mg/kg (dry weight of artificial soil)DWE/2005A-1KG ILD Draynzymetreatment group throughout the study duration in four replicates.

4. OBJECTIVE

The purpose of this study was to assess the acute toxicity of test item DWE/2005A-1KG ILD Draynzymein the earthworm, *Eisenia fetida*by artificial soil test method fora period of 14 days.

5. STUDY COMPLIANCE

The study was performed in accordance with the following:

1. OECD Principles of Good Laboratory Practice for the testing of chemicals as specified by International [C (97) 186/Final] Legislation.
2. OECD Test Guideline No. 207 - Earthworm Acute Toxicity Test.
3. The mutually agreed study plan and the Standard Operating Procedures of the test facility (CSIR-IITR/ECO/002, Revision 04).

6. AMENDEMENT AND DEVIATION

This study has one deviation and no amendment reported. Instead of 0.1, 1.0, 10.0, 100.0 and 1000.0 mg/kg we did the dose range finding study on 1.0, 10.0, 100.0 and 1000.0 mg/kg. As the range of calibration for balance was not available for 0.1 mg/kg. This deviation does not have any impact of the study.

7. MATERIALS AND METHODS

7.1. MATERIALS

7.1.1. Test Item Information

(As furnished by the Sponsor)

Test Item	:	DWE/2005A-1KG ILD Draynzyme
Common Name	:	Draynzyme
Chemical Name	:	Not Provided
Accession Number	:	Not Provided
Batch / Lot Details	:	01/01/24
Date of Manufacture	:	January, 2024
Date of Expiry	:	January, 2026
Physical Appearance	:	Mustard yellow gel encapsulated inside polyester sack
Recommended storage	:	Store away from heat
Purity	:	Not Provided
Solubility	:	Slowly dissolve into flowing water over a long period of time
Intended Usage	:	Treatment of contamination created by raw sewage exposure
Manufactured By	:	Dhara Biotech, Nr, Gaushala, Sarsa – Vasad chokdi, Bhalej Road, Sarsa, Anand, Gujrat - 388365

Supplied By	:	Quin Quent Industries Pvt Ltd S No. 131/2A Rajyog Colony Warje Pune - 411052

7.1.2. Identity of the Test Item

The Test Item information Sheet (TIIS), Certificate of Analysis (CoA) and Material Safety Data Sheet (MSDS) has been provided by the sponsor. The responsibility for the correct identity and purity of the test item rests with the sponsor. The authenticity of the test item was not being conducted at the test facility.

7.1.3. Test System& Test Conditions

Species	:	*Eisenia fetida*
Age	:	Adult earthworms with an individual weight of 300 to 600 mg
Source	:	Earthworm culture, Ecotoxicology LabCRK Campus,CSIR-Indian Institute of Toxicology Research, Lucknow.
Justification for the selection of species	:	Recommended by the regulatory guideline (OECD 207) for terrestrial toxicity assessment
Test Room Details	:	Room No: 46, 47 & 48
Test Method	:	Artificial Soil Test
Test Vessel	:	1-liter crystallizing glass jars covered with perforated plastic film.
No. of Replicates	:	Control: 4 Treatment: 4
Temperature (°C)	:	Acute Range Finding Study: 19.9 – 20.2°C Limit Test:19.7 – 20.4 °C
Light (Lux)	:	Acute Range Finding Study: 430 – 590 Limit Test: 450 – 572
Test Medium	:	Artificial Soil – 500g/replicate (10% Peat, 20% Kaolin clay and 70% Industrial sand)
Test Medium pH on Day zero	:	Acute Range Finding Study: 5.91 Limit Test: 5.98
Acclimatization	:	24 hours prior to test, earthworms were conditioned in the artificial soil
Test Duration	:	14days

7.2. Methods

7.2.1. Artificial Soil Preparation

10% sphagnum peat, 20% kaolin clay and 70% industrial sand were used for artificial soil preparation. The dry constituents were blended in correct proportions and hand mixed thoroughly.

7.2.2. Moisture Content

At the beginning and end of the test, the moisture content of the test medium was assessed. Approximately, 10 g of soil sample was dried in a previously weighed clean oven dried glass Petri plate in a thermostaticallycontrolled oven with a temperature range of 105 ± 2°C for approximately 3 hours. The beakers were placed in a desiccator and the moisture content of the artificial soil was calculated as percentage of the dry soil weight using the formula:

Moisture content (MC %) = (W2-W3) / (W3-W1) x 100

Where,

W1 = Weight of the glass Petri plate (g)

W2 = Weight of the moist soil + glass Petri plate (g)

W3 = Weight of the dried soil + glass Petri plate (g)

7.2.3. Acclimatization of Earthworms

Sufficient numbers of healthy earthworms were collected from the in-house laboratory culture breeding box, acclimatized for one day in artificial soil.

7.2.4. Weight of Earthworms

Prior to the acclimatization, approximate weight of earthworms was checked. 10 earthworms were approximately weighed between 300 and 600 mg on the day of acclimatization. Body weight of all earthworms (control and treatments) wasrecorded on day 0 and the remaining live worms onday 14 of exposure.

7.2.5. Vehicle

The test item is not soluble in distilled water and solvents, then the required quantity of test item will directly mix in the artificial soil and thoroughly mixed.

7.2.6. Dose Formulation

7.2.6.1. Range Finding Study

For acute range finding experiment, concentrations selected were 1.0, 10.0,100.0and1000.0mg/kg dry weight of artificial soil. This is equivalent0.52, 5.2, 52 and520mg for 520 g of artificial soil. A quantity 2.08 mg, 20.8 mg, 208 mg and 2080 mgadded in each group of artificial soil for 1.0, 10.0, 100.0 and 1000.0 mg/Kg concentrations. Control group consists of artificial soil without the test item.

7.2.6.2. Limit Test

Main study was conducted as a limit test by exposing the earthworms to a limit test concentration of 1000 mg/kg dry weight of artificial soil. For this, 2080 mg of DWE/2005A-1KG ILD Draynzyme is thoroughly mixed in 2.08 kg of artificial soil for 1000 mg/kg concentration. Control group consists of artificial soil without test item.

7.2.7. Treatment

For range finding study and limit test, 520 g of the test medium was used per replicate and 500 g of test item mixedsoil was carefully added into the respective test vessel. The earthworms acclimatized for 24 hours in artificial soil were washed with distilled water before use, weighed and placed on the test medium surface.
One litre crystallizing glass jar was used as test vessels covered with perforated plastic film to prevent the test medium from drying and kept under the test conditions in a cooling incubator.

8. OBSERVATIONS

8.1. pH

On the day of acclimatization and day 0, pH of artificial soil was checkedfor all the test groups during the range finding and main study in all replicates.

8.2. Light Intensity and Temperature

Light intensity and temperature were recorded on the day of acclimatization and once daily during the14-day exposure periodof range finding and limit test.

8.3. Moisture Content

Moisture content of artificial soil was checked on days 0 and 14 during the range finding study and limit test.

8.4. Body Weight

Body weight of earthworms was recorded on day 0 and live worms on day 14(Table 9 and 10).

