Evaluation of the impacts of model-based operation of SBRs on activated sludge microbial community

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Abstract Impact of model-based operation of nutrient removing SBRs on the stability of activated sludge population was studied in this contribution. The optimal operation scenario found by the systematic model-based optimisation protocol of Sin *et al.* (*Wat. Sci. Tech.*, 2004, **50**(10), 97–105) was applied to a pilot-scale SBR and observed to considerably improve the nutrient removal efficiency in the system. Further, the process dynamics was observed to change under the optimal operation scenario, e.g. the nitrite route prevailed and also filamentous bulking was provoked in the SBR system. At the microbial community level as monitored by DGGE, a transient shift was observed to gradually take place parallel to the shift into the optimal operation scenario. This implies that the model-based optimisation of a nutrient removing SBR causes changes at the microbial community level. This opens future perspectives to incorporate the valuable information from the molecular monitoring of activated sludge into the model-based optimisation methodologies. In this way, it is expected that model-based optimisation approaches will better cover complex and dynamic aspects of activated sludge systems.

Keywords Activated sludge; DGGE; modelling; nutrient removal; optimisation; SBR

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Introduction

The response of biological wastewater treatment systems to the environment is often difficult to predict since the microbial population may change its composition in a dynamic way depending on the different process conditions (Eikelboom, 2000). Consequently, there is still a limited understanding of the relationship between microbial community and process functionality despite the importance of the microbial community in wastewater treatment.

During the last decade, modern analytical methods based on molecular microbiology were developed, allowing for a more comprehensive analysis of the microbial communities. Denaturing gradient gel electrophoresis (DGGE) is among the most cited techniques as being very efficient in detecting population changes with an emphasis on the stability and dynamics of the microbial community (Boon *et al.*, 2002; Kaewpipat and Grady, 2002; La Para *et al.*, 2002). Fingerprinting of complex bacterial communities by DGGE was first published by Muyzer *et al.* (1993). The technique allows the separation of DNA fragments of the same length but with different base pair composition. The separation is based on the decreased electrophoretic mobilities of partially melted double-stranded DNA molecules in polyacrylamide gels containing a linear gradient of denaturants. This methodology allows a fast screening of multiple samples and gives additional information about changes within bacterial communities.

Activated sludge systems are usually operated under suboptimal conditions due to their highly variable and often unpredictable dynamics in the input characteristics. Modelling of these complex systems, e.g. by use of the activated sludge models (ASM) of Henze *et al.* (2000), was shown to be an effective tool to control operation, improve existing performance, and reduce costs of operation (Coen *et al.*, 1997; van Veldhuizen

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et al., 1999; among others). Recently a model-based systematic optimisation protocol was developed and successfully applied to improve the nutrient removal performance in sequencing batch reactors (SBRs) (Sin *et al.*, 2004).

In these model-based approaches, the activated sludge system is represented by a model, which is used to simulate a myriad of different operational scenarios. The results of these scenarios are later evaluated to find the best scenario that meets the targets of the optimisation. One important drawback with this approach is the fact that available models, e.g. ASMs, do not cover all aspects of activated sludge systems, particularly impacts of different operational scenarios on the activated sludge microbial community, activated sludge settling, etc. (Comas *et al.*, 2006; Sin *et al.*, 2006). To make up for that drawback, it was proposed to iterate the systematic protocol until the targets are reached (Sin *et al.*, 2004), which may require an unknown number of iterations.

The main objective of this contribution is to gain insight into and increase understanding of possible impacts of model-based operation of activated sludge systems on the activated sludge community. In this study, this objective will be tested on a pilot-scale nutrient removing SBR optimised following the systematic protocol of Sin *et al.* (2004). In the first part of the study, long-term molecular and process performance monitoring of the SBR is presented. This is followed by evaluation of the SBR performance under the model-based operation. In the third part, monitoring of the activated sludge community by DGGE is presented and evaluated. Finally, it is discussed whether the molecular monitoring techniques can be incorporated with the model-based optimisation approaches to improve the operation of dynamic activated sludge systems.

Material and methods

The pilot-scale SBR was previously described in detail (Sin *et al.*, 2004). Two operation configurations, which are shown in Figure 1, were applied to the SBR within this study. Synthetic wastewater was used as influent, the volumetric exchange ratio (VER) was fixed to 0.5, the HRT was 12 h, the SRT was 10 days and the total cycle time was fixed at 6 h. In total, three operation scenarios were applied in this study.

