Periodica Polytechnica
Chemical Engineering

61(3), pp. 149-155, 2017
https://doi.org/10.3311/PPch.10078

RESEARCH ARTICLE

Conditions Favoring Proliferation of Glycogen Accumulating Organisms for Excess Biological Carbon Removal in Treating Nutrient Deficient Wastewater

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Received 27 September 2016; accepted after revision 07 December 2016

Abstract

Impact of marginal availability and severe deficiency of nitrogen on the growth of glycogen accumulating organisms (GAOs) and performance of anaerobic/aerobic activated sludge systems treating nutrient deficient wastewater was investigated at marginal availability and severe deficiency of phosphorus.

Two continuous-flow lab-scale systems were operated simultaneously fed by model winery wastewater, one with marginal availability and the other one at severe deficiency of nitrogen. In the second experimental stage, marginal availability of P was converted into severe deficiency by interrupting external dosing.

Common practice of dosing N- and P-sources to marginal availability caused enhanced proliferation of filamentous bacteria leading to poor biomass settling and instable operation. At marginal N-availability accumulation of GAOs started when conditions became deficient for phosphorus. In severe lack of nitrogen GAOs overgrew filaments, and outcompeted phosphorous accumulating organisms (PAOs) initially present in the seed. Stable and good performance could be maintained even after withdrawal of phosphorous dosing.

Keywords

nutrient deficiency, GAO proliferation, marginal nitrogen availability, external nutrient dosing, activated sludge treatment

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1 Introduction

Several kinds of basically food-industrial wastewater, included the one discharged from wine production are in lack of nitrogen and phosphorus. Winery wastewater having been used in this study as a model for nutrient deficient influent may derive from a number of technological steps including cleaning of the basins, equipment and floor; rinsing the transfer lines, washing the barrels, bottling facilities and filtration units, etc.. Both the volume and the pollution load of winery effluents vary greatly in relation to the operational period (i.e. vintage, racking and bottling) and the kind of wine produced. Amount of wastewater of a winery ranges from 0.7 to 1.2 times of that of the wine produced [1], which draws an increased attention to the winery wastewater treatment. Several different processes are currently available for treating winery wastewater, such as physicochemical methods (coagulation, flocculation, electrocoagulation, etc.), membrane filtration and separation (nanofiltration and reverse osmosis) as well as advanced oxidation processes (ozonation, Fenton oxidation, etc.,) [2]. However, it is still the biological treatment being most commonly used in full-scale as it is considered to be the most environmental-friendly and cost-effective solution, although its control needs adequate engineering expertise.

In domestic activated sludge treatment plants basically low-S conditions, but also marginal nutrient availability can lead to the proliferation of filamentous microorganisms [3]. However, serious nutrient deficiency experienced rather in industrial wastewater treatment, may cause overproduction of extracellular polysaccharides (glycocalyx) required normally just for keeping the activated sludge flocs together. This ‘viscous bulking’ generally results in slimy, poorly settling biomass. Both viscous bulking and the overproduction of filaments in the biomass deteriorate activated sludge floc structure and lead to poor separability and compatibility [4].

The traditional way of overcoming nutrient deficiency through applying external nutrient sources does not only increase the cost of the treatment, but a possible overdosing likely occurring under the fluctuating wastewater characteristics may even create problems like necessity of N-removal, that would otherwise not exist. Therefore, it might seem to be safer to use external nutrient dosing for marginal availability based on ratios published in the literature [4].

Glycogen accumulating organisms (GAOs), known to be responsible for failures of enhanced biological phosphorous removal (EBPR) systems, are a group of microorganisms that take up volatile fatty acids (VFAs) and other readily biodegradable substrates (e.g. sugars) under anaerobic conditions and store them as polyhydroxyalcanoates (PHAs). They gain energy necessary for this process from glycogen degradation. Under aerobic condition, GAOs oxidize PHA for cell growth and replenishment of their glycogen pools. Due to the similarity of growth conditions required, GAOs can survive and grow in EBPR systems and directly compete with phosphorous accumulating organisms (PAOs) for carbon sources [5]. That is the reason why most of the scientific studies have been carried out aiming to find ways to efficiently suppress their growth. Factors such as organic load [6, 7], type of influent carbon source (e.g. acetate, propionate, etc.) [8, 9], influent phosphorous to carbon ratio (P/C) [10, 11], nitrite content [12], pH [13, 14], temperature [15], DO (dissolved oxygen) concentration and aerobic HRT (hydraulic retention time) [16] have been pointed out as determining factors to influence the PAO-GAO competition. Tu and Schuler [17] found in a long-term lab-scale sequencing batch reactor (SBR) experiment that growth of GAOs may not be a direct cause of PAO failure, but the consequence under high anaerobic acetate availability at lower pH values.

