BIOLOGICAL SCUM BY *Candidatus monilibacter* spp. IN A WASTEWATER TREATMENT PLANT IN NORTH-EASTERN SPAIN

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Abstract

During a scum study on a full-scale plant with carrousel aeration process treating domestic waste the dominant filament was a Gram and Neisser negative "Nostocoida limicola" morphotype. The "*N. limicola*" Gram and Neisser negative filaments fluoresced with the DF 198 probe designed target alphaproteobacterial *Candidatus Monilibacter batavus*-related organisms. Quantification of "*Ca. Monilibacter batavus*-related organisms" with a class index of 5 corresponded well with the detected morphotype. The fluorescence microscopy after Nile Blue staining made it also possible to observe which morphological types of bacteria were able to store PHB. Among the morphological types which were identified with the FISH probes (*Haliscomenobacter hydrossis*, *Chloroflexi*, Type 0092 variant A, "*Ca.* Alysiosphaera europaea", *Microthrix* and *Thiothrix*) only "*Ca. Monilibacter batavus*-related organisms were able to store PHA. The ability to form PHA is an obvious advantage, since storage material can be utilized as energy and/or as a reserve of carbon during periods of unbalanced growth due to unfavourable conditions. No exo-enzymatic activity were found for any of the ELF enzymes (ß-D-glucuronidase and phosphatase) tested.

Introduction

Filamentous micro-organisms are ubiquitous and conspicuous members of activated sludge wastewater treatment plant microbial communities. Excessive growth of filamentous bacteria in activated sludge wastewater treatment plants (WWTPs) can cause serious operational problems. During a study on a full-scale foaming activated sludge with carrousel processes plant treating domestic wastewater, the dominant filament was a Neisser negative *Nostocoida limicola* morphotype. Correct identification of the filamentous bacteria by targeted FISH probes and their association with particular operational features may provide a means of controlling foaming in WWTPs. Therefore this study focuses on identifying this filament, and with the FISH probes designed against it, to determine its distribution in full-scale treatment plants.

Methods

The sampling period was conducted between September 2009 and May 2010 in Empuriabrava WWTP (Northeastern Spain). The biological reactor volume was 7500 m^3 and presented occasionally discharge of septic tanks. During this period there have been two processing systems: biological reactor with the clarifier in the center controlled by ammonium setpoint and removal of sludge from the bottom (new line), and reactor carrousel in which the removal of sludge is done from the upper zone (old line), thus helping to eliminate foam. In this case the system is fully aerobic and controlled by setpoint oxygen.

Monthly samples of mixed liquor of mixed liquor from the aeration tank were taken for microscopic observation. Protozoa and small metazoan were counted under an optical microscope. The total extended filamentous microorganism length (TEFL) was calculated using the technique based on the "simplified filament counting technique" described by (Jenkins et al. 1993). The abundance of filamentous bacteria by FISH technique in the activated sludge samples was measured according to the subjective scoring method of Eikelboom (2000) where observations are rated on scale from 0 (none) to 5 (extensive growth). The hybridizations were performed at 46 ° C for 2 hours except for *Microthrix* that has extended the period of hybridization until 3 h. All the probes, labelled at the 5' end with Tamra, were purchased from TibMobiol, Germany. The ELF 97 glucuronide substrate (ELF 97 glucuronide) was used to detect the enzyme β-D-glucuronidase (glucosidase activity) and the ELF 97 phosphate reagent (ELF 97 phosphate) was used to detect acid and alkaline phosphatases.

Results and discussion

The "*N. limicola*" Gram and Neisser negative filaments fluoresced with the DF 198 probe (see Fig. 1) designed target alphaproteobacterial *Candidatus Monilibacter batavus*-related organisms (Nittami *et al*. 2009). Furthermore, they did not fluoresce with any of the probes previously described for the alphaproteobacterial "*N. limicola*" filaments. The fluorescence microscopy after Nile Blue A staining made it also possible to observe which morphological types of bacteria were able to store PHB. Among the morphological types which were identified with the FISH probes (*Haliscomenobacter hydrossis*, *Chloroflexi*, Type 0092 variant A, "*Candidatus* Alysiosphaera europaea", *Microthrix* and *Thiothrix*) only *Monilibacter batavus*-related organisms were able to store PHA (see Fig. 1). The ability to form PHA is an obvious advantage, since storage material can be utilized as energy and/or as a reserve of carbon during periods of unbalanced growth due to unfavourable conditions. No exo-enzymatic activity were found for any of the ELF enzymes (ß-D-glucuronidase and phosphatase) tested. The lack of ectoenzyme activity in *Monilibacter batavus* cluster III has been previously reported by McIlLroy *et al*. (2010) and these authors suggests that these populations are unable to use high-molecular-weight polymeric substrates.

