Integrating Metagenomic Profiling and Targeted Microbial Bioremediation: A Systems Approach to Process Optimization and Circularity in Wastewater Management

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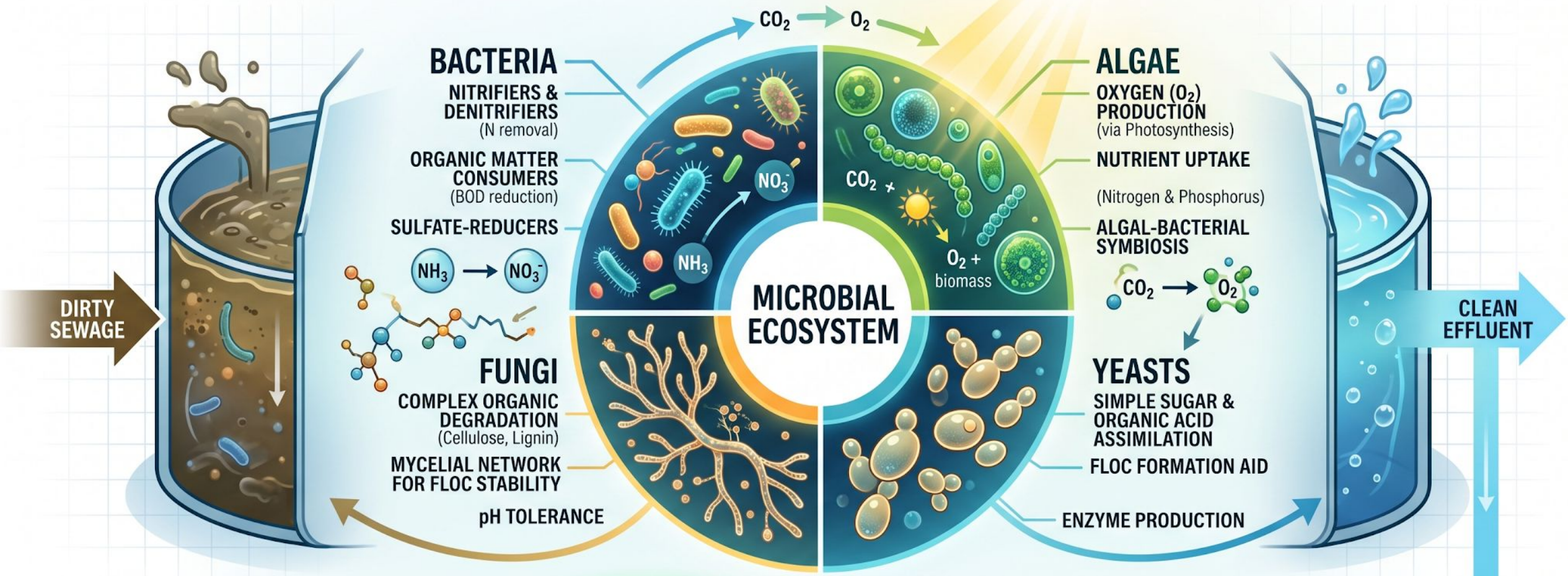
Wastewater as a resource.

Effective utilization of wastewater via microbial management..

Introduction & The Invisible Workers

- **The living ecosystem.**
- **The challenge: are we managing our microbes effectively?**
- **The Solution: microbial metagenomics and culturomics.**
- **The application: hollow ceramic beads**

A WASTEWATER ECOSYSTEM: MICROBIAL INTERACTIONS FOR EFFLUENT TREATMENT



THE EFFLUENT TREATMENT JOURNEY

1 PRIMARY INFLUENT
(Sewage)

2 BIOLOGICAL TREATMENT STAGE
Where the microbial ecosystem works
Key words: aeration, organic reduction

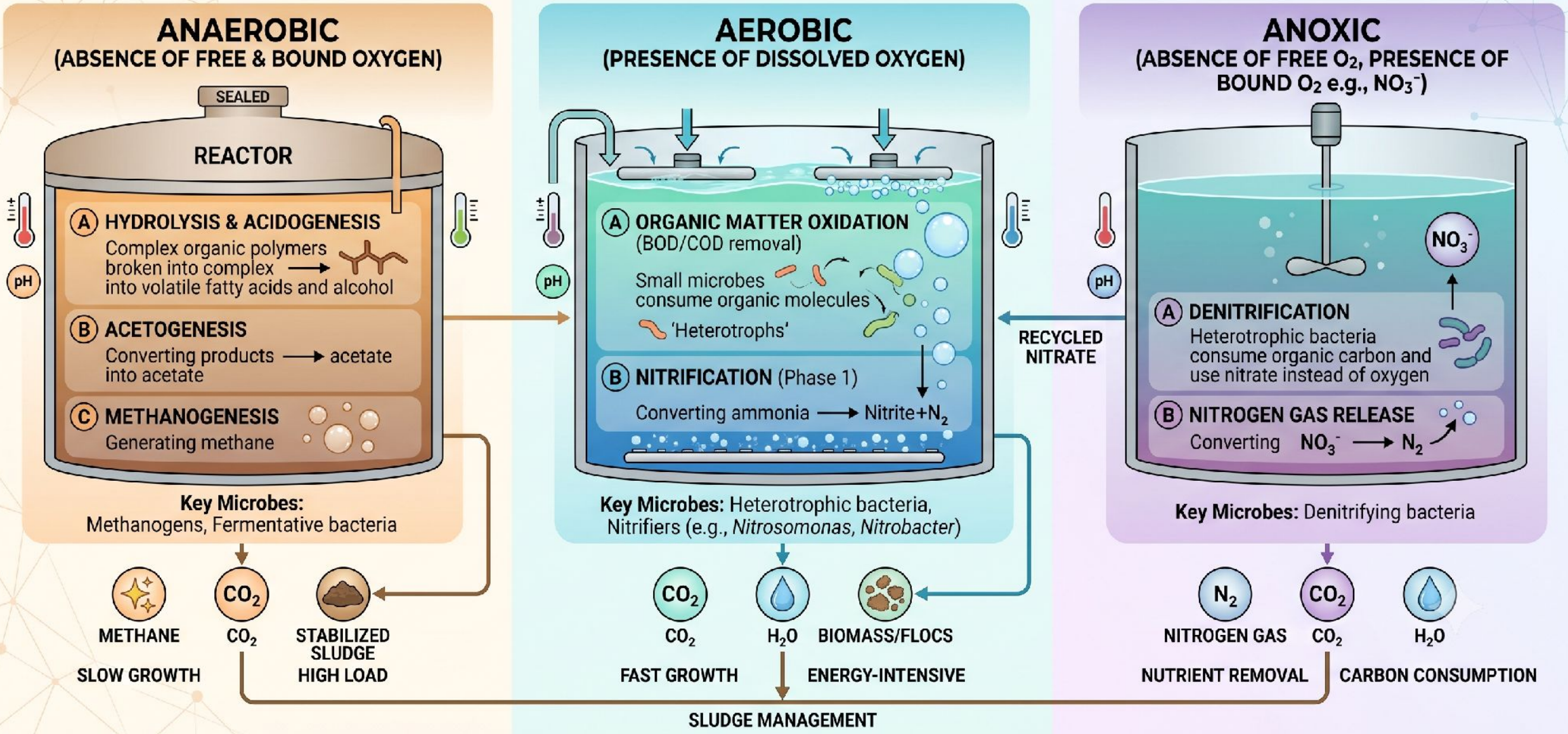
3 CLARNDARY
(Settling tank)

SECONDARY CLARIFICATION
(Settling tank)

Recirculation

4 TREATED EFFLUENT
(Clean water)

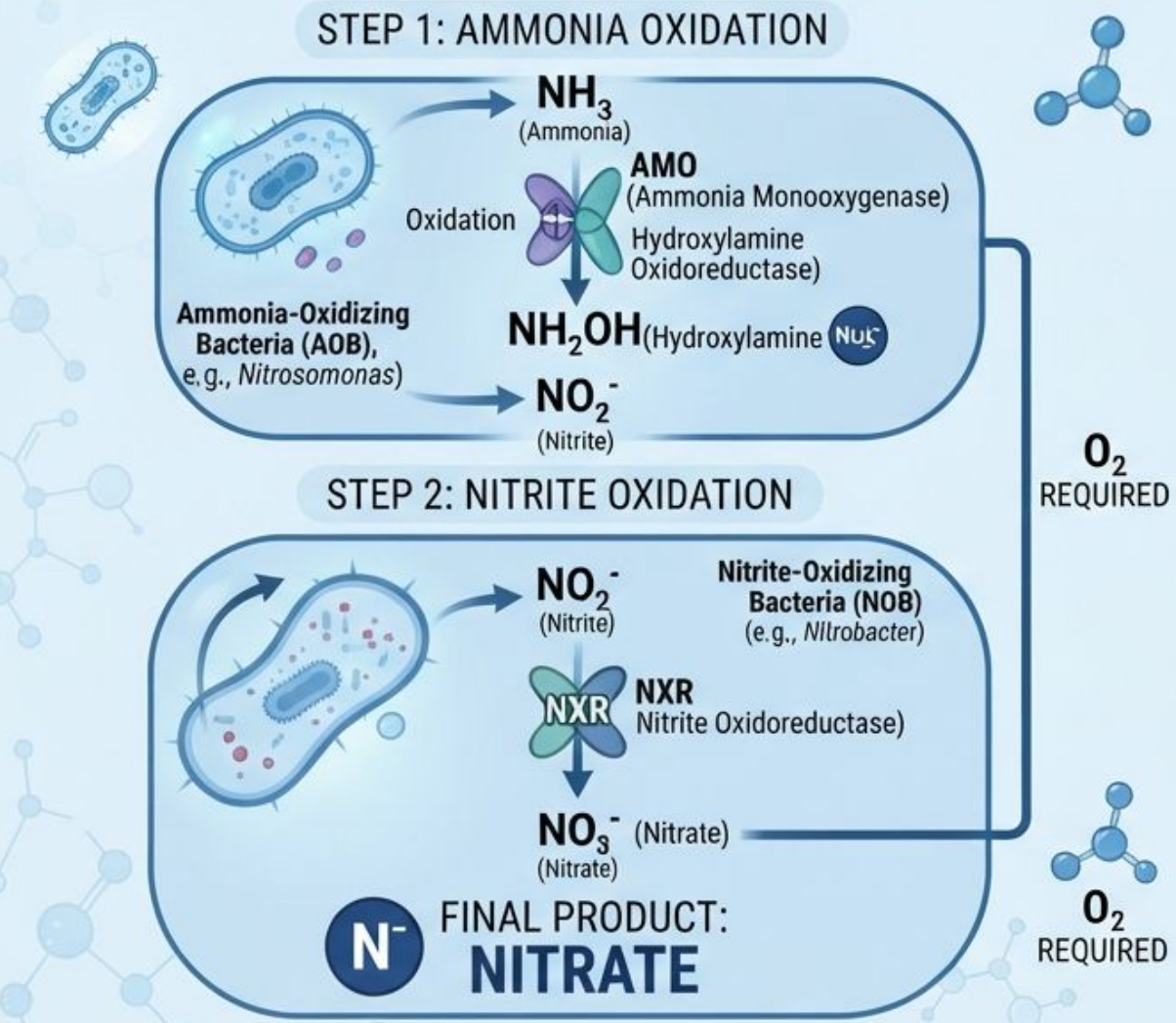
MICROBIAL WORKFLOW IN WASTEWATER TREATMENT: ANAEROBIC, AEROBIC, AND ANOXIC PROCESSES