8.5. Mortality Assessment

Earthworms were assessed for mortality, behavior and toxicity symptoms on days 7 and 14 during the limit test

9. RESULTS

9.1. pH of Artificial Soil

pH of artificial soil was found to be 5.91and 5.98 on the day of acclimatization during the acute range finding and limit test (Table 1 & 2).

During the acute range finding experiment, mean pH of the artificial soil was recorded as 5.93 ± 0.01, 5.93 ± 0.01, 5.96 ± 0.01, 5.98 ± 0.01 and5.99 ± 0.01for

control1.0, 10.0,100.0 and 1000.0 mg/kg (dry weight of artificial soil) test concentrations (Table 1).

During the limit test, mean pH of the artificial soil for DWE/2005A-1KG ILD Draynzyme exposure was recorded as 6.04 ± 0.01 and 6.19 ± 0.02respectively for control and 1000 mg/kg (dry weight of artificial soil) test concentrations (Table 2).

9.2. Light Intensity and Temperature

The light intensity range recorded during acute range finding and limit testwas between 430 to 590 lux and 450 to 570 lux respectively.Temperature was maintained between 19.9 to 20.2 and 19.7 to 20.4°C during range finding study and limit test.

9.3. Moisture Content

In Range finding study, mean moisture content of artificial soil on day zero was recorded to be 35.02 ± 1.29 in control group and 33.13 ± 1.45, 34.47 ± 1.84, 33.79 ± 1.58 and 34.43 ± 4.03 in treatment groups respectively (Table 3). On day 14, mean moisture content of artificial soil was recorded to be 32.35 ± 1.14 in control group and 31.16 ± 1.66, 32.57 ± 1.16, 31.40 ± 1.73 and 31.62 ± 1.03in treatment groups respectively (Table 3).

In Limit test, mean moisture content of artificial soil on day zero was recorded to be 32.91 ± 1.70 in control group and 35.15 ± 1.49, in 1000 mg/kg treatment groups respectively (Table 4). On day 14, mean moisture content of artificial soil was recorded to be 30.31 ± 1.62in control group and 32.46 ± 1.01 in1000 mg/kg treatment groups respectively (Table 4).

9.4. Body weight

No significant difference ($p>0.05$) in body weight was noticed between the control and DWE/2005A-1KG ILD Draynzymetreated earthworms.

9.5. Mortality and Toxicity Signs

During range finding study, no mortality was observed in control and worms exposed to 1.0, 10.0, 100.0 and1000.0mg/kg (dry weight of artificial soil) concentrations of DWE/2005A-1KG ILD Draynzyme on day 7 and day 14 (Table 5).

No behavioral or toxicity signs were observed in control and worms exposed to 1.0, 10.0, 100.0 and 1000.0 mg/kg(dry weight of artificial soil) concentrations throughout the experimental period (Table 7).

During the limit test, no mortality was observed in control and worms exposed to 1000 mg/kg (dry weight of artificial soil) concentration of DWE/2005A-1KG ILD Draynzymeon day 7 and day 14 (Table 6).

No behavioral or toxicity signs were observed in control and worms exposed to 1.0, 10.0, 100.0 and 1000.0 mg/kg(dry weight of artificial soil) concentrations throughout the experimental period (Table 8).

10. VALIDITY CRITERIA

A validity criterion wasmet, as there was no mortality found in the control group.

11. STATISTICAL ANALYSIS

Finney probit analysis was not carried out since the main study was conducted as a limit test. Paired sample t-test test was used to determine the significant difference in body weight between control and DWE/2005A-1KG ILD Draynzymetreated earthworms.

12. DATA COMPILATION

Data are summarized in tabular form for the following:
The number of earthworms used, average body weight of live worms (day 0 and 14), mortality and toxicity signs on days 7 and 14, pH of artificial soil on the day of acclimatization and day 0; moisture content data on days 0 and 14.

13. CONCLUSION

Based on the test results, the LC_{50} ofDWE/2005A-1KG ILD Draynzyme, *Eisenia fetida* was found to be greater than 1000 mg/kg dry weight of artificial soil.

14. ARCHIVING

The following has beenarchived at the test facility for 9 years after completion of the study: all raw data, study plan, draft and final reports. A representative sample of test Itemhas been sent from the TestItemControl Office to the Archives in the test facility. The sample shall be stored for a period of 9 years from the date of this final report. Before discarding of any archived study materials, the Sponsor will be contacted for the disposal.

15. REPORT DISTRIBUTION

The study report will be distributed as follows:
Test Facility : One signed final report in original (Copy No. 1/2) and an electronic copy in the PDF format.
Sponsor : One signed final report in original (Copy No. 2/2).

TABLE 1: pH (Range Finding Study)

Details	Replicate	pH Value
Acclimatization	--	5.91
Control	R1	5.94
	R2	5.92
	R3	5.92
	R4	5.93
Mean ± SD		**5.93 ± 0.01**
1.0 mg/kg	R1	5.93
	R2	5.94
	R3	5.93
	R4	5.93
Mean ± SD		**5.93 ± 0.01**
10.0 mg/kg	R1	5.95
	R2	5.96
	R3	5.95
	R4	5.96
Mean ± SD		**5.96 ± 0.01**
100.0 mg/kg	R1	5.98
	R2	5.99
	R3	5.98
	R4	5.97
Mean ± SD		**5.98 ± 0.01**
1000.0 mg/kg	R1	5.98
	R2	5.99
	R3	5.99
	R4	5.98
Mean ± SD		**5.99 ± 0.01**

TABLE 2. pH (Limit TEST)

Details	Replicate	pH Value
Acclimatization	--	5.98
Control	R1	5.97
	R2	5.98
	R3	5.95
	R4	5.97
Mean ± SD		**5.97± 0.01**
1000 mg/kg	R1	6.09
	R2	6.11
	R3	6.08
	R4	6.09
Mean ± SD		**6.09±0.01**

TABLE 3. MOISTURE CONTENT (RANGE FINDING STYDY

Concentration (mg/kg dry weight of artificial soil)		Moisture Content (%)	
	Replicates	Day 0 (Start of the experiment)	Day 14 (End of the experiment)
Control	R1	35.14	33.33
	R2	36.49	33.33
	R3	33.33	31.17
	R4	35.14	31.58
Mean ± SD		**35.02 ± 1.29**	**32.35 ± 1.14**
1.0	R1	33.33	29.87
	R2	31.17	29.87
	R3	34.67	33.33
	R4	33.33	31.58
Mean ± SD		**33.13 ± 1.45**	**31.16 ± 1.66**
10.0	R1	36.99	33.33
	R2	32.89	31.58
	R3	34.67	33.78
	R4	33.33	31.58
Mean ± SD		**34.47 ± 1.84**	**32.57 ± 1.16**
100.0	R1	31.58	29.11
	R2	35.14	33.33
	R3	34.67	31.58
	R4	33.78	31.58
Mean ± SD		**33.79 ± 1.58**	**31.04 ± 1.73**
1000.0	R1	29.87	28.21
	R2	36.49	31.58
	R3	38.89	35.14
	R4	32.47	31.58
Mean ± SD		**34.43 ± 4.03**	**31.62 ± 1.03**

TABLE 4. MOISTURE CONTENT (LIMIT TEST)