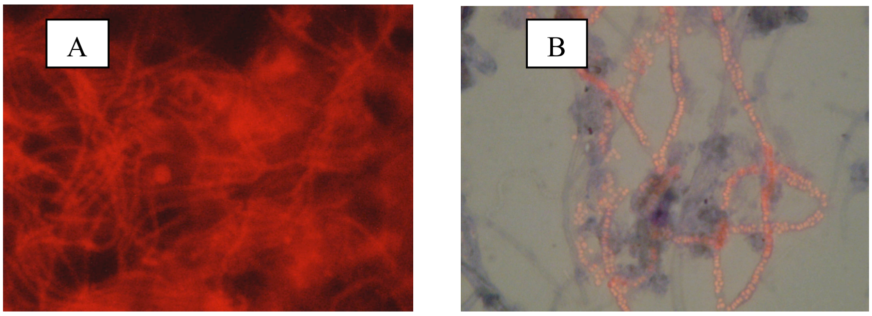

Figure 1. A) *Monilibacter batavus* hybridized with the DF198 probe, 1000x;
B) PHB storage of *Monilibacter batavus* by Nile blue A staining, 1000x

To understand the ecology of the dominant filamentous bacteria in almost all samples and test their patterns of development, and compounds that support growth, it is necessary to check that conditions prevailing in the plant, with special emphasis on the three study periods, since apparently the Carrousel system limits their growth and circular reactor operated by redox setpoint further limits the development of the organisms under setpoint of ammonium (Figure 1). On the one hand, the SS and BOD mean values were similar in the three periods, while the COD increased significantly in the third period. Similarly, the conductivity and chloride were significantly lower in the third period than in the other two. In the case of operational parameters, also were detected differences. The high concentration of MLSS during the first period, along with a very low F/M ratio (0.009 kg BOD/kg MLVSS.d) and sludge age of 77 days produced a highly stressed nutritional environment. Comparing the F/M ratio and sludge age of each period it can be seen that the F/M ratio of the second and third period are higher that the first period, 0.022 and 0.019 kg BOD / kg MLVSS d, respectively. The sludge ages of the second and third period were lower than the first period, 55 and 37 days, respectively. It is clear that the decrease of sludge age allowed a better distribution of nutrients. Comparing period 1 and 2, the growth of *Monilibacter batavus* can be related with low F/M ratio and high sludge ages. *Monilibacter batavus*, is associated with readily short chain fatty acids, acetic and propionic) (McIllroy *et al.*, 2010) and that its development is more competitive in situations of nutritional imbalance, it is obvious that conditions in the third period could limit their growth, along with operational conditions of this period.

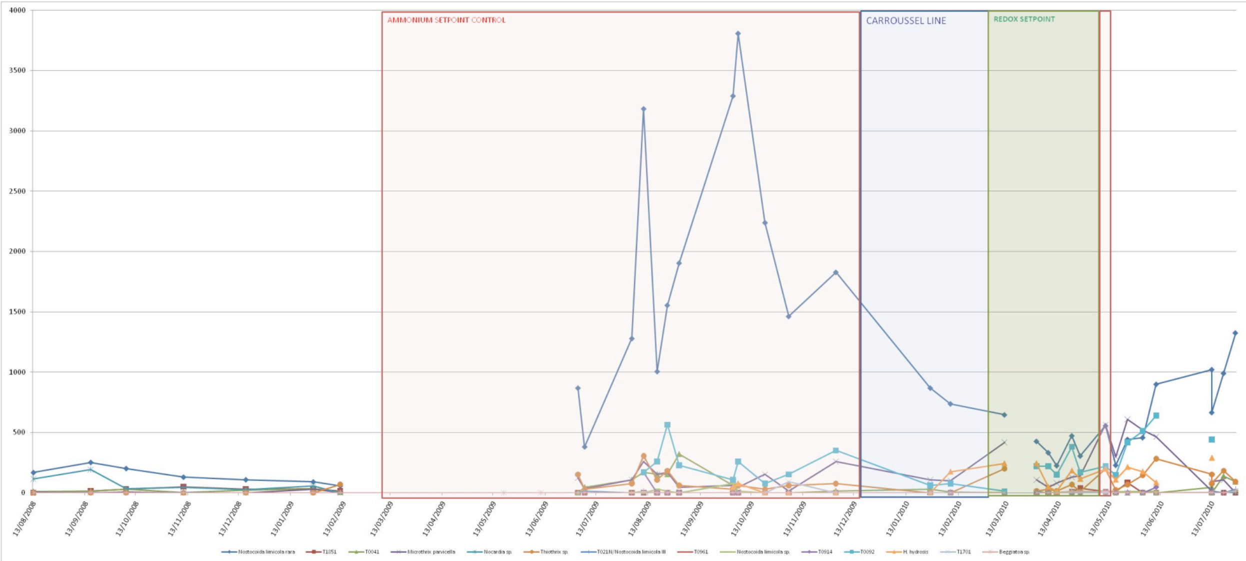

Figure 2. Abundance of the most important filamentous microorganisms in the study period

Conclusions

The oxygen control system under redox setpoint is more effective than ammonium setpoint, to avoid anaerobic conditions of the circular reactor. The control of nutritional conditions in the WWTP, operational measures such as reduction of MLSS, decreased sludge age and increased of the F/M ratio, it seems to help control the growth of this organism

References

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