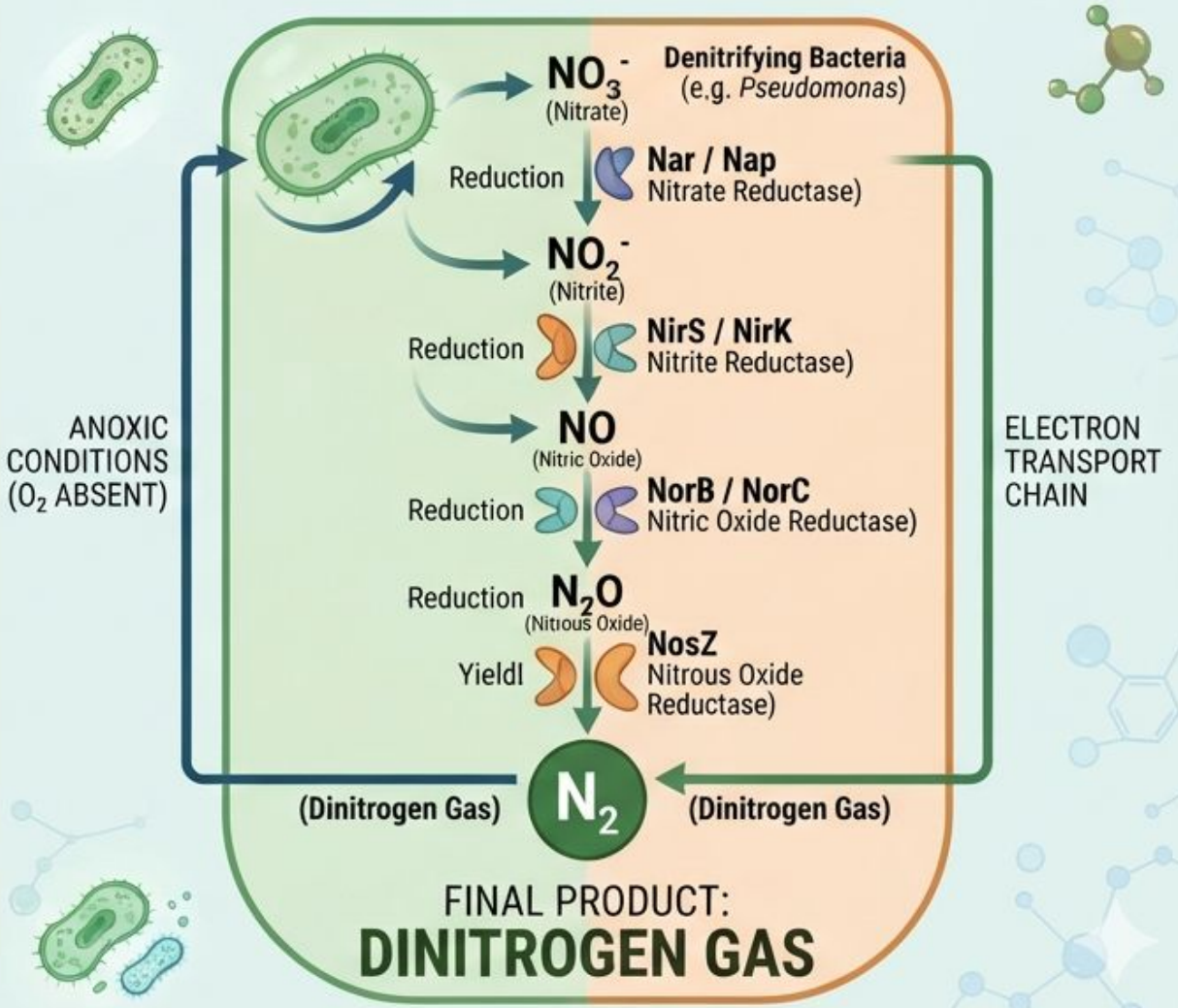
NITRIFICATION VERSUS DENITRIFICATION:

KEY ENZYMES AND END PRODUCTS

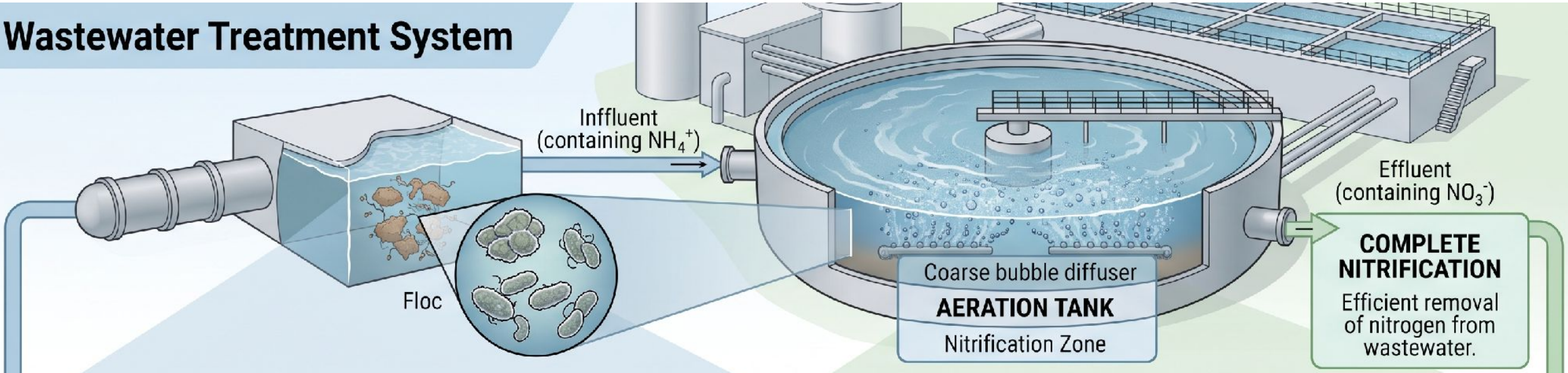
NITRIFICATION: OXIDATION PATHWAY



DENITRIFICATION: REDUCTION PATHWAY



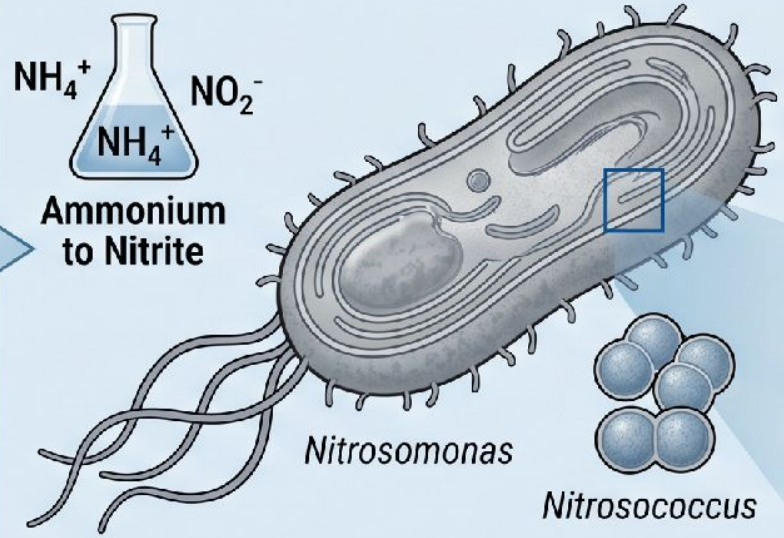
Wastewater Treatment System



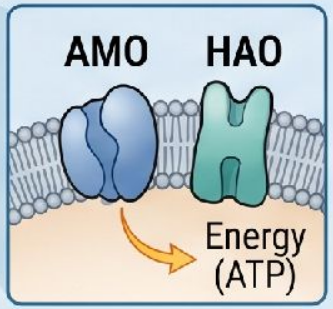
COMPLETE NITRIFICATION

Efficient removal of nitrogen from wastewater.

AMMONIA-OXIDIZING BACTERIA (AOB)

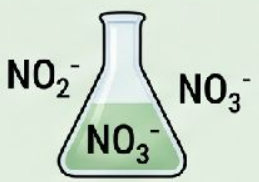


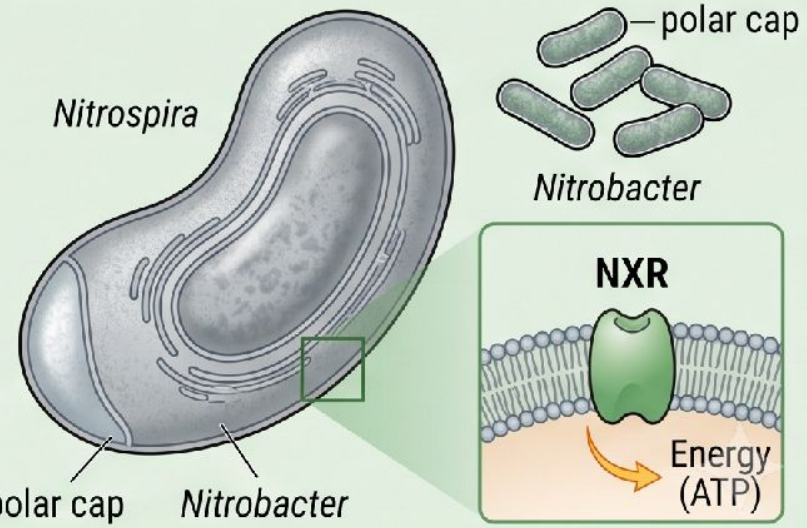
$3\ O_2$
$2\ H_2O \longrightarrow 2\ NO_2^- + 4\ H^+$



$2\ NH_4^+ + 3\ O_2 \longrightarrow 2\ NO_2^- + 2\ H_2O + 4\ H^+$ (Nitritation)

NITRITE-OXIDIZING BACTERIA (NOB)





$O_2 \longrightarrow 2\ NO_3^-$

$2\ NO_2^- + O_2 \longrightarrow 2\ NO_3^-$ (Nitratation)

Monitoring microbes

Conventional culture dependent methods

CULTURE-DEPENDENT METHOD FOR WASTEWATER MICROBIOLOGY: QUANTIFICATION OF VIABLE BACTERIA (CFU/mL)

1. SAMPLE COLLECTION & PREPARATION

A. COLLECT SAMPLE

WASTEWATER SOURCE
(e.g., Activated Sludge or Influent)

WASTEWATER SOURCE
Use sterile technique.
Mix well.