Concentration (mg/kg dry weight of artificial soil)		Moisture Content (%)	
	Replicates	Day 0 (Start of the experiment)	Day 14 (End of the experiment)
Control	R1	33.33	31.58
	R2	31.58	29.87
	R3	31.58	28.21
	R4	35.14	31.58
Mean ± SD		**32.91 ± 1.70**	**30.31 ± 1.62**
1000	R1	36.99	31.58
	R2	35.14	33.33
	R3	35.14	33.33
	R4	33.33	31.58
Mean ± SD		**35.15 ± 1.49**	**32.46 ± 1.01**

TABLE 5. MORTALITY DATA (RANGE FINDING STUDY)

Concentration (mg/kg, dry weight of artificial soil)	No. of Earthworms/ Replicate	Day: 7					Day: 14					Cumulative Mortality (%)
		Replicates				Average (%)	Replicates				Average (%)	
		1	2	3	4		1	2	3	4		
Control	10	0	0	0	0	0	0	0	0	0	0	0
1.0	10	0	0	0	0	0	0	0	0	0	0	0
10.0	10	0	0	0	0	0	0	0	0	0	0	0
100.0	10	0	0	0	0	0	0	0	0	0	0	0
1000.0	10	0	0	0	0	0	0	0	0	0	0	0

TABLE 6. MORTALITY DATA (LIMIT TEST)

Concentration (mg/kg, dry weight of artificial soil)	No. of Earthworms/ Replicate	Day: 7					Day: 14					Cumulative Mortality (%)
		Replicates				Average (%)	Replicates				Average (%)	
		1	2	3	4		1	2	3	4		
Control	10	0	0	0	0	0	0	0	0	0	0	0
1000	10	0	0	0	0	0	0	0	0	0	0	0

TABLE 7. TOXICITY SIGNS (RANGE FINDING STUDY)

Concentration (mg/kg, dry weight of artificial soil)	No. of Earthworms/ Replicate	Symptom Code	Day: 7				Symptom Code	Day: 14			
			Replicates					Replicates			
			1	2	3	4		1	2	3	4
Control	10	NAD	10	10	10	10	NAD	10	10	10	10
1.0	10	NAD	10	10	10	10	NAD	10	10	10	10
10.0	10	NAD	10	10	10	10	NAD	10	10	10	10
100.0	10	NAD	10	10	10	10	NAD	10	10	10	10

1000.0	10	NAD	10	10	10	10	NAD	10	10	10	10

TABLE 8. TOXICITY SIGNS (LIMIT TEST)

Test Item Concentration (mg/kg dry weight of artificial soil)	No. of Earthworms/ Replicate	Symptom Code	Day 7 Replicates				Symptom Code	Day 14 Replicates			
			1	2	3	4		1	2	3	4
Control	10	NAD	10	10	10	10	NAD	10	10	10	10
1000	10	NAD	10	10	10	10	NAD	10	10	10	10

Note: Symptom Code: NAD – No Abnormalities Detected

TABLE 9.BODY WEIGHT OF EARTHWORMS (MILLIGRAM):RANGE FINDIG STUDY

Concentration: ControlDay: 0				
S. No.	Replicate			
	1	2	3	4
1	310	418	328	379
2	322	303	341	340
3	329	376	335	325
4	337	343	326	333
5	326	334	330	361
6	310	320	371	341
7	328	349	362	333
8	303	358	331	315
9	322	346	326	309
10	342	315	350	371
Mean	**322.90**	**346.20**	**340.00**	**340.70**
S.D.	**12.32**	**33.13**	**15.94**	**23.16**

Concentration: 1.0mg/kg dry weight of artificial soil				Day:0
S. No.	Replicate			
	1	2	3	4
1	321	383	317	315
2	346	325	320	309
3	330	370	304	321
4	342	329	391	346
5	360	307	325	330
6	356	444	340	325
7	331	431	345	317
8	305	352	332	329
9	315	302	317	376
10	322	331	322	302
Mean	**332.80**	**357.40**	**331.30**	**327.00**
S.D.	**17.93**	**49.35**	**24.12**	**21.10**

Concentration: 10.0 mg/kg dry weight of artificial soil				Day: 0
S. No.	Replicate			
	1	2	3	4
1	342	338	323	320
2	315	329	333	303
3	378	370	332	318
4	306	325	346	309
5	467	340	302	483
6	320	321	319	315
7	329	317	300	349
8	322	368	314	388
9	364	402	320	351
10	325	342	329	334
Mean	**346.80**	**345.20**	**321.80**	**347.00**
S.D.	**47.77**	**26.88**	**14.17**	**54.10**

Concentration: 100.0 mg/kg dry weight of artificial soil				Day: 0
S. No.	Replicate			
	1	2	3	4
1	348	339	335	333
2	311	324	354	303
3	321	303	382	428
4	320	324	450	325
5	301	311	348	301
6	329	323	354	417
7	320	343	317	325
8	333	359	322	316
9	306	306	421	371
10	320	329	350	350
Mean	**320.90**	**326.10**	**363.30**	**346.90**
S.D.	**13.60**	**17.41**	**42.73**	**45.00**

Concentration: 1000.0 mg/kg dry weight of artificial soil				Day: 0
S. No.	Replicate			
	1	2	3	4
1	378	320	325	320
2	367	306	414	333
3	332	360	345	349
4	311	336	471	310
5	378	363	380	321
6	320	306	327	312
7	365	323	329	317
8	331	329	335	325
9	309	387	329	340
10	321	370	324	319
Mean	**341.20**	**340.00**	**357.90**	**324.60**
S.D.	**27.76**	**28.24**	**49.41**	**12.47**

TABLE 10. BODY WEIGHT OF EARTHWORMS (MILLIGRAM) (RANGE FINDING STUDY)

Concentration: Control				Day: 14
S. No.	Replicate			
	1	2	3	4
1	342	315	331	410
2	308	318	325	462
3	300	281	375	345
4	291	391	273	452
5	302	280	341	324
6	303	281	309	441
7	299	279	361	471
8	300	389	352	315
9	398	429	360	378
10	310	328	297	453
Mean	**315.30**	**329.10**	**332.40**	**405.10**
S.D.	**32.12**	**55.05**	**32.04**	**60.03**

Concentration: 1.0mg/kg (dry weight of artificial soil) Day:14				
	Replicate			
S. No.	1	2	3	4
1	395	321	317	345
2	365	431	342	413
3	321	462	278	321
4	511	519	278	341
5	290	308	423	426
6	501	386	483	395
7	323	351	302	358
8	473	432	387	329
9	410	407	321	301
10	303	310	293	328
Mean	**389.20**	**392.70**	**342.40**	**355.70**
S.D.	**82.81**	**70.64**	**68.09**	**41.88**

Concentration: 10.0 mg/kg (dry weight of artificial soil) Day:14				
	Replicate			
S. No.	1	2	3	4
1	387	297	448	471
2	371	334	368	382
3	309	319	361	333
4	368	311	371	270
5	372	382	384	372
6	278	412	394	401
7	342	297	309	409
8	273	271	310	281
9	481	391	379	345
10	469	302	339	301
Mean	**365.00**	**331.60**	**366.30**	**356.50**
S.D.	**70.35**	**47.22**	**41.05**	**62.97**

