



---- Again ---- Who are PAOs?

- A *Rhodocyclus* related group may be dominant in acetate-rich EBPR systems.
- The EBPR community appears to be diverse and composed of several major bacterial groups.
- Not a single group of bacteria but rather a complex microbial community as a whole should do the job.

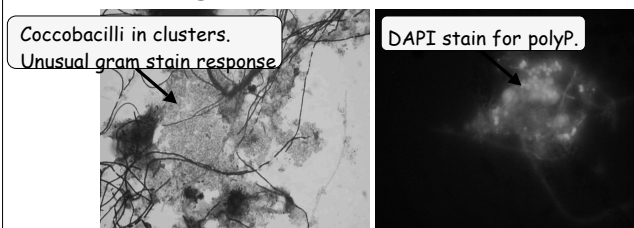


On-going Microbiological Studies

- Application of molecular tools to various EBPR communities
- Molecular isolation and identification
More information on gene sequences of the EBPR/activated sludge community members is needed.
- Metabolism/function vs identification
The dual staining technique/microautoradiography (MAR) may be useful.



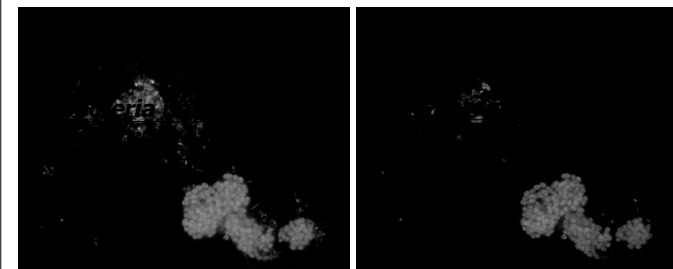
Large Coccobacilli in AS



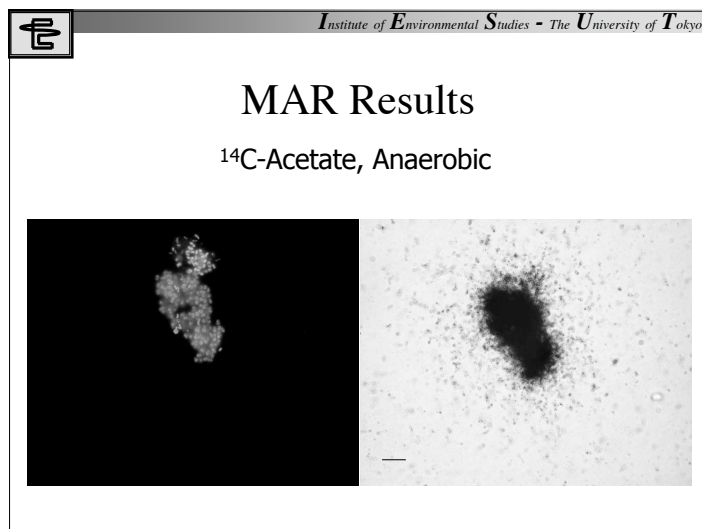
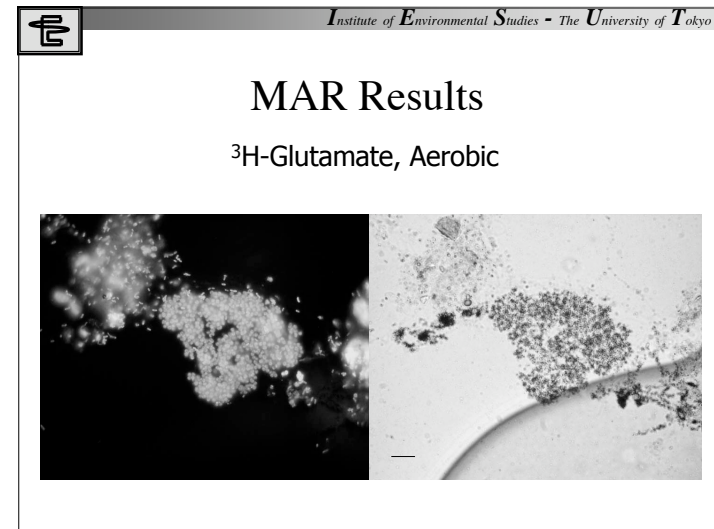
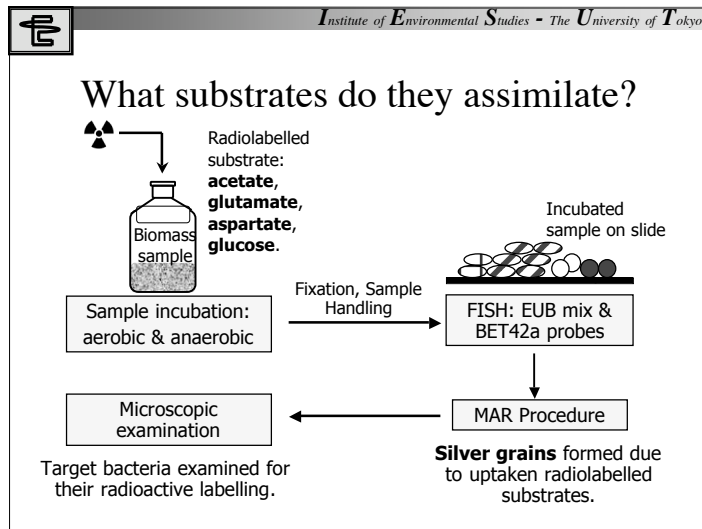
- ➔
- Cells with same features dominated in an Australian activated sludge WWTP.
 - Interested to know: are they yeast spores or *Rhodocyclus*-related PAOs?