Jobbágy et al. [18] verified that through application of an appropriate anaerobic-aerobic bioreactor combination growth of GAOs can be enhanced for a cost-effective treatment of both N and P deficient winery-type wastewater. These lab-scale results showing good sludge settleability and COD removal without any external nutrient dosing have also been verified in full-scale at an existing winery wastewater treatment plant in Hungary [19].

Influence of nutrient deficiency on the biomass settleability [20-23] and on the growth of GAOs [11, 24, 25] has already been the topic of a number of additional scientific studies as well. In laboratory scale SBR experiments Harper and Jenkins [26] investigated the impact of completely aerobic and anaerobic/aerobic conditions over a range of influent COD/P and COD/N ratios, using acetic acid as primary substrate and indicator for effluent quality as well. Results indicated that in P-deficiency biomass of the anaerobic/aerobic system used more influent carbon source for non-P-containing storage products while nitrogen limitation caused significant increases in both PHA and carbohydrate storage product contents in both of the systems.

The purpose of the current study has been to investigate the effect of different grades of nitrogen deficiency, i.e. marginal nitrogen availability and severe nitrogen deficiency, at both marginal availability and severe deficiency of P on the growth of GAOs and the performance of anaerobic/aerobic activated sludge systems treating characteristically nutrient deficient winery wastewater.

2 Materials and methods

Two continuous-flow experimental systems were operated simultaneously, receiving model winery wastewater influents with differently dosed N and P sources (see Table 1). Both of the systems contained a non-aerated selector (2.5 l), an aerated bioreactor (7 l) and a secondary clarifier (4.5 l). In System1 the concentration of the nitrogen source (NH_4Cl) added was set to a value regarded to possibly represent marginal N-availability due to relatively high yields for the readily biodegradable substrates applied, suggested by earlier experimental results [27].

In System2 there was practically no nitrogen source added externally so it was assumed that the conditions may largely support the growth of GAOs. According to the data of Table 1, in the first phase of the experiment phosphorus concentration was meant to maintain at a non-limiting level in both systems in order to examine just the impact of a sole nitrogen deficiency. Whereas in the second phase of the experiment was no amount of P dosed externally.

Table 1 Influent feed composition

COD : N : P ratio		
	System 1	System 2
31st May – 18th July (0-48. operational day ~7 weeks)	100 : 5 : 1	100 : ~0 : 1
19th July- 7th August (49-72. operational day ~3 weeks)	100 : 5 : ~0	100 : ~0 : ~0

The synthetic wastewater contained 6.8 cm^3/l 'BB Szürkebarát demi sec' type wine (~800 mg/l COD), 1182 mg Sodium-Acetate/l (~400 mg/l COD) and 0.6 g/l sugar (sucrose) (~700 mg/l COD) as carbon source giving a total of ~1900 mg/l COD. NH_4Cl was used as nitrogen source in a concentration of ~90 mg/l TN in System1, whereas in System2 the total influent nitrogen concentration was ~ 2 mg/l, originating solely from wine. Phosphorus derived from the externally added K_2HPO_4 in a concentration of 19 mg/l in the first seven weeks of the experiment (31st May – 18th July). Despite stopping external P-dosing in the second phase of the experiment the feed still had some phosphorus content (0.6 mg/l) deriving from the wine. Minerals in the feed were 36 mg/l $CaCl_2$ and 16.5 mg/l $MgCl_2$.

The systems were seeded by an activated sludge mixture deriving in a ratio of 1:1 from a GAO-based winery and a municipal wastewater treatment plant.