Concentration: 100.0 mg/kg (dry weight of artificial soil) Day:14				
S. No.	Replicate			
	1	2	3	4
1	385	291	401	415
2	305	400	402	291
3	338	419	407	311
4	341	287	497	369
5	301	282	337	281
6	381	347	237	272
7	362	322	317	278
8	339	342	285	252
9	418	436	318	376
10	342	358	369	268
Mean	**351.20**	**348.40**	**357.00**	**311.30**
S.D.	**36.17**	**55.40**	**74.02**	**55.42**

Concentration: 1000.0 mg/kg (dry weight of artificial soil) Day:14				
S. No.	Replicate			
	1	2	3	4
1	341	327	340	331
2	338	325	276	296
3	319	341	336	294
4	358	268	296	331
5	291	272	310	346
6	303	371	330	291
7	349	281	271	254
8	271	343	256	269
9	261	272	229	272
10	318	321	246	298
Mean	**314.90**	**312.10**	**289.00**	**298.20**
S.D.	**33.01**	**36.29**	**39.46**	**29.82**

TABLE 11.BODY WEIGHT OF EARTHWORMS(MILLIGRAM) (LIMIT TEST)

Concentration: Control				Day: 0
	Replicate			
S. No.	1	2	3	4
1	338	423	397	429
2	412	343	345	351
3	384	357	367	328
4	316	356	325	305
5	350	364	344	303
6	323	375	305	306
7	303	352	300	428
8	345	333	424	329
9	420	394	390	354
10	345	406	311	364
Mean	**353.60**	**370.30**	**350.80**	**349.70**
S.D.	**39.52**	**28.92**	**42.56**	**46.70**

Concentration: 1000 mg/kg (dry weight of artificial soil) Day: 0				
	Replicate			
S. No.	1	2	3	4
1	312	388	309	315
2	407	328	328	307
3	362	351	338	304
4	345	401	303	358
5	349	328	306	319
6	312	371	318	388
7	381	412	323	305
8	401	322	336	351
9	309	337	360	309
10	368	300	329	319
Mean	**354.60**	**353.80**	**325.00**	**327.50**
S.D.	**36.00**	**37.43**	**17.30**	**28.41**

Table 12. BODY WEIGHT OF EARTHWORMS (MILLI GRAM): LIMIT TEST

Concentration: Control				Day:14
S. No.	Replicate			
	1	2	3	4
1	302	465	305	393
2	314	310	309	378
3	352	291	352	366
4	382	302	428	302
5	309	346	325	331
6	287	324	304	396
7	264	306	275	418
8	346	352	328	265
9	332	366	293	306
10	364	319	318	321
Mean	**325.20**	**338.10**	**323.70**	**347.60**
S.D.	**36.61**	**50.60**	**42.16**	**49.71**

Concentration: 1000 mg/kg dry weight of artificial soil				Day: 14
S. No.	Replicate			
	1	2	3	4
1	322	398	318	394
2	316	294	394	350
3	370	383	304	380
4	356	400	296	392
5	256	364	333	305
6	300	283	345	320
7	298	286	333	280
8	249	317	351	312
9	350	348	349	336
10	380	292	298	312
Mean	**319.70**	**336.50**	**332.10**	**338.10**
S.D.	**45.14**	**47.66**	**29.99**	**39.57**

ANNEXURE-I
RESULT OF REFERENCE STUDY

Study No.: CSIR-IITR/GLP/381

SUMMARY

Acute toxicity study in Earthworm (*Eisenia fetida*) was performed as per the OECD Guideline for the testing of Chemicals, Number 207 and EC Directive 88/303/EEC C.8. with the test item, 2-chloroacetamide. The earthworms were acclimatized one day prior to the exposure in the artificial soil. All earthworms were normal during the acclimatization period. The test item was formulated with distilled water.

Initially, a range finding study was conducted with widely spaced test concentrations (0.1, 1.0, 10.0 & 100.0 mg/Kg, dry weight of artificial soil) of test item along with a concurrent control group. Four replicates were maintained for each treatment and control group with 10 earthworms per replicate. The test item was formulated in distilled water. After the test item exposure, earthworms were observed for mortality and toxicity signs on days 7 and 14. No mortality and toxicity signs were observed in earthworms treated between 0.1 to 10.0 mg/Kg test concentrations on day 7 and day 14. 100 % mortality was observed in 100 mg/Kg treatment group on day 7 in all four replicates.

Based on the results of range finding study, the main study was carried out with 10.00, 16.00, 25.60, 40.96, 65.54 & 104.86 mg/Kg (dry weight of artificial soil) test concentrations along with a concurrent control group. Four replicates were maintained for each treatment and control group with 10 earthworms per replicate. On day 7, no mortality was observed in 10.00, 16.00, and 25.60 mg/Kg treatment groups however 25%, 100%, and 100% mortality was noticed in worms exposed to 40.96, 65.54 & 104.86 mg/Kg treatment groups respectively. No mortality was noticed on day 14 in 10.00, 16.00, and 25.60 mg/Kg treatment group however 5% mortality was noticed in 40.96 mg/Kg test concentration. Thus, the cumulative mortality at the end of 14-day exposure was 0% in control, 10.00, 16.00 & 25.60 mg/Kg (dry weight of artificial soil) test concentrations, 30%, 100%, and 100% mortality in 40.96, 65.54, and 104.86 mg/Kg (dry weight of artificial soil) test concentrations respectively.

No toxicity signs were observed in control and worms exposed to 10.00, 16.00 and 25.60mg/Kg (dry weight of artificial soil) concentrationswhereas, earthworms exposed to 40.96 mg/Kg (dry weight of artificial soil) concentration appears to be sluggish (lack of alertness) on day 7 and day 14.

Based on the test results, the LC_{50} of 2-chloroacetamide for earthworms observed for a period of 14 days was found to be 44.58 mg/Kg (dry weight of artificial soil) with lower confidence limit of 41.10 mg/Kg (dry weight of artificial soil) and the upper confidence limit of 48.47 mg/Kg (dry weight of artificial soil). The NOEC and LOEC was found to be 25.60 mg/Kg (dry weight of artificial soil) and 40.96 mg/Kg (dry weight of artificial soil) respectively.