B. TRANSPORT & STORAGE

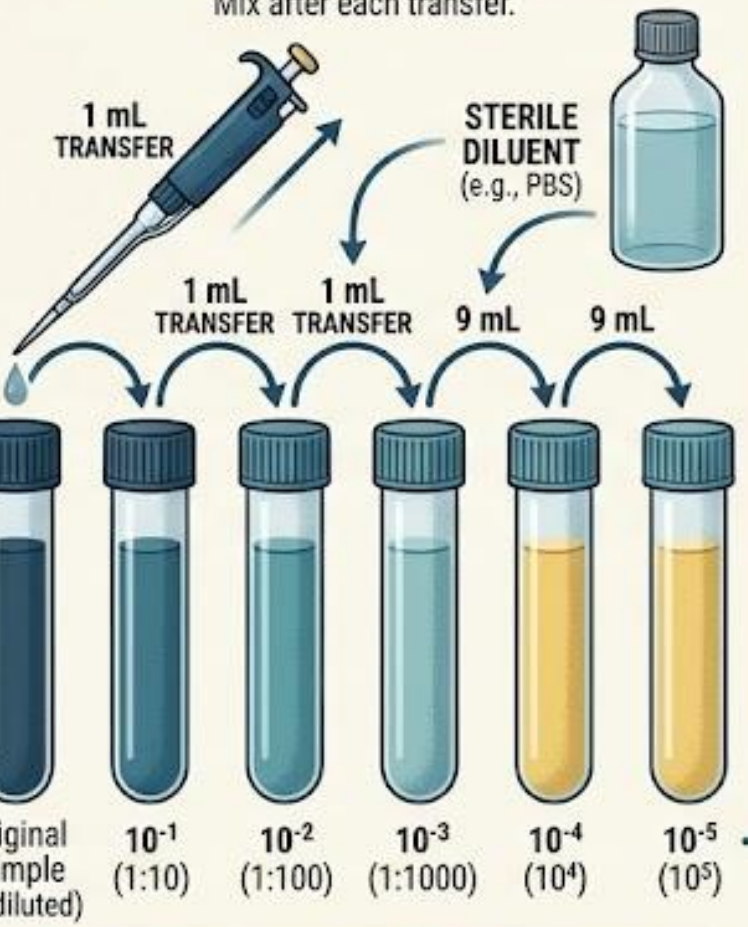
4°C, process within 24h

C. HOMOGENIZE

Original Undiluted Sample

2. SERIAL DILUTION (REDUCING MICROBIAL LOAD)

Example: 1 mL Sample into 9 mL Diluent.
Mix after each transfer.



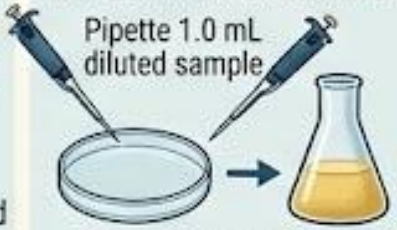
Sterile Technique Viable Cell (CFU)

3. PLATING, INCUBATION & COLONY COUNT

A. SPREAD PLATE METHOD

Predominantly for aerobes

B. POUR PLATE METHOD



C. INCUBATION

30-37°C for 24-48h
Plates inverted

D. COLONY COUNT & CFU CALCULATION

10^{-2}
Too Numerous to Count (TNTC)

10^{-4}
Countable Range (30-300 CFU)

10^{-5}
No / Low Colonies

$$\text{MICROBIAL COUNT (CFU/mL)} = \frac{\text{Number of Colonies} \times \text{Dilution Factor}}{\text{Volume Plated (mL)}}$$

Example: 120 colonies on 10^{-4} plate, 0.1 mL plated.
$(120 \times 10^4) / 0.1 = 1.2 \times 10^7$ CFU/mL


63


NA

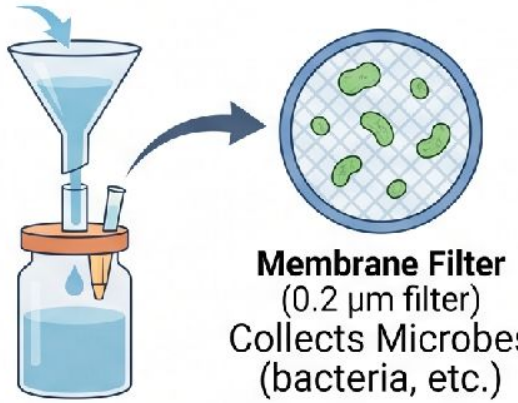
Culture Independent Methods

Metagenomics, Shotgun sequecning, Culturomics, Metabolomics

MICROBIAL METAGENOMICS: WORKFLOW FOR WATER SAMPLES

1 WATER SAMPLE COLLECTION

2 FILTRATION

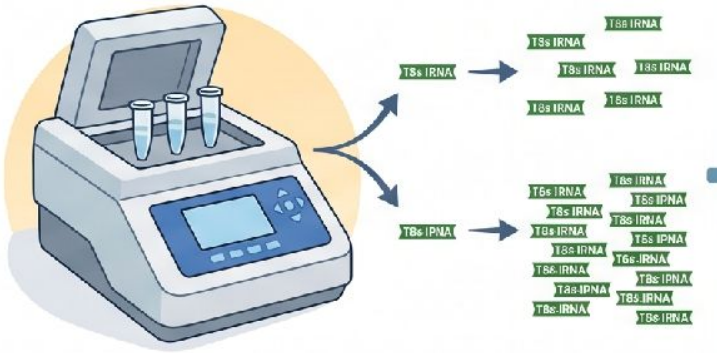


Membrane Filter
(0.2 µm filter)
Collects Microbes
(bacteria, etc.)

3 DNA EXTRACTION

ISOLATE GENOMIC DNA

4 PCR of 16s rRNA



AMPLIFY TARGET GENES (e.g., 16s rRNA)

5 DNA SEQUENCING

READ THE GENETIC CODE

6 BIOINFORMATICS

MICROBIAL IDENTIFICATION & POPULATION DIVERSITY ANALYSIS

TAXONOMIC CLASSIFICATION	ALPHA & BETA DIVERSITY
bacteria	
specnamus	
spephatis	
bacteria	
bacteria	

Which microbes? How diverse?

How do we interpret data?

- Microbial diversity
- Microbial DNA barcodes
- Copy number
- Changes in copy number across an ETP
- Changes in composition across an ETP
- Associations with water quality.

A typical profile of microbial diversity in a single water sample.

The story...

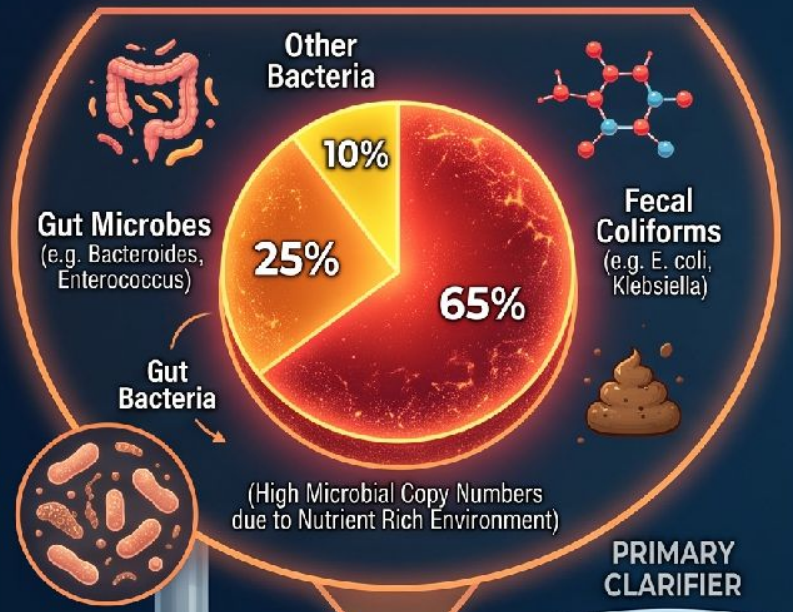
What do microbes tell us about the health of an WTP

MICROBIAL METAGENOMICS: VISUALIZING POPULATION SHIFTS ACROSS A WASTEWATER TREATMENT PLANT

1. INFLUENT (PRIMARY TREATMENT)

RAW WASTEWATER: HIGH PATHOGEN & GUT

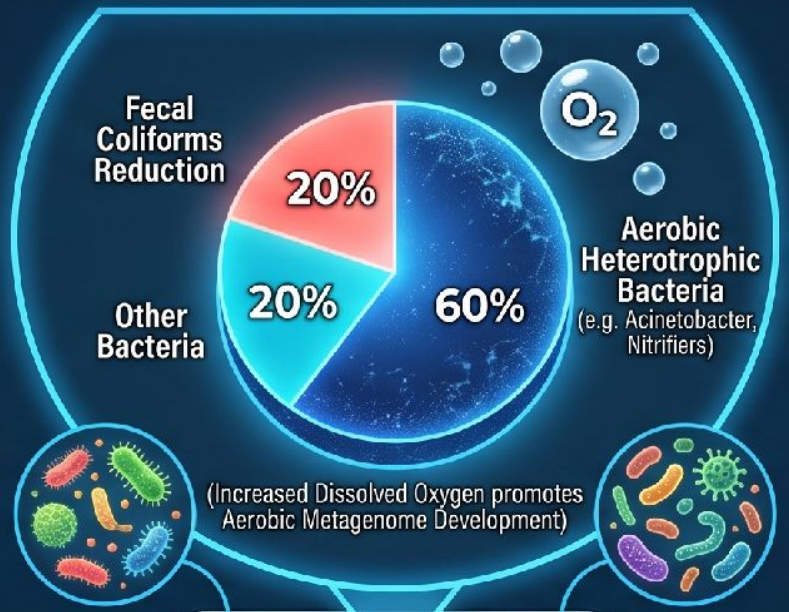
(High Microbial Copy Numbers due to Nutrient Rich Environment)



