

## Future of Activated Sludge Relies on Deeper Understanding of Its Microbial Community

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Since activated sludge process was invented in early 20<sup>th</sup> century, engineers have been made efforts to improve the performance and functions. Various functions of activated sludge have been explained by assuming groups of functionally distinct bacteria. The functions of such bacterial groups have been partially clarified by isolated strains. However, our knowledge is still very limited for microbes in activated sludge as the isolates are the tip of an iceberg in it. Microbial community and ecophysiological studies are essential for further progress of the process.

As an initial approach, bacterial compositions of activated sludge in 14 municipal wastewater treatment plants (WWTPs) were investigated by using fluorescent in situ hybridization (FISH) at a group level in Japan<sup>1)</sup>.  $\alpha$ - or  $\beta$ - proteobacteria were the most predominant bacterial groups in all the samples. The other groups, such as  $\gamma$ -proteobacteria and *Actinomycetes* did exist but occupied small fractions. Statistical analysis could not delineate influences of operating conditions like temperature, location, influent water quality and so on, on bacterial composition. The predominance of  $\beta$ - proteobacteria in municipal WWTPs was also reported in other countries. Activated sludge treating municipal wastewater seems to have common composition at its group-level, though the reason is not clear.

Populations of ammonia oxidizing bacteria (AOB) in 12 activated sludge samples from municipal WWTPs in different seasons. AOB communities were analyzed by specific PCR amplification followed by denaturing gel gradient electrophoresis (DGGE), cloning, and sequencing of 16S rRNA genes. Four real-time PCR primer sets were developed for the groups of AOB clones, and AOB populations were quantified. Sequence type 6a-3&5 of *N. oligotropha* cluster was the dominant AOB in every system and every studied season<sup>2)</sup>. Further experiments clarified that the ammonia and nitrite concentration levels have strong effect on predominant AOB species<sup>3)</sup>.

Estrone (E1) and 17 $\beta$ -estradiol (E2) are natural estrogens excreted by humans and cattle in urine. Although natural estrogens are biodegradable in biological treatment processes, insufficient estrogen removal caused the concentration of estrogens in sewage effluents to reach a level that leads to feminization of male aquatic organisms. To date, a number of bacterial strains that can utilize E1 and E2 as growth substrates have been isolated from activated sludge. We used microautoradiography-fluorescent in situ hybridization (MAR-FISH) to identify estrogen degrading bacteria in activated sludge.  $\beta$ -proteobacteria were most abundant among MAR

positive cells, and they accounted for 1 to 2% of the total microbial community<sup>4)</sup>. By using previously designed probes, the E1-assimilating bacteria were further identified by probe Cte, which targets family *Comamonadaceae*. We have further designed probes to narrow down our target in these bacterial groups, and a small bacterial group which is closely related to genus *Sphaerotilus* was found to be E1-assimilating bacteria. The importance of in situ investigation was demonstrated with this result, as they are phylogenetically far from isolated estrogen degrading bacteria.

## References

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