ANNEXURE-II
STUDY PLAN

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

STUDY PLAN

STUDY No.: CSIR-IITR/GLP/397

EARTHWORM, ACUTE TOXICITY TEST OF DRAYNZYME

TEST ITEM: DRAYNZYME

SPONSOR
PUNE MUNICIPAL CORPORATION
SHIVAJI NAGAR, PUNE 411005

TEST FACILITY
VISHAKTATA PARIKSHAN: GLP ANUROOP SUVIDHA
CSIR-INDIAN INSTITUTE OF TOXICOLOGY RESEARCH
CRK CAMPUS, GHERU, SAROJINI NAGAR INDUSTRIAL AREA
KANPUR ROAD, LUCKNOW-226008
INDIA

Study No. CSIR-IITR/GLP/397
Copy No. 1/2
Page 1/13

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

TABLE OF CONTENTS

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

VishaktataParikshan: GLP AnuroopSuvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

1. **STUDY DETAILS**

Study Title	:	Earthworm, Acute Toxicity Test of Draynzyme
Test Item	:	DWE/2005A-1KG ILD Draynzyme
Study Number	:	CSIR-IITR/GLP/397
Sponsor	:	Pune Municipal Corporation Shivaji Nagar, Pune 411005
Sponsor's Representative	:	Mangesh Dighe Environment Officer, Pune Municipal Corporation E Mail: indradhanushya@punecorporation.org
Test Facility	:	Vishaktata Parikshan: GLP Anuroop Suvidha CSIR-Indian Institute of Toxicology Research CRK Campus, Gheru, Sarojini Nagar Industrial Area Kanpur Road, Lucknow-226008, India
Study Director	:	Ms Vijaya Shree Ecotoxicology Laboratory Vishaktata Parikshan: GLP Anuroop Suvidha CSIR-Indian Institute of Toxicology Research CRK Campus, Gheru, Sarojini Nagar Industrial Area Kanpur Road, Lucknow-226008, India Contact Details: +91-522-2476051, +91-7052335668 E Mail: vijayashree0208@gmail.com
Study Personnel	:	Ms Priya Maurya Mr Sudhanshu Mishra
Study Schedule **Study Start Date**	: :	 05/02/2025
Range finding Study **Experiment Start Date** **Acclimatization** **Dosing** **Day 7 Observation** **Day 14 Observation** **Experimental Completion Date**	: : : : : : :	 06/02/2025 06/02/2025 07/02/2025 14/02/2025 21/02/2025 21/02/2025
Main Study/Limit Test **Experiment Start Date** **Acclimatization** **Dosing**	: : : :	 24/02/2025 24/02/2025 25/02/2025

Vishaktata Pankshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

Day 7 Observation	:	04/03/2025
Day 14 Observation	:	11/03/2025
Experimental Completion Date	:	11/03/2025
Study Completion Date	:	DD/04/2025

2. **QUALITY ASSURANCE**
The Quality Assurance Unit of the Test Facility will inspect the study, the raw data, the draft and final reports. Findings of all inspections will be reported to the Management and to the Study Director. The details of phase inspected, Inspection dates and reporting dates will be entered as QA-statement in the study report.

The Quality Assurance Unit has reviewed the study plan and will receive a copy thereof.

3. **STUDY COMPLIANCE**
The study will be performed in accordance with the following:

- OECD Principles of Good Laboratory Practice for the testing of chemicals as specified by International [C (97) 186/Final] Legislation.
- OECD Test Guideline No. 207 – Earthworm, Acute Toxicity Test.
- The mutually agreed study plan and the Standard Operating Procedures of the test facility (CSIR-IITR/ECO/002, Revision 04).

4. **AMENDMENT PROCEDURES**
This study plan may be amended or subjected to alterations. In each case, any amendment to the approved study plan and the reasons for such amendments will be documented and realized only after written / e mail consent from the study Sponsor and review by the Quality Assurance Unit and Test Facility Management. If immediate action is necessary, verbal agreement from the Sponsor will be confirmed as soon as possible by study plan amendment followed by written consent from the sponsor. Minor changes (unplanned) of the study plan which do not influence the procedures, or the outcome of the study may be subject

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

to the discretion of the Study Director but will be mentioned in the report as deviations.

5. SAFETY PRECAUTIONS

Gloves, face mask and goggles (if required) will be used in addition to protective body garments and shoes to ensure adequate personal health and safety. In case of eye contact, the eye will be washed thoroughly with water and medical treatment will be sought. In case of skin contact, it will be washed with soap and water with subsequent medical aid.

6. OBJECTIVE

The purpose of the study is to determine the acute toxicity potential of test item, DWE/2005A-1KG ILD Drayzyme by artificial soil test method to the earthworm, *Eisenia fetida*. Results of a recent reference study will be attached in the final report towards the responsiveness of test system.

7. MATERIALS AND METHODS

7.1 Materials

7.1.1 Test Item Information

(As furnished by the Sponsor)

Test Item	:	DWE/2005A-1KG ILD Drayzyme
Common Name	:	Not Provided
Chemical Name	:	Not Provided
Accession Number	:	Not Provided
Batch / Lot Details	:	01/01/24
Date of Manufacture	:	January,2024
Date of Expiry	:	January,2026
Physical Appearance	:	Mustard yellow gel encapsulated inside polyster sack
Recommended storage	:	Store away from heat
Purity	:	Not Provided
Solubility	:	Slowly dissolve into following water over a long period of time
Intended Usage	:	Treatment of contamination created by raw sewage exposure
Manufacturer By	:	Dhara Biotech, Nr, Gaushala, Sarsa – Vasad Chokdi, Bhalej Road, Sarsa Anand, Gujrat - 388365
Supplied By	:	Quin Quent Industries Industries Pvt Ltd

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

		S. No. 131/2A Rajyog Colony Warje Pune- 411052

7.1.2 Identity of the Test Item

The Test Item information Sheet (TIIS), Certificate of Analysis (CoA) and Material Safety Data Sheet (MSDS) has been provided by the sponsor. The responsibility for the correct identity and purity of the test item rests with the sponsor. The authenticity of the test item was not being conducted at the test facility.

7.1.3 Test System & Test Conditions

Species	:	*Eisenia fetida*
Age	:	Adult earthworms with an individual weight of 300 to 600 mg.
Justification for the selection of species	:	Recommended by the regulatory guideline (OECD) for the terrestrial toxicity assessment.
Source	:	Earthworm culture, Ecotoxicology Lab CRK Campus, CSIR-Indian Institute of Toxicology Research, Lucknow.
Test Room Details	:	Room No: 46, 47 & 48
Test Method	:	Artificial Soil Test
Test Vessel	:	1litre crystallizing glass jars covered with lid.
Number of Replicates	:	**Range Finding Study:** 4 replicates for each test concentration and control. **Main Study:** 4 replicates for each test concentration and control.
Light	:	Continuous light (400-800 Lux)
Test Medium	:	Artificial soil - 500g/replicate (10% Peat, 20% Kaolin clay, 70% Industrial sand)
Test Medium pH Range	:	5.50 - 6.50 (Measured at the start of the test)
Test Duration	:	**Range Finding Study** Acclimatization: 01 day Dosing & Observation: 1 day dosing in soil followed by 14 days observation. **Main Study** Acclimatization: 01 day

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

	Dosing & Observation: 1 day dosing in soil followed by 14 days observation.

7.2 Methods

7.2.1. Artificial Soil Preparation

Artificial soil is a mixture of 10 percent sphagnum peat, 20 percent kaolin clay and 70% industrial sand. The dry constituents will be blended in the correct proportions and hand mixed thoroughly and stored for use. pH of the medium will be adjusted to 6.0 ± 0.5 (if needed) by addition of calcium carbonate. The dry mixture will be moistened before use but not so wet that water appears when the artificial soil is compressed.

7.2.2. Moisture Content

Moisture content will be determined in all test concentration replicates including control during range finding and main study at the beginning and end of the test. Determined quantity of distilled water will be added to give an overall moisture content of about 35 percent of the dry weight.

A clean oven dried glass beaker will be taken, and its weight will be taken approximately 10 g of soil will be dried in thermostatically controlled oven with a temperature range of 105 ± 2°C for approximately 3 hours. The beaker will be placed in a desiccator and the moisture content of the soil is calculated as a percentage of the dry soil weight.

