Task 5: Molecular ecology analysis for bioreactors

Beatriz Gil Pulido
PhD student
University College Cork

NUI Galway
OÉ Gaillimh

Trinity College Dublin

ucc
Coláiste na hOllscoile Corcaigh, Éire
University College Cork, Ireland

eagasc
Agriculture and Food Development Authority

Department of
Agriculture,
Food and the Marine
An Roinn
Talmhaíochta,
Bia agus Mara

AIT
Institiúid Teicneolaíochta
Bhaile Átha Luain
Athlone Institute
of Technology

Ryan
Institute

DairyWater

Objectives

- To investigate changes in the **microbial ecological structure** of laboratory-scale and meso-scale intermittently aerated sequencing batch reactors (**IASBRs**) treating dairy influents and in **water reuse systems**.
- To correlate **ecological shift** with the **biological nutrient removal profiles** in the bioreactors (efficiency and stability).
- PCR cloning, sequencing and analysis of **relevant functional gene** diversity during high efficiency nutrient removal.

DairyWater

Literature review

- Biological nutrient removal. Nitrogen and phosphorus.

Key nutrients causing **eutrophication** in waterways.

Nitrogen removal: simultaneous nitrification-denitrification (SND), partial nitrification, anammox.

AOB/AOA (ammonia-oxidizing bacteria/archaea) NOB (nitrite-oxidizing bacteria)

Phosphorus removal: enhanced biological phosphorus removal (EBPR).

PAOs (phosphorus accumulating organisms)

SND and phosphorus removal in one single reactor.

DPAOs (denitrification phosphate accumulating organisms)

References: S. Tsuneda (2005), Raymond J. Zeng (2003).

DairyWater

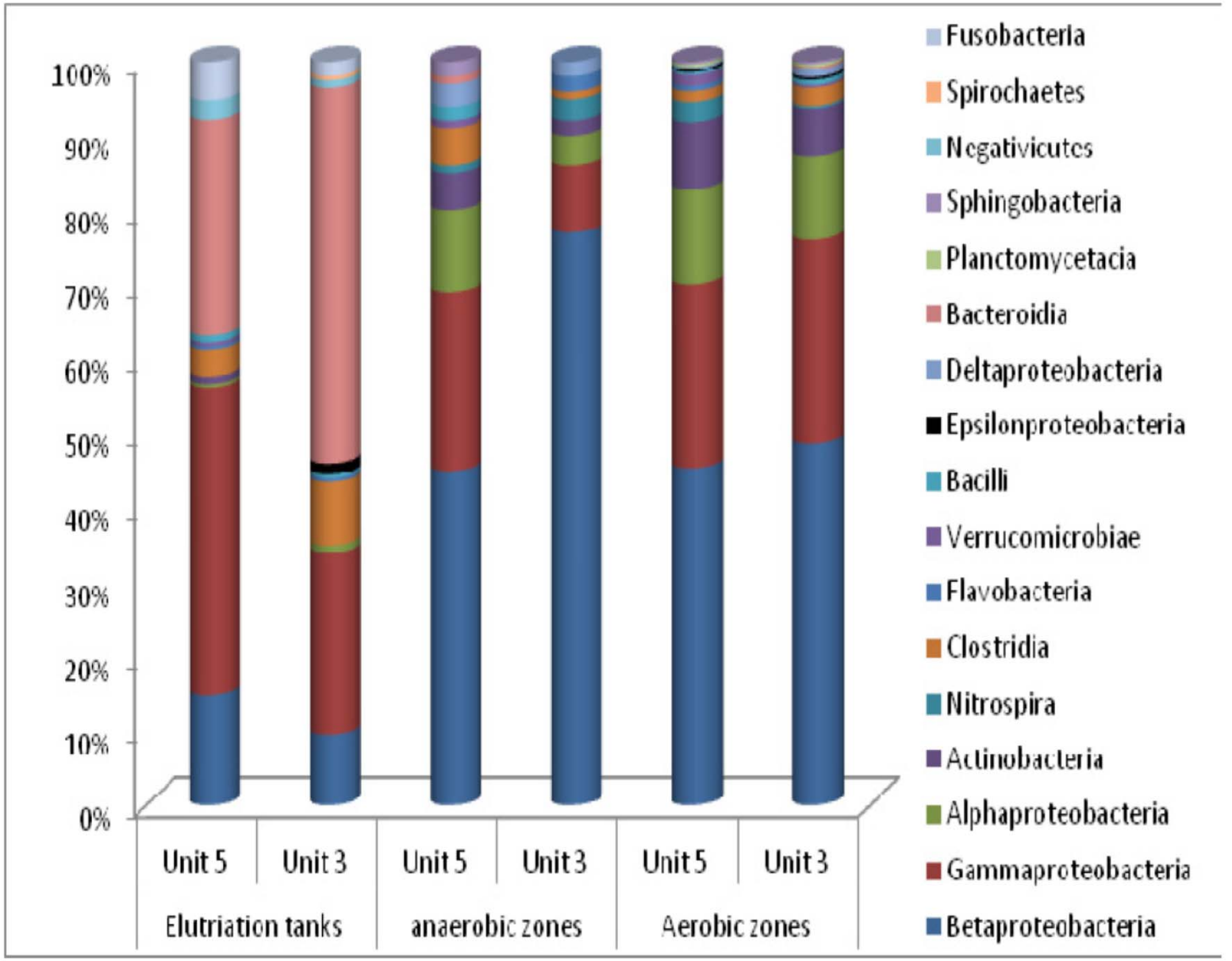
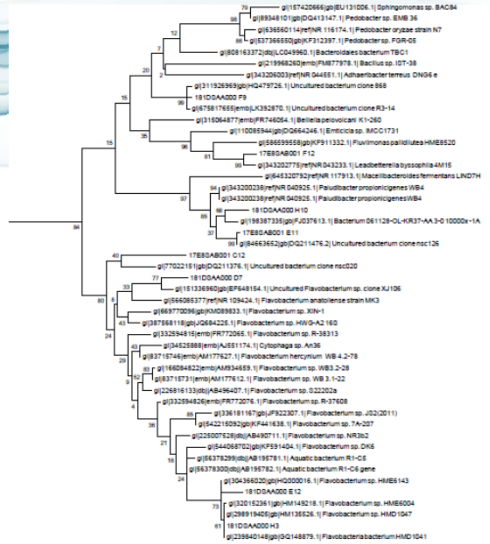