Reference operation scenario. The configuration of one cycle is shown in Figure 1a. The total volume of the SBR was 80 L and the 40 L of influent was supplied to the reactor during fill/anaerobic phase. The DO set-point was 2 mgO_2/l. Further detail is given in Sin *et al.* (2004).

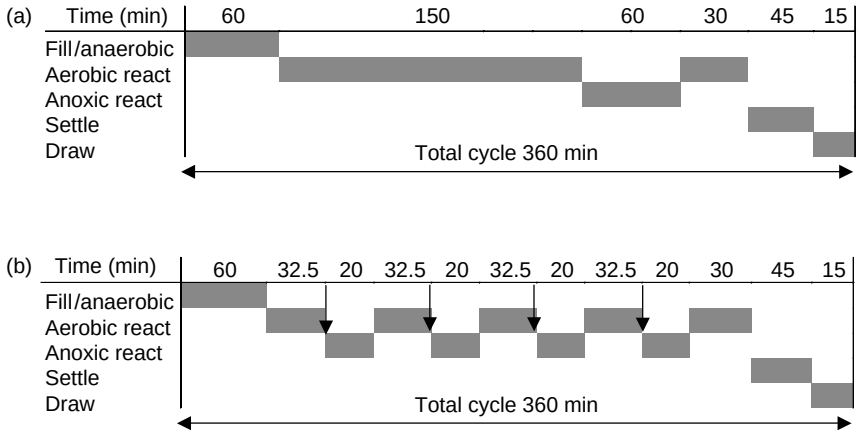

Figure 1 Two operation configurations of the SBR: The reference operation configuration (a), the optimal operation scenario with IAF4 configuration. The arrow indicates the instant of step-feed (b)

Optimal operation scenario. The configuration of one cycle is given in Figure 1b. The optimal DO set-point was 0.5 mg/l. The total volume of the SBR was 68 L. 24 L of the influent was supplied during the fill/anaerobic phase of the cycle and the remaining 10 L was equally step-fed to the anoxic phases, i.e. 2.5 L per each anoxic phase (see Figure 1b).

Third operation scenario. The configuration of one cycle is the same as the optimal operation. However, two degrees of freedom of the optimal operation scenario configuration were changed. First, the DO set point was increased to 1.0 mgO_2/l and second the concentrations of the divalent cations (Ca^{2+} and Mg^{2+}) were increased in the influent composition (Table 1) to approximate equilibrium between monovalent and divalent cations concentrations. It was considered that this addition would help to induce a better flocculation process and an improvement of the settling process (Higgins and Novak, 1997; Cousin and Ganczarczyk, 1998).

DNA extraction, PCR and DGGE. Total DNA was extracted from the sludge sample based on the protocol presented by Boon *et al.* (2002). A 100 μL aliquot of the crude extract was further purified using Wizard PCR preps (Promega, Madison, WI, USA). The cleaned DNA was stored at −20 °C. 1 μL of the extracted DNA was amplified by PCR with the bacteria specific 16S rRNA forward primer 338f (ACT CCT ACG GGA GGC AGC AG) and the reverse primer 518r (ATT ACC GCG GCT GCT GG) (Muyzer *et al.*, 1993). The PCR product contains a GC-clamp of 40 bases, added to the forward primer. PCR products were subjected to DGGE as described previously (Boon *et al.*, 2002). In brief, PCR samples were run for 17 h at 38 V on 8% (wt/vol) polyacrylamide gel with a denaturing gradient ranging from 45–60% (where 100% denaturant contains 7 M urea and 40% formamide). After electrophoresis the gels were stained with SYBR Green I nucleic acid gel stain (1:10,000 dilution; FMC BioProducts, Rockland, ME, USA) and photographed. Cluster analysis (WARD algorithm) of the DGGE patterns was performed with the Bionumerics software 2.0 (Applied Maths, Kortijk, Belgium). The calculation of the similarities is based on the Pearson correlation coefficient and results in a distance matrix (Boon *et al.*, 2002).