Moisture content (%) = (W2-W3) / (W3-W1) x 100
Where,
W1 = Weight of the glass beaker (g)
W2 = Weight of the moist soil + glass beaker (g)
W3 = Weight of the dried soil + glass beaker (g)

7.2.3 Acclimatization of Earthworm

Before start of the experiment, enough earthworms with well-developed clitellum will be collected from the stock culture, washed with distilled water, and acclimatized for minimum of one day in artificial soil. Bodyweight of minimum 10 earthworms will be weighed and recorded on the day of acclimatization.

7.2.4 Earthworm Weighing

Prior to the release of earthworms on the test medium surface, body weight of 10 earthworms per replicate will be checked and recorded in

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

the raw data. Earthworm weighing between 300 and 600 mg will be released into the test vessel containing test medium.

7.2.5 **Vehicle**
The test item is not soluble in distilled water and solvents, then the required quantity of test item will directly mix in the artificial soil and thoroughly mixed.

7.2.6 **Test Item Preparation**
Required quantity of test item will be added to the respective test vessel directly. Adequate volume of distilled water will be added to each replicate test vessel containing the test item to moistened the soil. The contents were thoroughly mixed with the water and the details of test item preparation for exposure will be recorded in the raw data file and mentioned in the study report.

7.2.7 **Treatment**
During range finding and main study/limit test, four replicates will be maintained for each test concentration including control and each replicate will contain 10 earthworms. For each replicate, 500g of the artificial soil will be placed in the respective test vessel. Ten earthworms in the body weight ranges between 300 and 600 mg will be placed on the test medium surface. The test containers will be covered with lid to prevent the test medium from drying and will be kept under the test conditions for 14 days.

7.2.8 **Range Finding Study**
A preliminary range finding will be conducted by exposing the earthworms to 0.1, 1.0, 10, 100 and 1000 mg/kg concentrations of Urja Infra in artificial soil along with control groups.

7.2.9 **Main Study**
Based on the cumulative mortality obtained from the range finding study, main study will be conducted by exposing the earthworms to at least five different test concentrations in a geometric series with a separation factor preferably not exceeding 2.0. The details of test concentrations selected for the main study will be mentioned in the raw data and study report.

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

7.2.10 Limit Test

If no earthworm mortality is noted during the range finding study at all the test concentrations, then the study will be restricted to limit test. During limit test, earthworms will be exposed only to the highest test concentration of 1000 mg/Kg DWE/2005A-1KG ILD Drayzyme along with control and solvent control (if used) groups.

8. OBSERVATIONS

pH of the blended artificial soil will be recorded on the day of acclimatization and in all test concentration replicates including control on the day 0 of treatment during range finding and main study.

Moisture content of artificial soil will be checked on day 0 and day 14 in all test concentration replicates including control during range finding and main study.

Light intensity will be recorded on acclimatization and everyday (day 0 to day 14) during the range finding and main study.

Mortality and toxicity signs will be recorded on day 7 and 14 after treatment. The mortality will be assessed by emptying the test medium onto a tray, sorting worms from the medium and testing their reaction to a mechanical stimulus at the front end. After the 7th day assessment, worms and test medium will be replaced in the corresponding test vessel for another 7 days. Toxicity symptoms such as sluggish, rigidity, sores, coiling, fragmentation, and dead will be observed and recorded in the raw data.

Body weight of live earthworms will be recorded on day 0 and day 14 in control and treatment groups during the range finding and main study.

9. VALIDITY CRITERIA

The mortality in controls should not exceed 10 percent at the end of the test.

10. STATISTICAL ANALYSIS

The mortality/concentration data will be used to calculate the median lethal concentration (LC_{50}) and its confidence limits from main study data. Finney's Probit Analysis will be applied to calculate the LC_{50} with 95% confidence limits and graph showing concentration/effect curve will also be plotted. No Statistical analysis will be performed for the range

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

finding study and limit test. SPSS software (Version 25) will be used to for the probit analysis.

11. DATA COMPILATION

Data will be summarized in a tabular form, the number of earthworms used, average live weight and number of live worms per treatment at start and end of the experiment. Soil pH, light intensity, moisture content and mortality data will also be tabulated.

12. FINAL REPORT

The final report will be prepared in compliance to the principles of GLP and normally include, but not limited to the following:

- A descriptive title.
- The name and address of the Sponsor and the test facility along with the details of study schedule.
- The names of all personnel involved in the study.
- A compliance statement signed by the Study Director that all applicable GLP regulations were followed in the conduct of the study.
- Quality Assurance (QA) statement; that states that the report accurately reflects the raw data obtained during the performance of the study and including the dates of QA activities and the dates reported to study director and management.
- The Test Item and its code, composition and other appropriate characteristics and vehicle with identification by name.
- Complete description of the test system including species, source, number, test conditions, photoperiod, and acclimation.
- Statistical analysis of the results (if applicable).
- Method of preparation of stock and test solutions.
- Graph of the concentration mortality curve at the end of the test.

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

- LC_{50} values, with 95% confidence limits at each of the recommended observation times (day 7 and 14), if applicable.
- Highest concentration causing no mortality, lowest concentration causing 100% mortality.
-
- Moisture content of artificial soil at the start and end of the test, pH at start of the test, light intensity details etc.
- A description of the results; discussion and conclusion.
- A description of all study plan deviations, if any.
- A description of all circumstances that may have affected the quality or integrity of the study.
- The storage locations of all raw data, specimens, reports, Test Item reference sample and the archiving period.

13. **ARCHIVING**
The following will be archived at the test facility for at least 9 years (3 cycles of GLP) after completion of the study: study plan, all raw data, draft and final reports, a representative sample of Test Item (approximately one gram), etc. Before discarding of any archived study materials, the Sponsor will be contacted for the disposal.

14. **STUDY PLAN DISTRIBUTION**
The final study plan (original copies) will be distributed as follows:
Test Facility: One signed study plan in original (Copy No. 1/2)
Sponsor : One signed study plan in original (Copy No. 2/2)
Document Control: One controlled copy
Quality Assurance Unit: One controlled copy
Study Personnel: One controlled copy

15. **REPORT DISTRIBUTION**
The study report will be distributed as follows:
Test Facility : One signed final report in original (Copy No. 1/2) and an electronic copy in the PDF format.
Sponsor : One signed final report in original (Copy No. 2/2)

Vishaktata Parikshan: GLP Anuroop Suvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

16. AGREEMENT

This study plan for Study No.: CSIR-IITR/GLP/397, "Earthworm, Acute Toxicity Test of Draynzyme" has been mutually agreed:

for **TEST FACILITY**	for **STUDY SPONSOR**
1. _____	1. _____
STUDY DIRECTOR	SPONSOR REPRESENTATIVE
Date: 05/02/2025	Date:
	Email Consent Received on: - 07/02/2025
2. _____	
QUALITY ASSURANCE UNIT	
Date: 05/02/2025	
3. _____	
TEST FACILITY MANAGEMENT	
Date: 05/02/2025	