Figure 1: Microbial diversity within different zones of the treatment in a WWTP.
Source: I.Kamika et al., 2014. (Int. J. Environ. Res. Public Health 2014,11,2876-2898)

Molecular analysis of IASBR



16S Clone library

- Material and Methods

1. Three samples, one from each reactor: **R1, R2, R3** in day 63 since starting.
2. **DNA extraction**: Mobio PowerSoil Isolation kit.
3. **PCR amplification** with the pair of primers: 27F/1492R (1500bp length).
4. **Cloning** and screening: Qiagen PCR Cloning^{Plus} kit. Kanamycing and ampiciline selection.
5. **Sequency**: Sanger sequencing (Macrogen).

- Results

Groups (phyla) of species (85-99%similarity, excluding uncultured bacteria):

- Actino
- **Bacte** *nticicia sp. , Leadb*
- Firmic
- Planct
- **Proteobacteria**: ***Arcobacter sp.,*** *Campylobacteraceae sp., Comamonas sp.,* ***Acidovorax sp.,*** *Rhodoferax ferrireducens, Actinetobacter sp., Shewanella sp., Thauera sp., Sphingomonas sp., Ferribacterium sp., Castellaniella sp., Alcaligenes sp., Delftia sp.,*

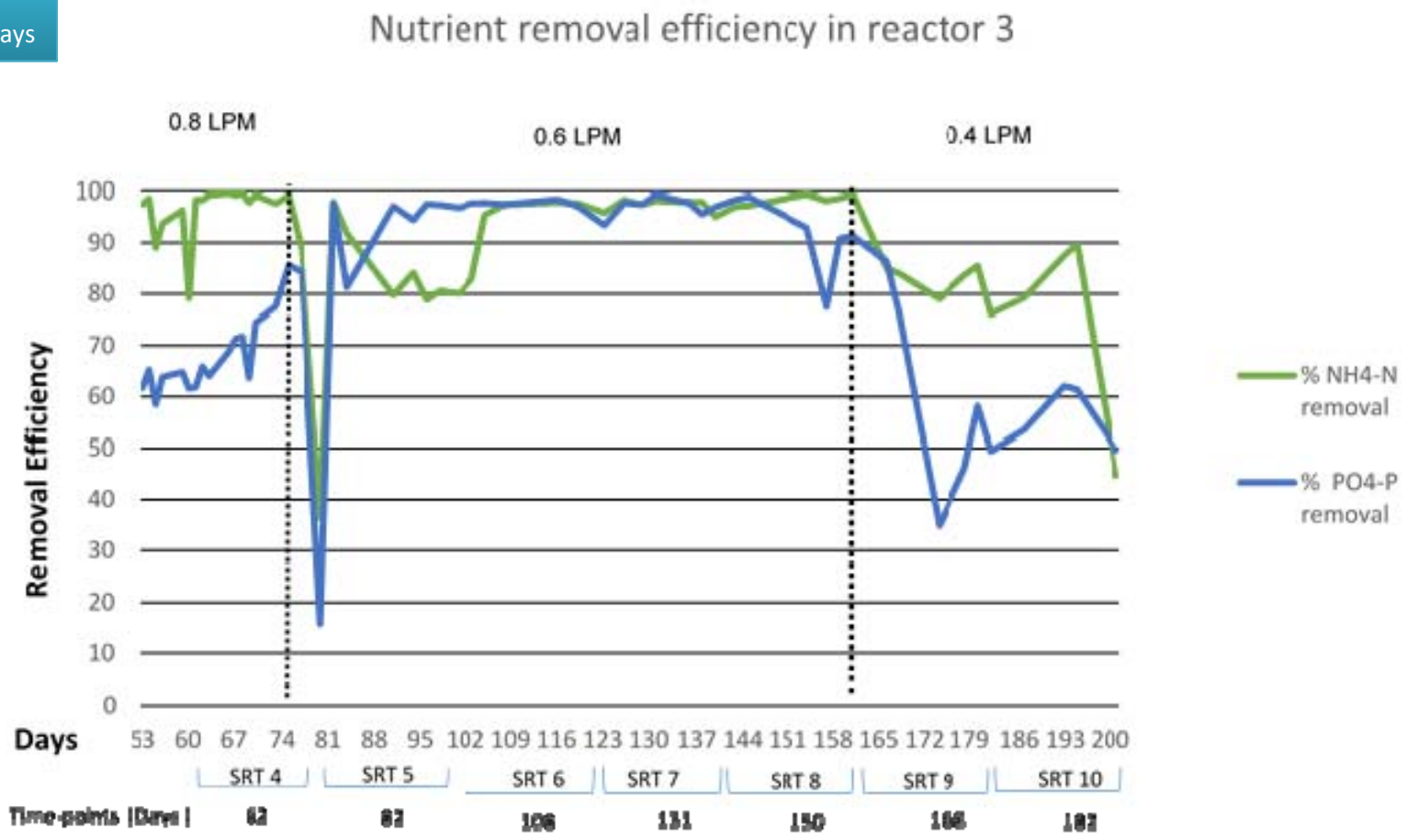
- Time consuming and laborious.
- Many clones have to be sequenced to ensure most of individual species in the sample are covered.

Successful identification of know species but inconsistent for the purpose of comparative assessment of reactors overtime.

Molecular analysis of IASBR

HRT : 4 days

SRT: 20 days



Molecular analysis of IASBR

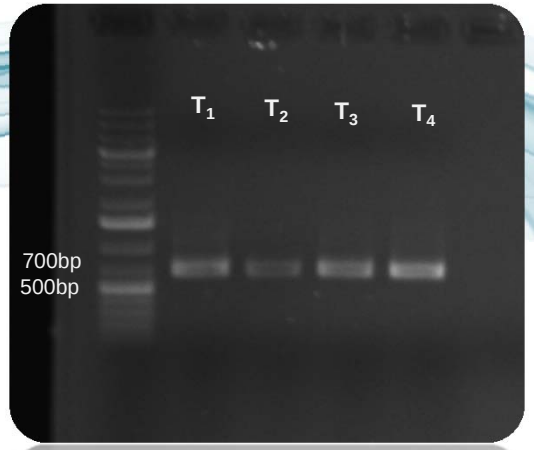