Results and discussion

History of the SBR monitoring

The initial purpose of the SBR was to operate it under stable environmental conditions and to evaluate whether these conditions will lead to stable microbial composition and properties of the activated sludge. Accordingly, a *reference operation scenario* (see Figure 1a) was set up and applied. The SBR performances were monitored over a period of 226 days and the results (Govoreanu *et al.*, 2003) revealed that stable operation conditions of the SBR did not imply stability in microbial community and settling performances. It was shown that long-term monitoring of the SBR led to a highly dynamic microbial

Table 1 Cations concentration in the influent before and after increasing the bivalent cations

Cation	Cation concentration (meq/l)	
	Before addition	After addition
Na^+	6.1	6.1
K^+	0.4	0.4
Ca^{2+}	0.6	3.5
Mg^{2+}	0.9	1.5

population (Figure 2a), but a link between microbial community evolution, settling and structural properties of the sludge was found.

By contrast, further evaluations, showed that short-term stable sludge properties characterised by a relatively stable microbial communities were possible to be obtained (Figure 2b). However, these periods were in general limited in time (about 30–40 days) and any reactor failure created disturbances of the microbial populations. It was also observed that the effect of these failures on the microbial populations becomes significant, leading to shifts in the microbial populations, after about 30 days (3 × SRT) from the occurrence of the failure.

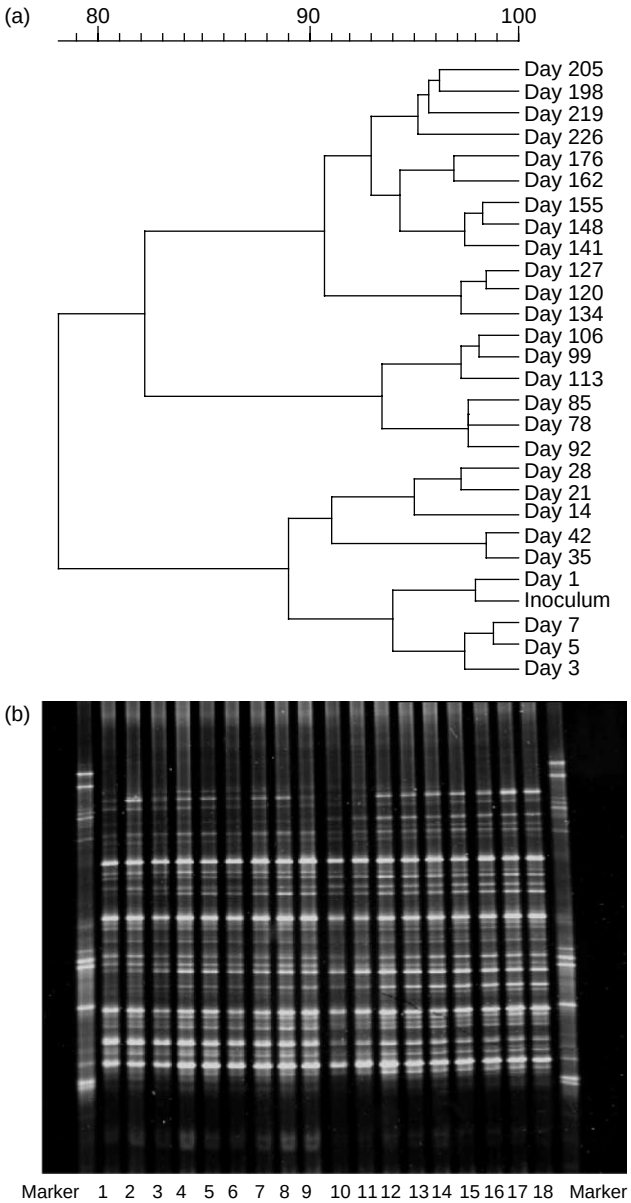

Figure 2 Cluster analysis for long-term SBR monitoring (a) (Govoreanu *et al.*, 2003) and DGGE profiles for activated sludge samples collected over one-month period (b)

During the above-mentioned monitoring period, the nutrient removal performances of the SBR were rather unsatisfactory except for the COD removal, which was complete as expected (see Table 2). However, total nitrogen and phosphorus removal efficiencies of the SBR were rather poor around 70% and 48% respectively. To improve this situation, a model-based optimisation approach was chosen and a systematic methodology was developed and applied (Sin *et al.*, 2004). In what follows, the resulting performances of the SBR under the model-based operation are discussed.