2. AERATION (SECONDARY TREATMENT)

REDUCTION & AEROBIC BACTERIA GROWTH

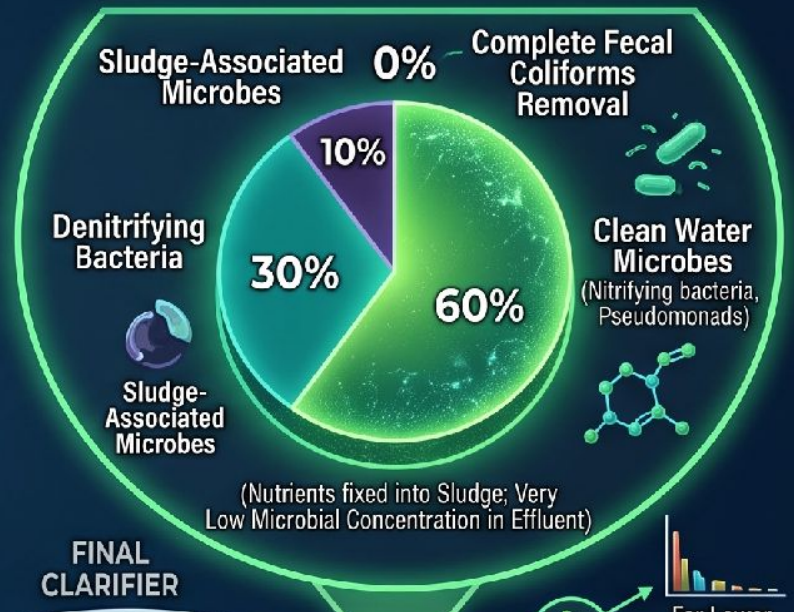
(Increased Dissolved Oxygen promotes Aerobic Metagenome Development)



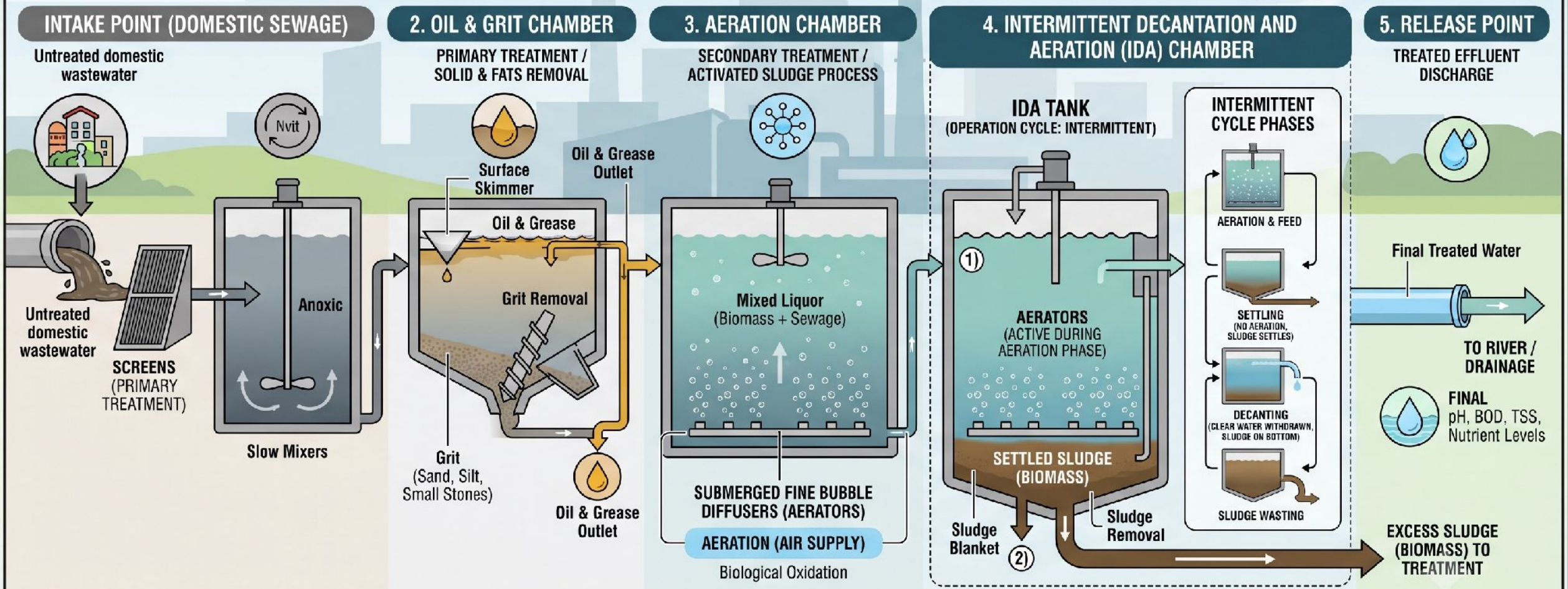
3. EFFLUENT (TERTIARY TREATMENT)

PATHOGEN REMOVAL & STABILIZED COMMUNITY

(Nutrients fixed into Sludge; Very Low Microbial Concentration in Effluent)



STANDARD EFFLUENT TREATMENT PLANT (ETP) - SCHEMATIC PROCESS FLOW



Clean
Untreated
Untreated

1. ANOXIC CHAMBER
(Nitrification/Denitrification)

2. OIL & GRIT CHAMBER
(PRIMARY TREATMENT / SOLID & FATS REMOVAL)

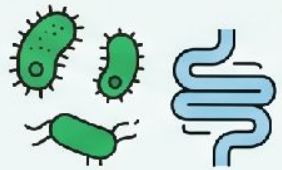
3. AERATION CHAMBER
(SECONDARY TREATMENT / ACTIVATED SLUDGE PROCESS)

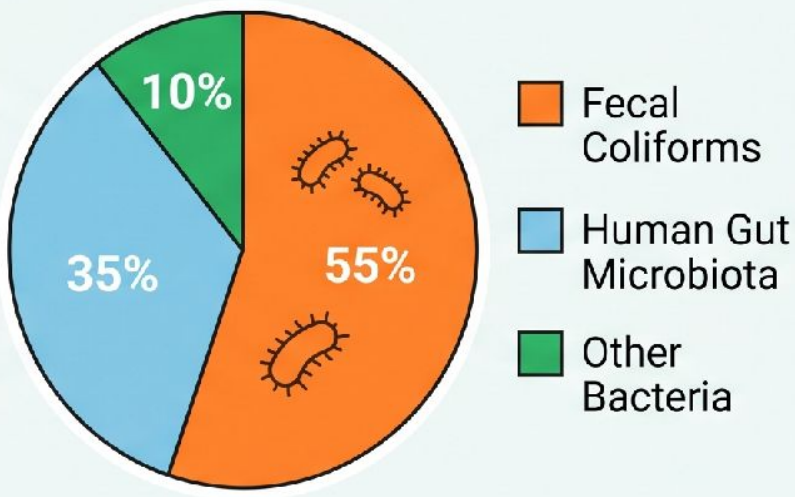
4. INTERMITTENT DECANTATION AND AERATION (IDA) CHAMBER

5. RELEASE POINT
(TREATED EFFLUENT DISCHARGE)

1 Intake Point

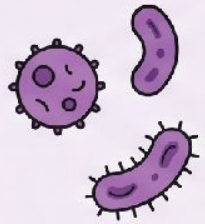
High Population of **Coliforms** and **Human Gut Microbiota**