ANNEXURE – III
CERTFICATE OF ANALYSIS

Green Leaf

Test Report
Water Sample Analysis Report

Client's Name & Address: **M/s. Dhara Bio-tech** Near SarasChokdi, Near Gaushala, Kunjrav Road, Sarsa, Anand 388365, Gujarat. **Contact Person:** Mr. Vasantlal Patel	**Report No:** GLEPL/240124/01 **Issue Date:** 01/02/2024

Lab ID Code	:	GLEPL/240124/WL1			
Sample Description	:	Product Sample	Purpose	:	As per Client requirement
Date of Sampling	:	Submitted by Client (Dhara Bio-tech)	Sample collected/Submitted by	:	Submitted by Client (Dhara Bio-tech)
Date of Sample Received	:	24/01/2024	Test Parameters	:	As per Client requirement
Date of starting Analysis	:	25/01/2024	Quantity/No. of sample	:	1No. Batch No. 01/01/24
Date of completion Analysis	:	31/01/2024	Packed/Seal	:	Sealed (13/01/24)

Result Table

Sr. No.	Test Parameters	Test Method	Unit	Results
1	Drayzyme for Bacteria Count	ISO 16649-3	MPN/100 mL	Absent

Remark: In accordance to G.S.R 613(E)-This product does not contain any Bacteria, thus does not require 'Appraisal by genetic Engineering Appraisal Committee).

Chemist

Authorized Signatory
Rekha Dare

Notes: (1) The results pertain to tested items only.
(2) This report shall not be reproduced, except in full, without written approval of the laboratory.
(3) Authenticity of this Report could be validated with office copy at Greenleaf Evirotech Pvt. Ltd.
(4) Perishable samples will be destroyed after testing, others after 7 days from the date of issue of the report, unless otherwise agreed with the customer or as required by the applicable regulations.

CIN: U74140GJ2010PTC059798
GreenleafEnvirotechPvt. Ltd., Nr. RangoliFlats,Radhanpur Road, Mehsana – 384002. Gujarat, India.
Tel : +91-9725519974, E-mail: info@glepl.com, Web: www.glepl.com
Branch Office: 304, Kankavati Complex, Singanpor-Causway Road, Katargam, Surat– 395004

VishaktataParikshan: GLP AnuroopSuvidha
Toxicity Testing: GLP Test Facility, CSIR-IITR, India

ANNEXURE – IV
TEST SYSTEM CHARACTERIZATION CERTIFICATE

PG & RESEARCH DEPARTMENT OF ZOOLOGY
THE NEW COLLEGE (Autonomous)
Sponsored by: THE MUSLIM EDUCATIONAL ASSOCIATION OF SOUTHERN INDIA
(AFFILIATED TO THE UNIVERSITY OF MADRAS & ACCREDITED BY NAAC WITH 'A' GRADE)
"Association Gardens", 87, Peters Road, Royapettah, Chennai - 600 014. India.
Phone : 2835 1269, 2835 0386 Fax : 044 - 2835 2883

M. Saiyad Musthafa, M.Sc., M.Phil.,
Assistant Professor

Date 20.03.2015

Identification Certificate

This is to certify that the specimens sent by **Dr. Anbumani Sadasivam**, Scientist, Ecotoxicology Division of CSIR-Indian Institute of Toxicology Research (IITR), Lucknow belongs to ***Eisenia fetida*** (Savigny, 1826). The specimens are identified and authenticated based on the following external morphological characters:

- Segments 80-120;
- first dorsal pore between segments 4/5 (sometimes 5/6);
- clitellum over segments 24,25, 26-32;
- tubercula pubertatis on segments 28-30;
- seminal vesicles, four pairs on in 9-12;
- spermathecae, two pairs in 9/10 and 10/11

CLASSIFICATION

Kingdom	:	Animalia
Subkingdom	:	Eumetazoa
Phylum	:	Annelida
Class	:	Oligochaeta
Family	:	Lumbricidae
Genus	:	*Eisenia*
Species	:	*fetida*

M. SAIYAD MUSTHAFA, M.Sc., M.Phil.,
ASSISTANT PROFESSOR
P.G. & RESEARCH DEPT. OF ZOOLOGY
THE NEW COLLEGE (AUTONOMOUS)
CHENNAI - 600 014

2/24 Muthamil Nagar, Thalakkudi, Lalgudi Taluk, Tiruchirappalli - 621 216, India.
E-mail : saiyad_musthafa@rediffmail.com 99651 24466

ANNEXURE – V
GLP Certificate

National Good Laboratory Practice (GLP) Compliance Monitoring Authority (NGCMA)
Department of Science and Technology
GOVERNMENT OF INDIA

Certificate of GLP Compliance

This is to certify that

Toxicity Testing: GLP Test Facility, CSIR-Indian Institute of Toxicology Research
CRK Campus, Gheru, Sarojini Nagar Industrial Area
Lucknow-226008, Uttar Pradesh (India)

is a GLP certified test facility in compliance with the NGCMA's Document No. GLP-101 "Terms & Conditions of NGCMA for obtaining and maintaining GLP certification by a test facility" and OECD Principles of GLP.

The test facility conducts the below-mentioned tests/ studies:

- **Toxicity Studies**
- **Mutagenicity Studies**
- **Environmental Toxicity Studies on Aquatic and Terrestrial Organisms**
- **Analytical and Clinical Chemistry Testing**

The specific area(s) of expertise, test item(s) and test system(s) are listed in the annexure overleaf.

Validity: June 5, 2023 – June 4, 2026

Certificate No. : GLP/C-213/2023
Issue Date : 07-12-2023

(Dr. Ekta Kapoor)
Head, NGCMA

Technical Review Report on “Drynzyme”

Reviewer:
Dr. Banwari Lal, PhD

Former Senior Director TERI, New Delhi

---

1. Background

I have reviewed the Technical Report on “Drynzyme” provided by the manufacturer. The manufacturer claims that Drynzyme is a cocktail of enzymes intended for:

- Treatment of sewage water
- Treatment of wastewater in drainage systems
- Remediation of contaminated river water
- Control of water hyacinth growth in ponds and stagnant water bodies

The product is being used by Municipal Corporations and other user industries for large-scale environmental applications.

---

2. Claims Made by the Manufacturer

According to the technical report, Drynzyme contains multiple enzymes, including:

- Urease
- Xylanase
- Cellulase
- Amylase
- Ammonia monooxygenase
- Acid phosphatase
- Lipase
- Protease

The functions of these enzymes, as described in the report, appear to have been compiled from published scientific literature available in the public domain. However, no product-specific experimental validation data are provided.

---

3. Scientific Concerns

3.1 Enzyme Production and Purification

The manufacturing process described for Drynzyme is vague and scientifically inadequate. Critical details are missing, including:

- Source organism(s) used for enzyme production
- Fermentation conditions
- Enzyme extraction procedures
- Purification methods
- Stabilization strategy in final formulation

Without these details, the scientific validity of the product cannot be assessed.

3.2 Formulation Inconsistency

The manufacturer states that Drynzyme contains:

- 70% hydrated silica
- 29% lignocellulosic material
- 15% enzymes

The total percentage equals 114%, which is mathematically incorrect and scientifically unacceptable. This raises serious concerns regarding formulation accuracy and documentation integrity.