Process performance of the model-based SBR operation

The so-called ASM2dN model was used in the previous study to describe the SBR system and evaluate a multitude of different operation scenarios (Sin *et al.*, 2004). The optimal operation scenario (see Figure 1b) found using this model was implemented on December 17th, 2003 and was run till March 1st, 2004. The daily monitoring of effluent COD, TN, PO_4-P and settling properties activated sludge as monitored by measurement of sludge volume at 30 min (SV_{30}) are shown in Figure 3.

The optimal operation was indeed observed to considerably improve the nitrogen and phosphorus removal efficiency of the SBR (see Figure 3 and Table 2). For example, the total nitrogen removal was improved from 56% to 86% and P-removal was increased from 18% to 65% (Table 2). The system operated under the optimal scenario was observed to have considerably drifted from the previous state (Sin *et al.*, 2006). Particularly, nitrite build-up was observed in the system indicating 2-step nitrification under aerobic conditions and 2-step denitrification under anoxic conditions, i.e. the so-called nitrite route prevailed in the system (Sin *et al.*, 2006).

Moreover, settling problems with the activated sludge were observed. Microscopic investigations showed that excessive filamentous bulking was provoked in the system after changing into the optimal operation. This can also be clearly observed in the plot of sludge volume (SV) in Figure 3. Finally, when confronted with the new behaviour obtained under the optimal operation, the ASM2dN model failed (Sin *et al.*, 2006). In short, it can be said that the model-based operation led to changes in the SBR system to an extent where the model no longer holds. The underlying reason was thought to be changing of the activated sludge microbial community in the system (Sin *et al.*, 2006), which is discussed in detail in the following section.

To overcome the settling problems provoked by the optimal operation, on the other hand, it was decided: (i) to increase the oxygen set point in the aerobic condition that is known to reduce filamentous bulking and (ii) to increase cation concentration in the influent to improve flocculation process. The so-called third operation phase (see above) was applied starting from March 1st, 2004 and onwards. The removal efficiencies for total nitrogen and phosphorus of the SBR were sharply dropped (see Table 2 and Figure 3). It is noteworthy that the P-removal performance of the system was the most influenced by this operation change. However, activated sludge settling quality was observed to improve gradually following 3 times the SRT (see SV_{30} in Figure 3).

Table 2 Performance of SBR under different operation scenarios

	COD mgCOD/l	Total nitrogen mgN/l	PO_4-P mgP/l
Influent	410	60	11
Removal efficiency			
Reference operation (1 year average)	91%	70%	48%
Optimal operation (2.5 months average)	94%	86%	65%
Third operation (2 months average)	92%	72%	20%

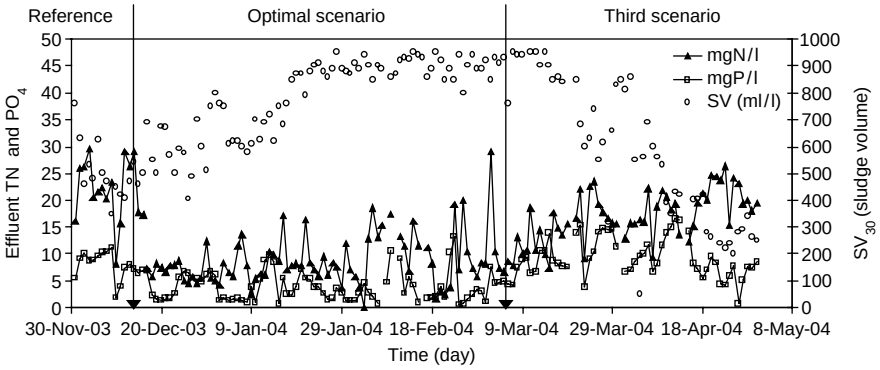

Figure 3 SBR operation with different scenarios: effluent total nitrogen, effluent phosphorous and sludge volume measurements

Impacts of the model-based operation on the activated sludge microbial community

The DGGE patterns of the sludge samples evaluated during the *optimal operation scenario* and the *third operation scenario* were compared and analysed first by cluster analysis, revealing four major groups (Figure 4a). The differences in similarity between the microbial population on December 19 (first days of the SBR operation under the optimal scenario) and the other evaluated periods are illustrated in Figure 4b. Figure 4c shows the correlation coefficients calculated between two consecutive samples, indicating the relative rate of change of the composition of the microbial community.