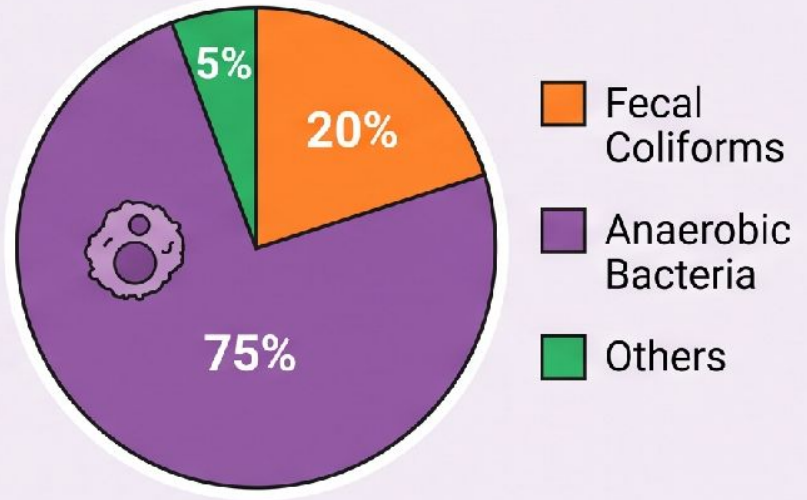




2 Anoxic Chamber

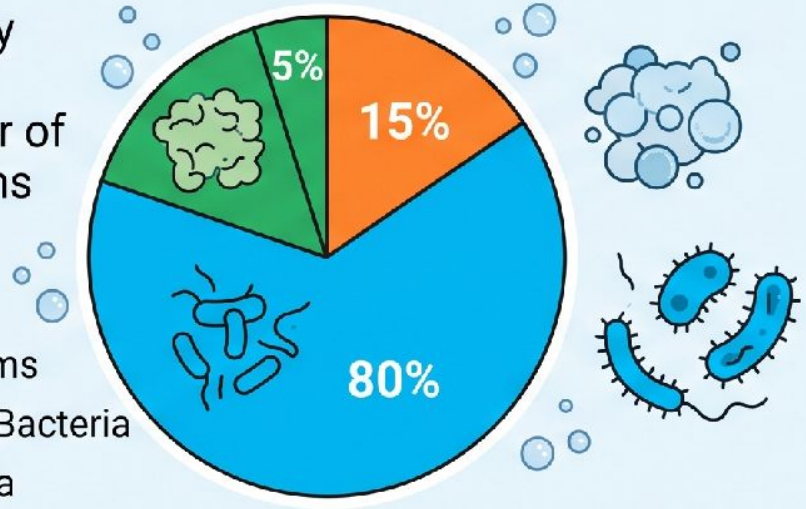
Dominated by **Anaerobic Bacteria**





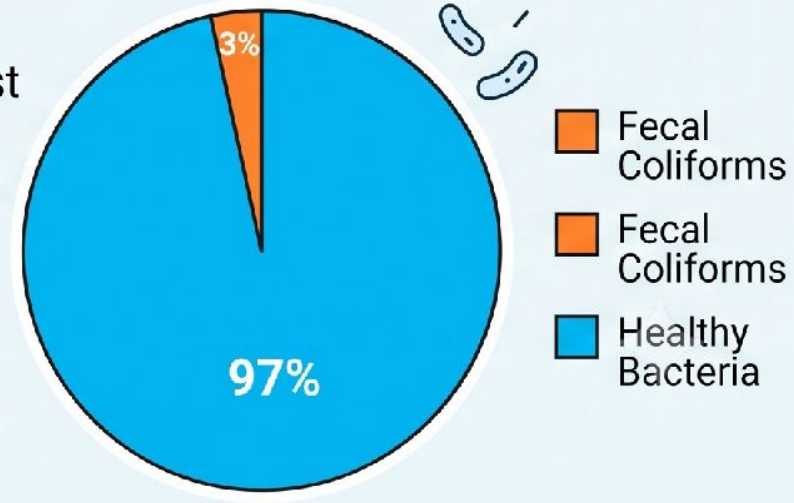
3 Aerobic Chamber

Higher Healthy Bacteria and Lower Number of Fecal Coliforms



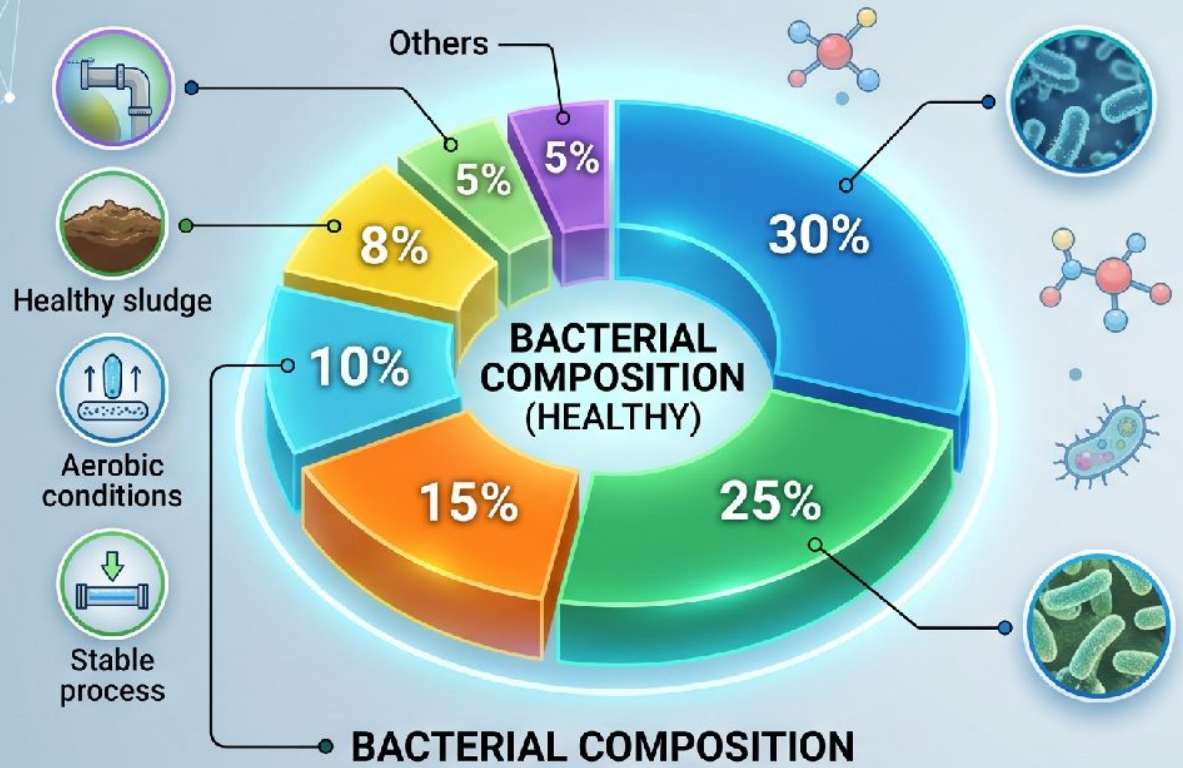
4 Decantation and Intermittent Aeration Chamber

Coliforms Almost Eliminated and Healthy Bacteria



COMPARISON OF METAGENOMIC BACTERIAL COMPOSITION IN EFFLUENT TREATMENT PLANTS: HEALTHY VS. UNHEALTHY

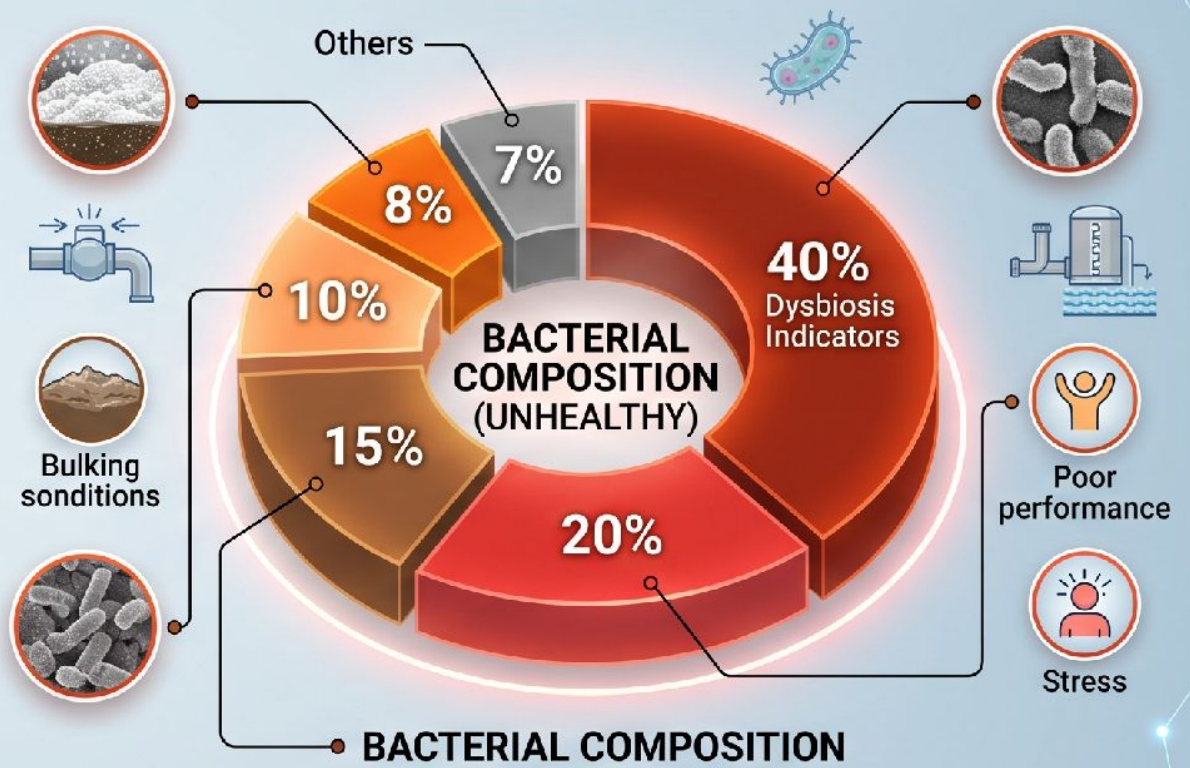
HEALTHY EFFLUENT TREATMENT PLANT (ETP)



BACTERIAL COMPOSITION (HEALTHY)

- **Proteobacteria** (e.g., Alpha-, Beta-, Gamma-)(30%)
- **Bacteroidetes** (e.g., Flavobacteria)(25%)
- **Actinobacteria**
- **Firmicutes** (e.g., Clostridia)
- **Planctomycetes**
- **Verrucomicrobia**
- **Others**

UNHEALTHY EFFLUENT TREATMENT PLANT (ETP)



BACTERIAL COMPOSITION (UNHEALTHY)

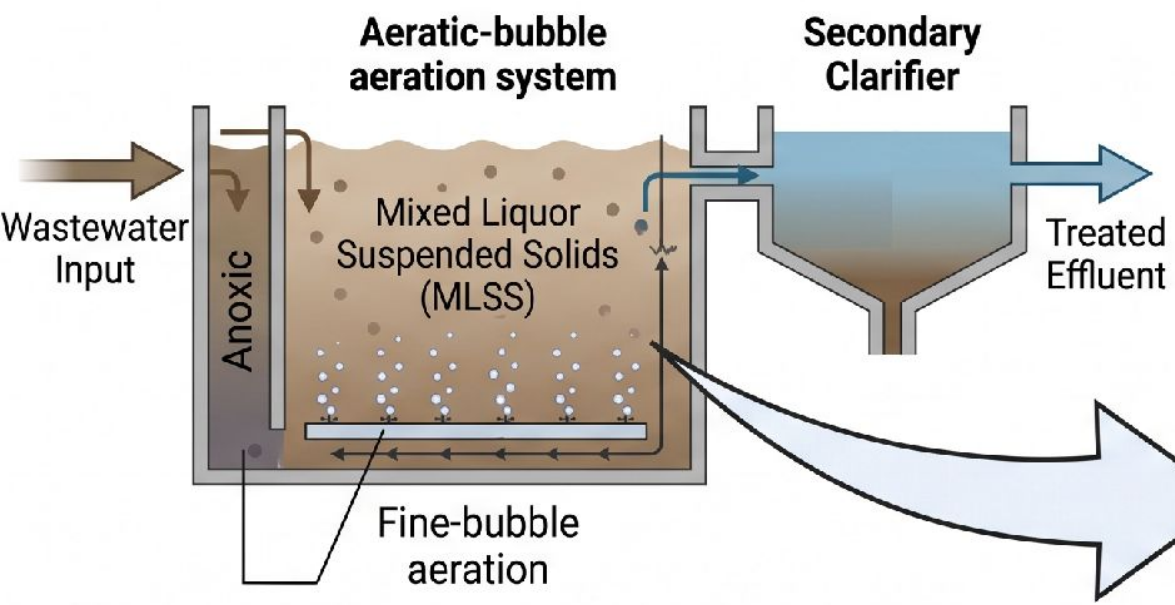
- **Dysbiosis Indicators** (e.g., Nocardia, Microthrix
- **Firmicutes** (e.g., filamentous, bulking)(20%)
- **Pathogenic/Stress Groups**
- **Proteobacteria** (e.g., reduced diversity)(10%)
- **Actinobacteria**
- **Others**