Figure 2: PCR product for samples T_1 to T_4

Microbial ecology structure in R3

- Material and Methods

1. Nine samples from **R3** along the 201 days setup: **T_0, T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8.**
2. **DNA extraction**: Mobio PowerSoil Isolation kit.
3. **454-Pyrosequencing** using the universal primers: U905F/U1492R for the regions V5-V9 in the 16S (Wang&Qian, 2009; Gao et al., 2015). 35 nucleotide barcodes were added in each pair of primers (~650 bp length).
4. **Bioinformatics analysis**: QIIME.

Regular parameters:

-Cycle and aeration period lenghts
-Temperature (11ºC)
-Hydrolic retention time (HRT)
-Solid retention time (SRT)

Variable parameter:

- Air flow

DairyWater

Future Prospects

- To link physicochemical parameters with the molecular microbiolgy studies of IASBR systems.
- To understand how the microbial communities do perform and recover after breakdown events.
- Microscopy and clone specific probes for FISH (fluorescence *in situ* hybridisation) analysis.
- To determine the ecology in meso-scale IASBR.

DairyWater

Acknowledgements

- Funding provided by the Department of Agriculture, Food and the Marine for the DairyWater project (Ref.: 13-F-507).
- Collaborators in NUI Galway.
- Colleagues in the lab 442 in the Department of Microbiology at University College Cork.
- Professor Alan Dobson and Niall O'Leary, supervisors of the thesis.

Thanks for your attention

DairyWater