The first group (I) corresponded to the period in which the *optimal operation scenario* was implemented. A high degree of similarity (Figure 4b) was observed between the band patterns analysed before changing the operation scenario (9/12/03 and 16/12/03) and the first 10 days after the new operating scenario become effective (19/12/03 and 29/12/03) suggesting that changing of the operation parameters did not create any shock effect to the microbial community. A second group (II) developed in the next 40 operating days. The appearance of diverse populations was observed especially for days 4/01/04 and 9/01/04, which clustered together. The group II could be assigned to a transition period characterised by dynamic microbial community. This is in agreement with the previous findings where a shift in the microbial community is usually observed only after about 3 × SRT following a change that occurred in the SBR operation.

Two new ribotypes (arrows 1 and 2 in Figure 4a) developed and become dominant during the third group (III) characterized by a rather stable microbial community. The corresponding species, which seems to be adapted well to the new SBR conditions, could be one of the developing filamentous organisms responsible for the occurrence of the bulking phenomena or one of the species responsible for the removal of phosphorous or the nitrogen from the system. These hypotheses are sustained further until the last group (IV) are analysed, since these ribotypes disappeared again from the system while the process performances showed an improvement of the settling properties and fewer filaments but also a deterioration of the removal capacities. This needs further validation by determining the sequence of the DNA fragments. The observed changes on the microbial population in the group IV occurred after approximately one month following the implementation of the third operation scenario. This led again to a new transition period characterised by very dynamic microbial community.

As shown in Figure 4c, the relative rate of changes between two microbial communities present in two consecutive samples (the sampling time is in the order of 1–2 weeks) is slow indicating a slow dynamics in microbial populations within 1–2 weeks

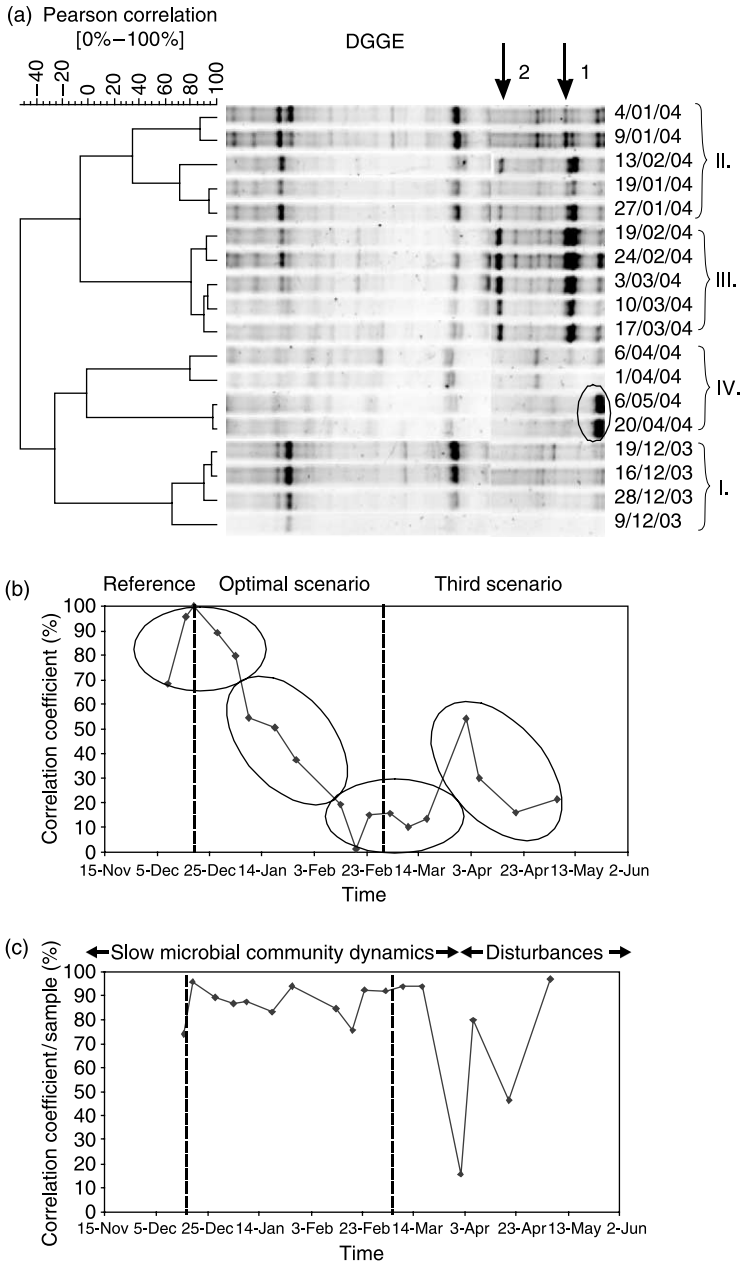

Figure 4 DGGE profiles and cluster analysis during optimal and third operation scenario (a) and the correlation coefficient between microbial population of the first day after the implementation of the optimal scenario (b) and microbial community dynamics comparison of correlation coefficients of the samples on time basis (c)