DATA ARE REPRESENTATIVE & ILLUSTRATIVE

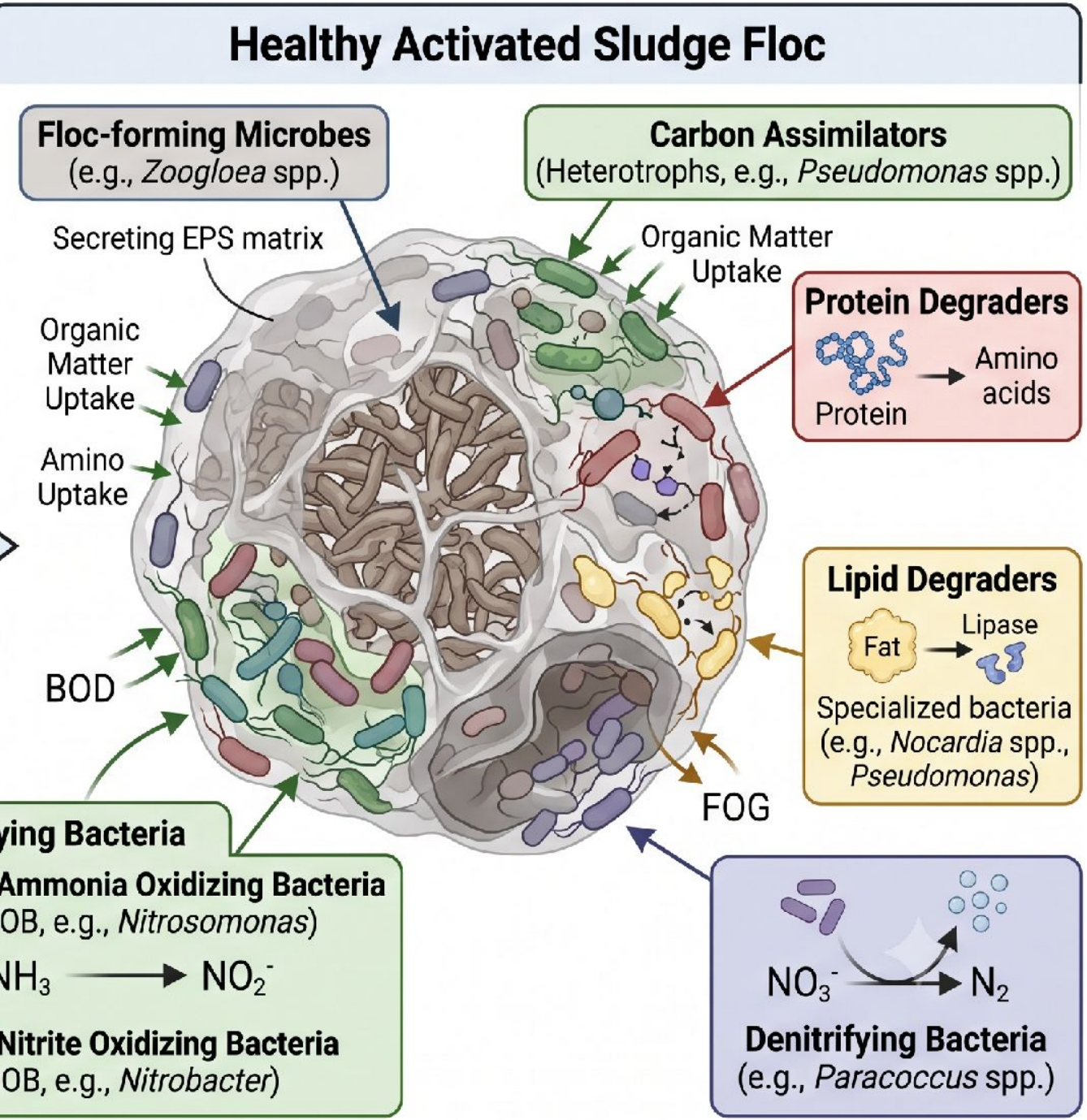
Application

Hollow Ceramic Beads

Well-maintained Activated sludge
Effluent Treatment Plant (ETP)



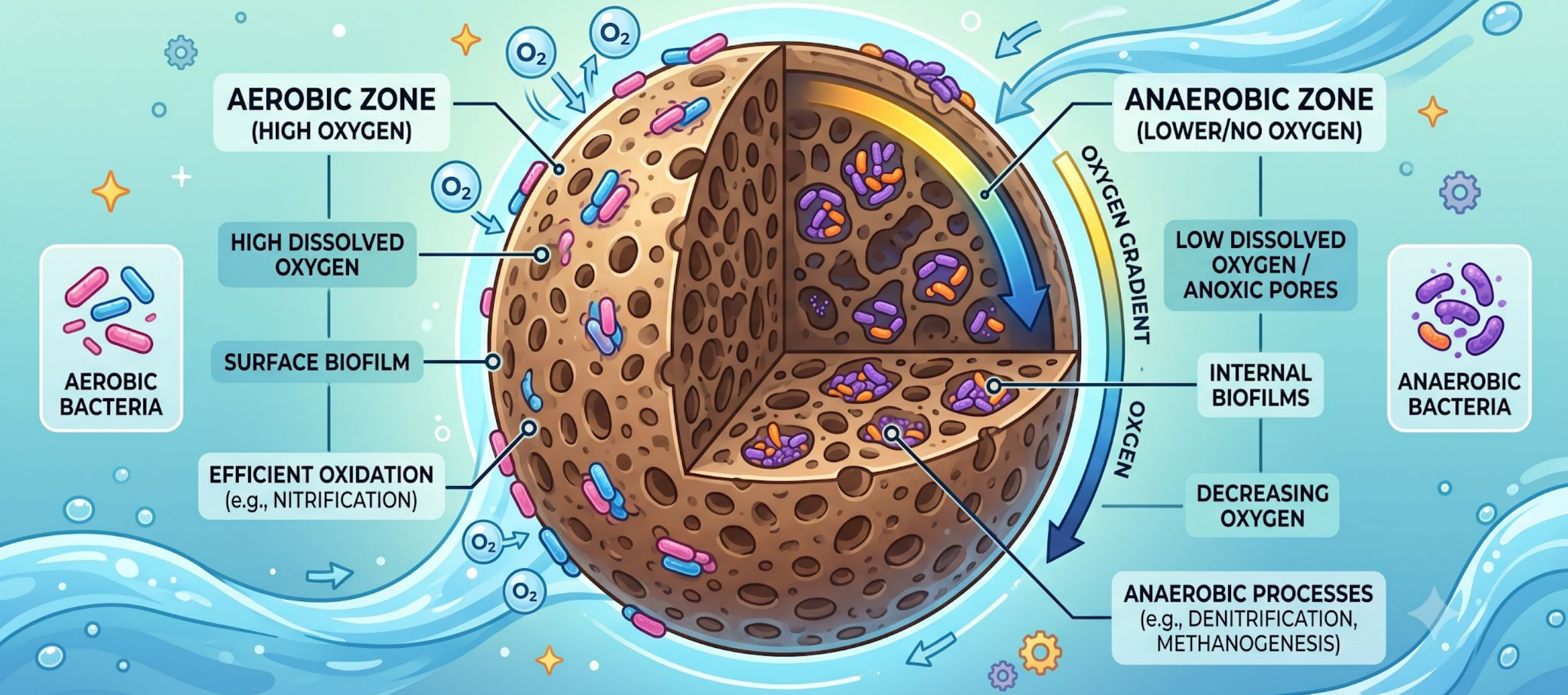
Healthy Activated Sludge Floc



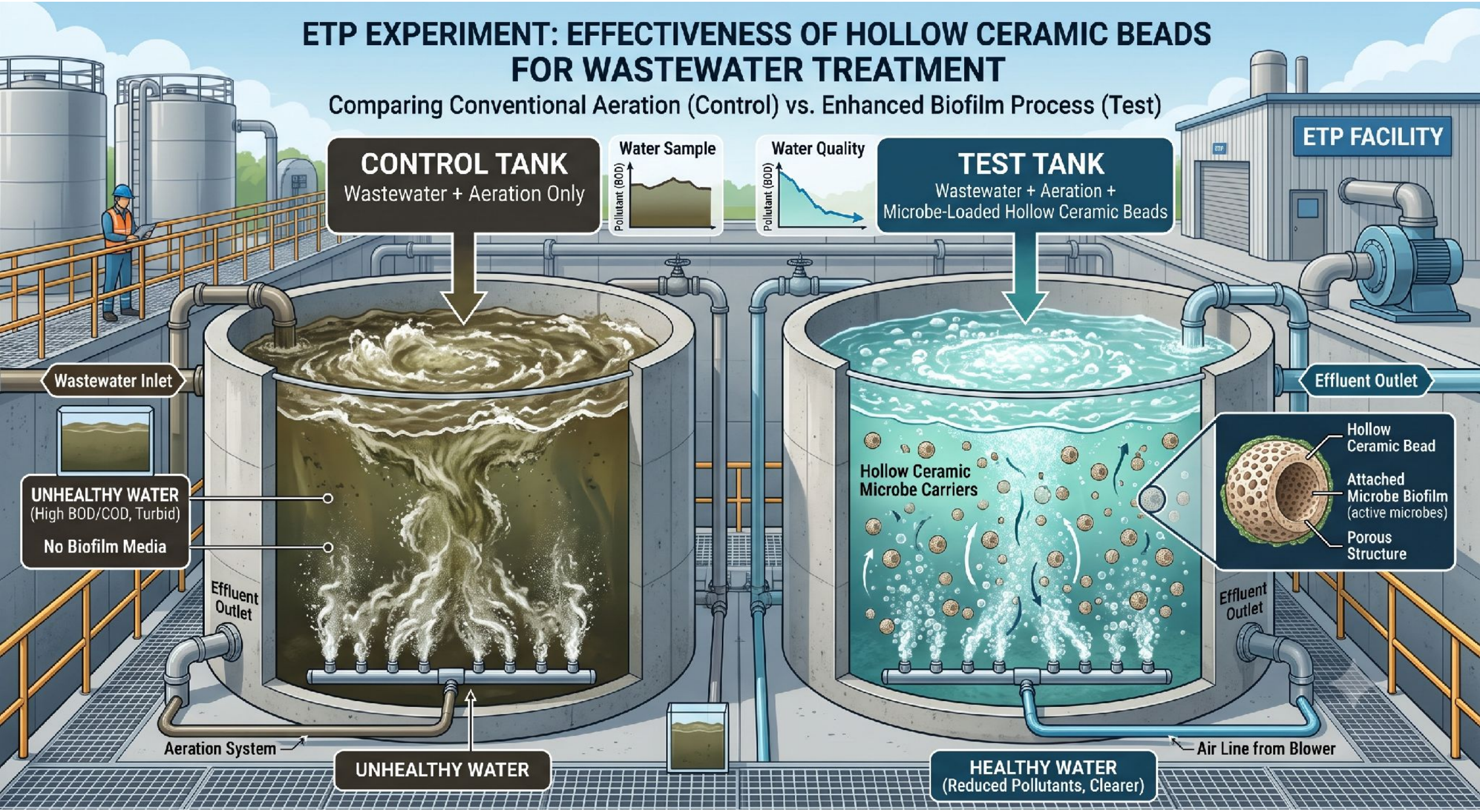
Well-maintained ETP indicators wito:

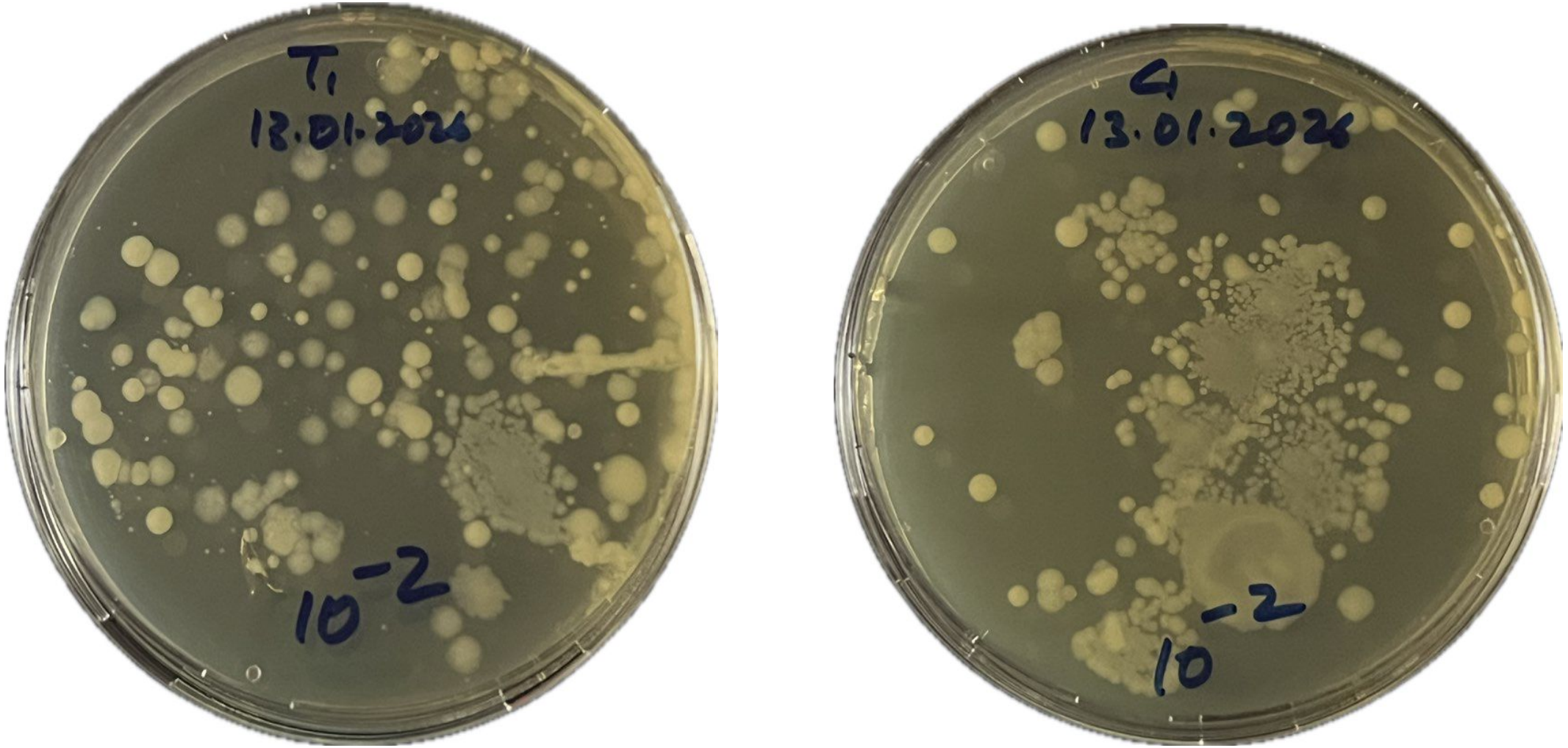
- Efficient COD/BOD Removal
- Complete Nitrification
- Low Effluent TSS
- Good Sludge Settling (SVI <120 mL/g)
- Stable pH and DO levels

POROUS CERAMIC BEAD:
DUAL MICROBIAL ENVIRONMENTS (CROSS-SECTION)

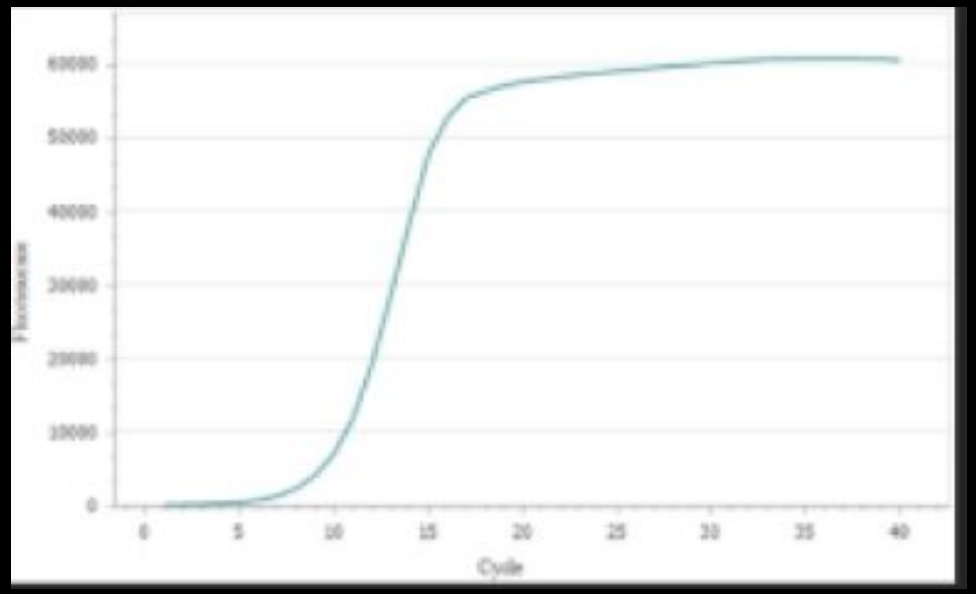


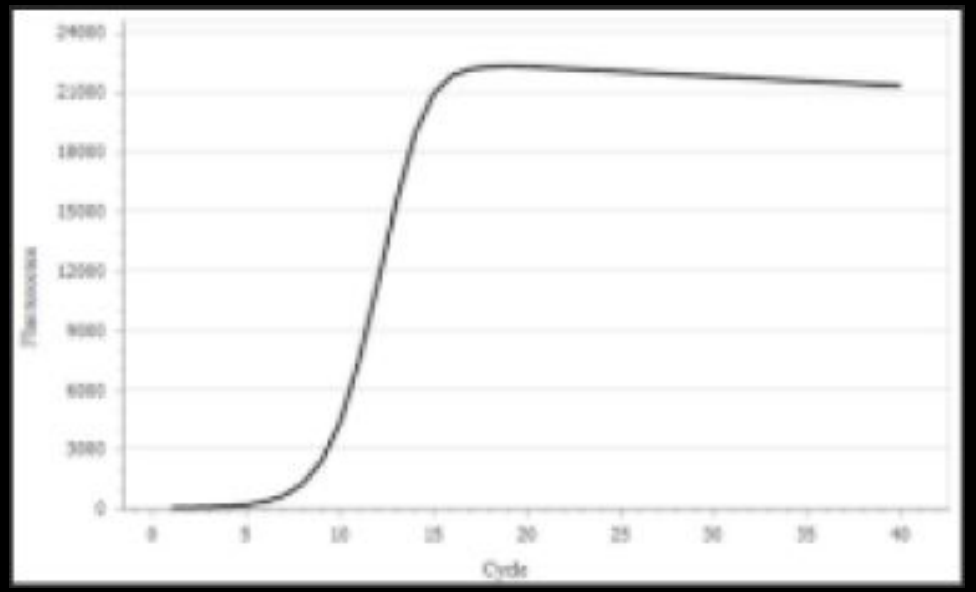
A BIOLOGICAL WATER TREATMENT MEDIA


ETP EXPERIMENT: EFFECTIVENESS OF HOLLOW CERAMIC BEADS FOR WASTEWATER TREATMENT
Comparing Conventional Aeration (Control) vs. Enhanced Biofilm Process (Test)
CONTROL TANK
Wastewater + Aeration Only
Water Sample
Pollutant (BOD)
Water Quality
Pollutant (BOD)
TEST TANK
Wastewater + Aeration + Microbe-Loaded Hollow Ceramic Beads
ETP FACILITY
Wastewater Inlet
UNHEALTHY WATER
(High BOD/COD, Turbid)
No Biofilm Media
Effluent Outlet
Hollow Ceramic Microbe Carriers
Hollow Ceramic Bead
Attached Microbe Biofilm
(active microbes)
Porous Structure
Effluent Outlet
Effluent Outlet
Aeration System
UNHEALTHY WATER
Air Line from Blower
HEALTHY WATER
(Reduced Pollutants, Clearer)