Furthermore:

- Hydrated silica and lignocellulosic material are not water-soluble.
- IITR reports that Drynzyme is water-soluble and prepared as a stock solution in water for toxicity test.

This discrepancy requires clarification.

3.3 Lack of Enzyme Activity Data

The manufacturer has not provided:

- Specific activity (U/mg or equivalent) of each enzyme
- Total enzyme activity per gram of formulation
- Units of enzyme activity
- Stability profile under storage conditions
- Shelf-life of each enzyme
- Stability of enzyme activity in the formulated product

Without quantitative enzyme activity data, the claim that Drynzyme is a functional enzyme cocktail cannot be scientifically validated.

3.4 Physical Form Uncertainty

The product is described as:

- A solid material
- Packed in polyester bags

However, it is unclear:

- Drynzyme is powder, but enzymes used in Drynzyme are powder or liquid
- How enzyme activity is preserved in this matrix
- How enzyme release occurs in aqueous systems

---

4. Review of IITR Toxicity Testing

I have reviewed the IITR test protocol and test reports. It appears that:

- Standard protocols were used
- GLP laboratory practices were followed

However, major concerns remain:

1. It is unclear whether IITR verified that the tested sample actually contained the enzymes claimed by the manufacturer.
2. There is no indication that enzyme composition or activity was confirmed before toxicity testing.
3. The physical form of the Drynzyme is solid, however silica and lignocellulose material in Drynzyme are not water soluble, and how the stock solution of non-water soluble Drynzyme was prepared, it is not clearly described.

Most importantly, IITR does not appear to have certified that the tested material was a verified enzyme-based formulation.

---

5. Key Scientific Gap

Before conducting toxicity studies, the following should have been established:

1. Whether Drynzyme contains all listed enzymes
2. Functional activity of each enzyme
3. Units and measurable activity levels

4. Stability and shelf-life of enzyme activity
5. Batch-to-batch consistency

Ideally, IITR should have:

- Collected independent samples from municipal corporations using the product
- Verified enzyme composition and activity
- Then conducted toxicity evaluation

Without verification of product identity and functionality, toxicity testing alone is insufficient to validate environmental safety.

---

6. Conclusion

Based on the information provided:

- Drynzyme does not appear to be a scientifically validated enzyme cocktail.
- The formulation data are inconsistent and mathematically incorrect.
- Enzyme activity and stability data are absent.
- There is no confirmation that the product tested for toxicity actually contains the claimed enzymes.

Therefore, the toxicity tests conducted by IITR cannot be considered fully valid in the absence of product characterization and enzyme activity verification.

---

7. Recommendation

In view of the lack of scientific data regarding:

- Enzyme production
- Purification
- Activity units
- Stability
- Verified composition

I strongly object to the large-scale application of Drynzyme in natural ecosystems for wastewater treatment, river remediation, or water hyacinth control until comprehensive scientific validation is conducted.

Large-scale environmental application without proper biochemical characterization and ecological safety assessment poses potential risks to natural ecosystem

Expert Comments on the Use of “Draynzyme” in Natural Water Bodies

Submitted by:
Prof. Rup Lal
INSA Senior Scientist

Background and Context

I have gone through the documents provided. The present comments are based on my critical examination of toxicity assessment reports submitted by CSIR–Indian Institute of Toxicology Research (IITR), information provided by the manufacturer regarding the composition and claimed functionality of the product “Draynzyme,” and the context of its proposed or trial use in natural water bodies.

Concerns Regarding Product Composition and Characterisation

The manufacturer describes Draynzyme as an enzyme-based formulation comprising multiple enzymes. However, no product-specific experimental data have been provided to conclusively demonstrate enzyme presence, stability, or activity under environmental conditions.

Inconsistencies in Formulation Details

Declared constituents exceed 100%, and the presence of insoluble materials contradicts aqueous testing protocols. These inconsistencies raise serious concerns regarding product transparency and quality control.

Evaluation of IITR Toxicity Studies

The IITR studies primarily focus on acute toxicity endpoints. While useful as an initial screening, such tests are insufficient to assess ecological safety in complex natural water bodies, particularly without independent verification of product composition.

Ecological and Environmental Considerations

Introduction of enzyme-based or undefined biological additives into natural ecosystems may alter nutrient cycling and microbial community structure. No long-term ecological impact data have been provided.

Regulatory and Scientific Gaps

Independent biochemical characterisation, enzyme activity validation, batch-to-batch consistency analysis, and chronic ecological risk assessments are required before environmental application can be considered.

Conclusions

Based on the available information, Draynzyme cannot presently be regarded as a scientifically validated enzyme-based remediation product suitable for application in natural water bodies.

Recommendations

Large-scale or repeated application should not be permitted until comprehensive laboratory validation, controlled field trials, and long-term ecological impact studies are completed.

348

Use of DraynZyme: Points of Concern

Atya Kapley

Prof of Practice, Raisoni Group of Institutes, Nagpur
Former Chief Scientist, CSIR NEERI

With reference to the email from Ms Deepa Kumari, Senior Scientific Assistant, CPCB to the undersigned, regarding my comments on the use of DraynZyme in rivers and lakes to control water hyacinth, the following are my concerns:

1. Water hyacinth is a sturdy free floating plant that has roots going upto 1-3 feet below water. It does not seem feasible that a collection of enzymes sprayed on the plant will be able to eradicate it from a target niche since enzymes, as proteins, have a very short shelf life without proper stabilising factors. The manufacturers information on the cocktail does not indicate any such stabilizers. Besides, enzymes work at a particular temperature and concentration that is not defined in the documents attached.
2. There are multiple enzyme inhibitors in polluted water. What is the acting point of the enzyme? What part of the plant does it act on?
3. Chemical composition of the product, DraynZyme is not provided, nor is concentration of the enzyme cocktail, nor any information of dosage required for different stages of growth of the weed.
4. Product is described as 'slowly dissolving into water over a period of time'. How can silica and lignocellulosic material dissolve?
5. The cocktail of enzymes listed may not act in synergy with one another. For example, the action of the enzyme urease increases the pH of the medium while acid phosphatase works at an acidic pH. Some enzymes are NADH- or NADPH-dependent for their action and hence not viable in cell free forms. There seems no purpose of urease in plant growth control.
6. Enzymes need time to act and hence there has to be a holding time to take effect, they require specific temperature too, even if the cocktail is compatible. The growth of the plan will overtake the bioremediation option of only spraying the bioremediating agent.
7. What is the half life of the cell-free enzymes?
8. Toxicity reports from IITR: I would like to state here that this is not my area of expertise, however, as a person who has worked in the environmental remediation field for 30 years, I would like to point out some red flags:
    - There seems to be no dose dependent study carried out, so it is not possible to discern if higher doses could be lethal or toxic.
    - No statistical analysis was done

- If, as mentioned in point 3, the product slowly dissolves, then how long was the toxicity test period?
- No long term effect of aquactic flora and fauna can be predicted with the current tests

Conclusion: The information provided is not sufficient to state if this product is safe to use in the environment. The composition of the product needs to be specified with concentrations, half life of enzymes, stabilizing material etc. And most importantly, long term toxicity is necessary to understand effect on aquatic flora and fauna.