FISH Results



- They are α -Proteobacteria;
- But not *Rhodocyclus*-related PAOs.
- Poses doubt about role of yeast spores in EBPR.

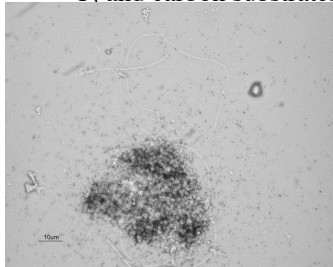


- Institute of Environmental Studies - The University of Tokyo*
- ### MAR Results
- Coccobacilli take up acetate, glutamate and aspartate under both aerobic and anaerobic conditions.
 - They do not take up glucose under both conditions applied.



P accumulation

- Incubated under anaerobic-aerobic condition with $^{33}\text{P}_i$ and carbon substrates.



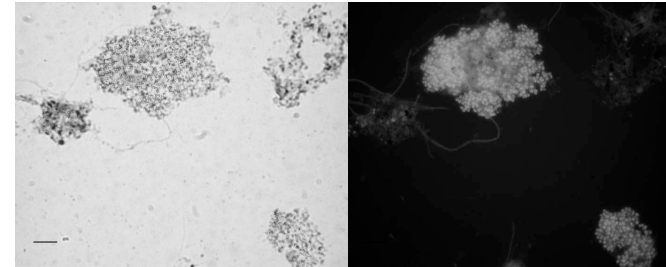
Results:

- Coccobacilli accumulate $^{33}\text{P}_i$
 - possibly a novel PAO candidate of □-Proteobacteria.



PHA staining/MAR Results

^3H -Glutamate, Anaerobic



- Unable to deplete the pre-existing PHA:
 - Correlation between substrate uptake and PHA is not definite.



PHA staining/MAR Results

- Coccobacilli contains PHA:
 - they are prokaryotes.
- PHA staining/MAR: useful technique for correlating substrate uptake and PHA storage of PAOs.
 - eliminate pre-existing PHA in biomass.



Summary from FISH/MAR/PHA staining

- Large coccobacilli in clusters are □-*Proteobacteria*.
- Phylogenetically different from *Rhodocyclus*-related PAOs.
- Selective for acetate, glutamate, and aspartate.
- Store PHA.
- Accumulate polyP.

A novel PAO candidate!



5. Final Remarks



Future of the EBPR process (1)

From technological points of views:

- The EBPR process is a promising alternative for phosphate removal from wastewaters.
- Sludge treatment must be optimized for EBPR.
- Phosphorus recovery will be considered in combination with EBPR.



Future of the EBPR process (2)

From microbiological points of views:

- The EBPR microbial community is still like a difficult microbiological puzzle.
- Molecular approaches will bring about significant amount of new fundamental information, but its practical usefulness is still unclear.
- The EBPR process is a very interesting target of microbial community analysis, leading to new insight into the complex microbial world.



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