time frame. However, a fast change in the microbial populations was observed after approximately 25 days from the implementation of the third scenario. Based on the stable operating conditions of the SBR the disturbing effect could be seen as a consequence of the performed changes, i.e. the DO level or the cations addition in the influent. Similar observations were reported by Boon *et al.* (2003) showing that even under an imposed shock effect shifting of the microbial community was observed following a certain delay

(8 days). These differences could also be explained by the occurrence of a new dominant species (Figure 4a encircled bands).

Perspectives on combination of molecular monitoring techniques with activated sludge modelling

Mechanistic models, ASMs of Henze *et al.* (2000), are based on the-state-of-the-art understanding of activated sludge systems from the process kinetics and stoichiometry point of view. Further, the ASMs represent activated sludge by several functional groups, such as heterotrophs (aerobic COD oxidation + denitrification), autotrophs (nitrification) and phosphorus accumulating organisms (PAOs) (P-uptake and P-release). The molecular monitoring results presented here combined with the experiences obtained with the model-based optimisation of the SBR (Sin *et al.*, 2006) suggest that a useful potential exists in utilisation of the information from the advanced molecular techniques.

Fingerprinting techniques, like DGGE, TGGE, and T-RFLP are generally used to examine the microbial diversity of all bacteria or of specific phylogenetic groups (Dejonghe *et al.*, 2001). The relative distribution of the different species in a community can be an indication of the population stability. The molecular quantitative techniques, like FISH or real-time PCR can be used to determine the number of cells of one particular microbial group or species, e.g. ammonia oxidisers, nitrite oxidisers, PAOs, denitrifying PAOs (DPAOs), glycogen accumulating organisms (GAOs), etc. (Yuan and Blackall, 2002). Isotopic-labelling methods combined with some molecular techniques, e.g. MAR-FISH among others, provide insight into microbial-community function (Wagner and Taylor, 2005).

In view of modelling, the latter methods seem more appropriate as they offer simultaneous identification and quantification of microbial communities. Practically, this provides direct indications of certain functional groups described in ASMs. That information can be used to support the choice of an *appropriate* model structure during the model calibration step, e.g. depending on the absence or presence of PAOs or DPAOs one can choose ASM2 or ASM2d. The same information is also useful during model validation to have an a priori idea on which functional groups are present in the system. Further *direct* use of the information of quantitative molecular techniques in modelling will be quite valuable but is currently still subject to serious challenges as outlined by Yuan and Blackall (2002). Among others much depends on: (i) the development of appropriate methods to translate these data into quantities employed by the models, e.g. expressed as mgCOD/l of organisms; and (ii) finding relationship between these molecular data and the functions of the observed microbial groups. In this context, it is worth remembering Pareto's law (80:20) that states that a minority (20%) of the microbial community governs the majority (80%) of the energy flux of the system (Dejonghe *et al.*, 2001).

Conclusions

The activated sludge microbial population was observed to be rather dynamic during long-term operation of a nutrient removing SBR with a reference scenario under stable environmental conditions. For short-term periods (30–40 days), however, a stable activated sludge community could be observed in the system. When the SBR system was optimised for nutrient removal following the model-based systematic optimisation protocol of Sin *et al.* (2004), it was observed that this operation led to changes in the activated sludge community as monitored by DGGE measurements during ca 2.5 months. It is noteworthy that a clear transient could be observed in the similarity coefficient of microbial populations indicating a shift in the microbial community parallel to the shift in the operation of the SBR. Further, it was observed to cause settling problems in

the system. Increasing the oxygen set point and the cation concentration in the influent improved the settling quality of the sludge. Finally, the model used to represent the system became invalid after imposing the different operation scenarios (results shown in Sin *et al.*, 2006), overlapping with the above-mentioned change in microbial community. The results also contributes to the perspectives on integrating the advanced molecular techniques with the model-based optimisation methodologies for better understanding and improving operation of activated sludge systems.

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