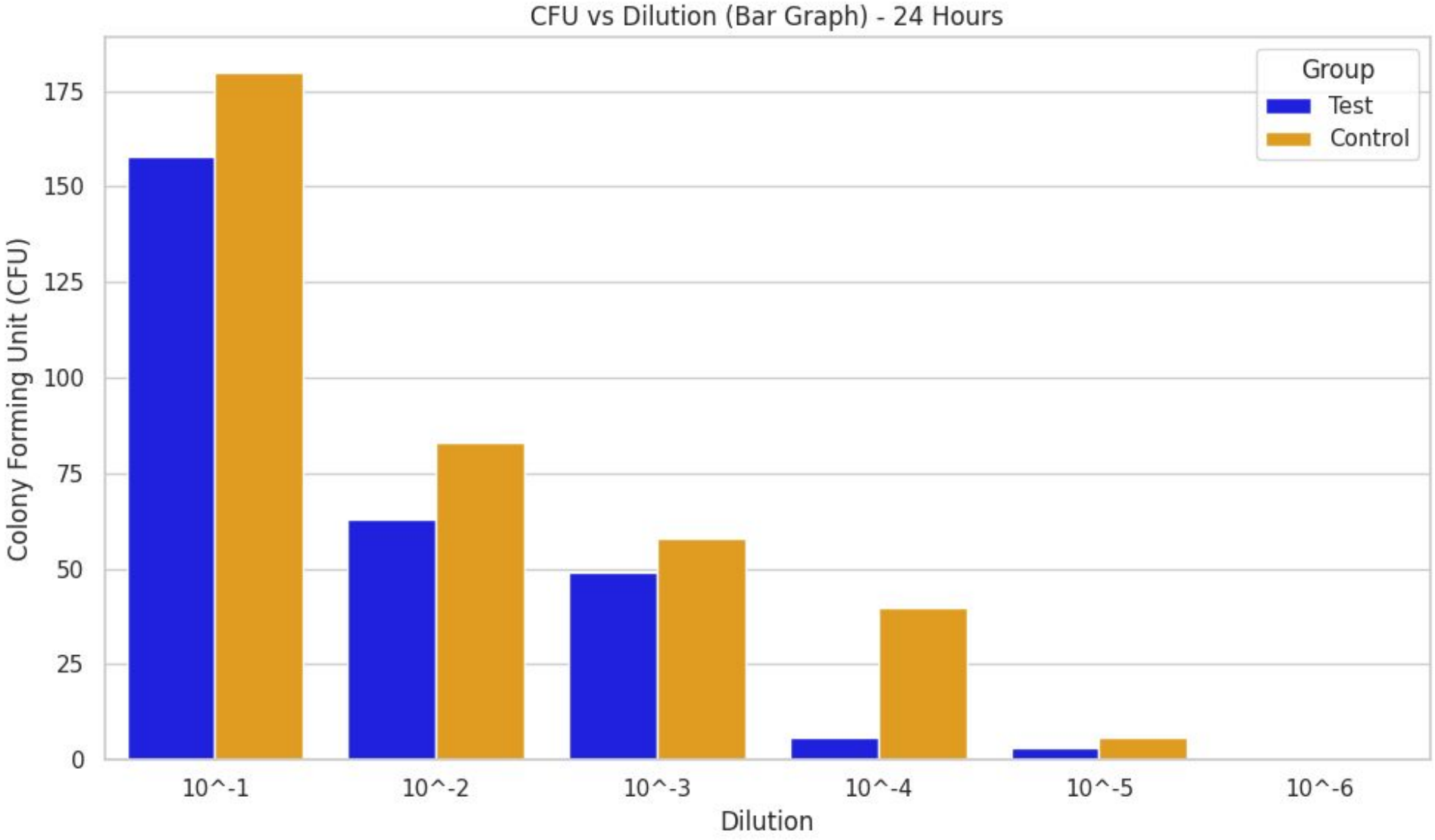


Microbial viability in the Test (T) and Control (C) after 72 hours

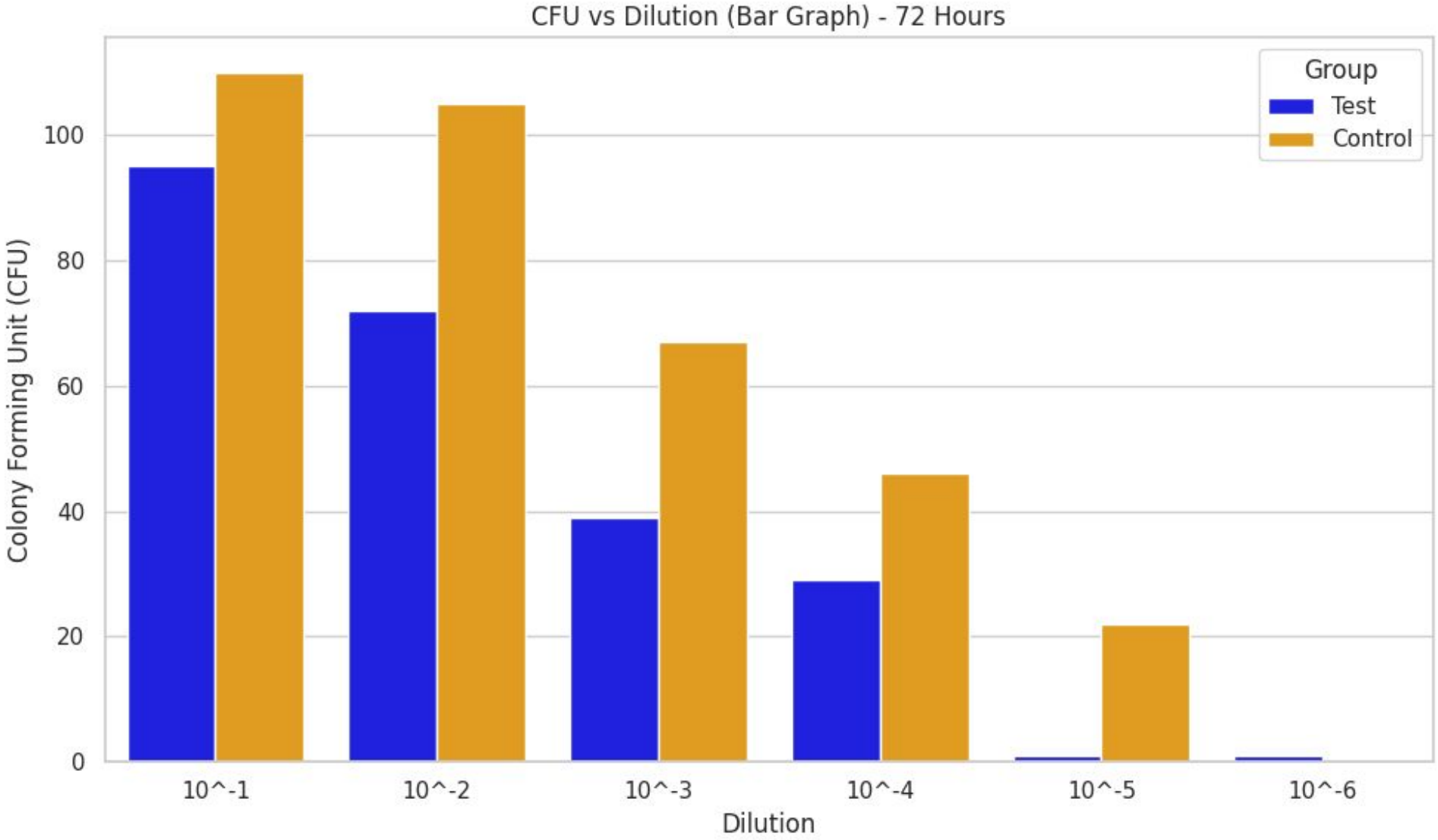




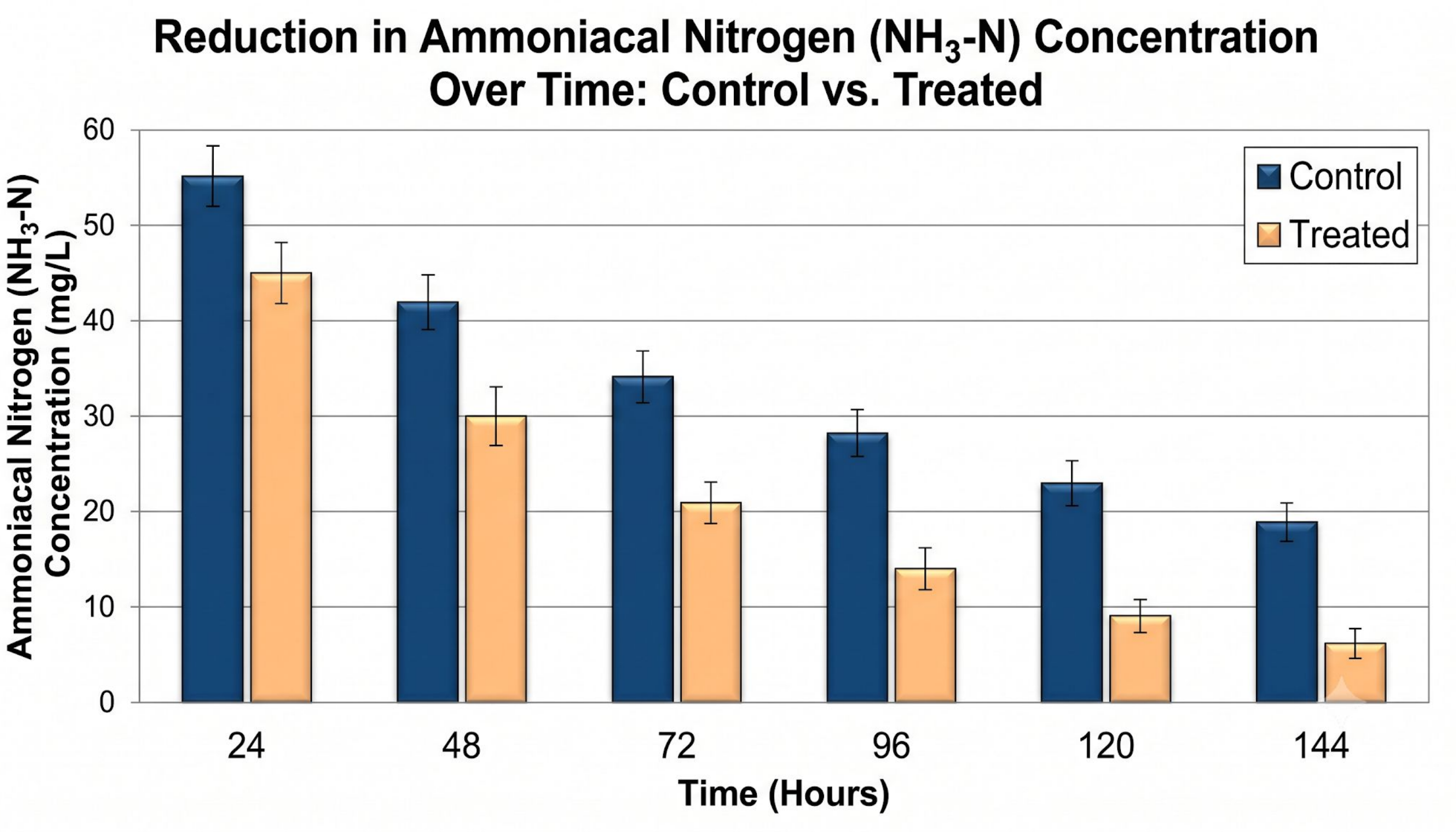
Quantitative Real Time Polymerase Chain Reaction (qRT-PCR)



Microbes remain viable one week after incorporation into the system



Microbes remain viable 72 hours after incorporation into the system


Reduction in Ammoniacal Nitrogen (NH_3-N) Concentration Over Time: Control vs. Treated
Ammoniacal Nitrogen (NH_3-N) Concentration (mg/L)
60
50
40
30
20
10
0
Control
Treated
24
48
72
96
120
144
Time (Hours)

Summary

- Improvement in water quality parameters.
- Maintenance of a viable bacterial culture via incorporation into porous ceramic beads.
- Simulated the activated sludge.

Conclusion

The future of clean water relies on looking at the microscopic level. By understanding and partnering with microbes, we can build a truly circular, zero-waste society.

Acknowledgements

JABATAN PERKHIDMATAN PEMBETUNGAN SABAH

WEITENGEN SDN. BHD.

BIWWEC Borneo International Water & Wastewater Exhibition & Conference 2026

SARAWAK WATER
Empowering Every Drop

Thank you

Sarawak Maju Makmur

placeborneo
BUSINESS EVENTS • INTEGRATED PR

INTER BORNEO TOURS SDN BHD