The operation was monitored through the daily determination of temperature, pH and Dissolved Oxygen (DO) concentration, Mixed Liquor Suspended Solids (MLSS) concentration and Diluted Sludge Volume Index (DSVI). The concentrations of the Chemical Oxygen Demand (COD), dissolved COD, Total Organic Carbon (TOC), Total Nitrogen (TN), ammonia, nitrate and nitrite were measured three times a week, while phosphate and total phosphorous concentrations were measured

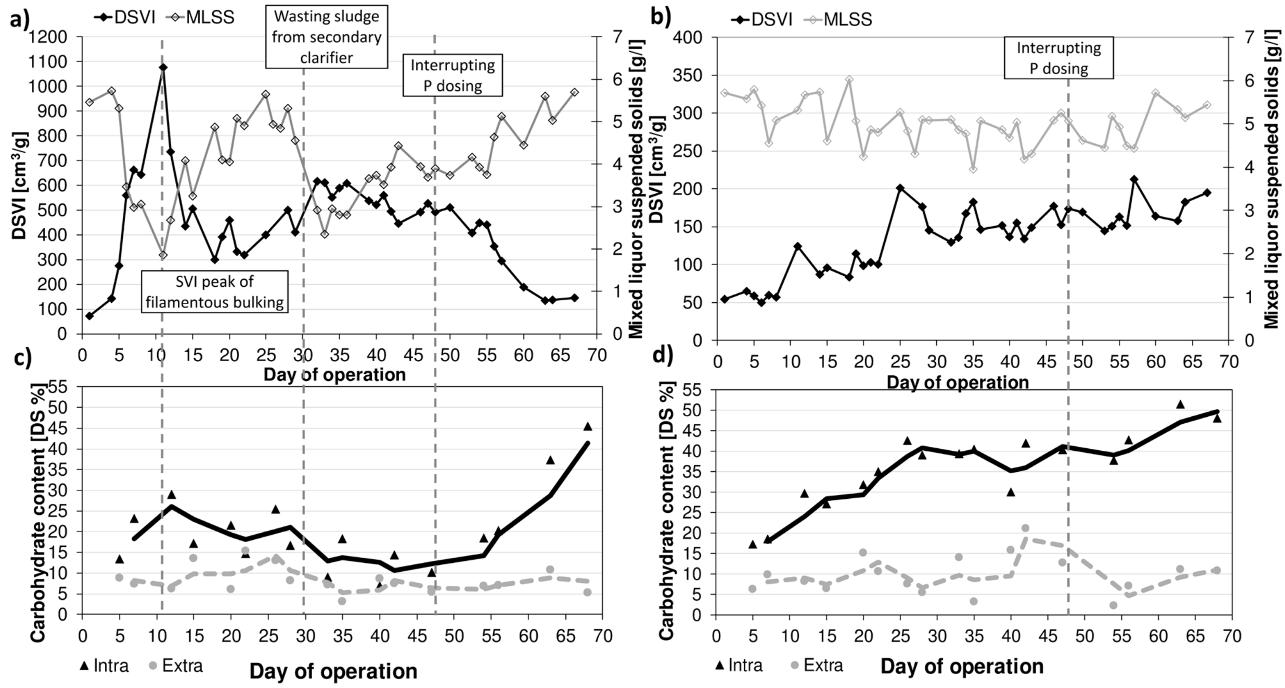

Fig. 1 Sludge concentration (MLSS) and Diluted Sludge Volume Index (DSVI) of the biomass (a) in System1 (COD: N_{dosed} = 100:5) and (b) in System2 (without N-dosing); and intra- and extracellular carbohydrate concentration of the biomass (c) of System1 and (d) of System2

twice a week according to Hungarian standard methods (MSZ 260/3:1973, MSZ ISO 6060:1991, MSZ ISO 7150-1:1992, MSZ 260/10:1985, MSZ 260/11:1971, MSZ EN 25663:1998, MSZ 260/20:1980). In addition to these commonly investigated parameters, total as well as intra- and extracellular carbohydrate contents of the biomass were also investigated applying the procedure developed by Jobbágy et al. [18]. In order to follow the changes in the biomass structure and composition microscopic observations were carried out using a direct light microscope (OLYMPUS CX41) under phase contrast and applying India Ink staining for the sludge samples taken from the aerated reactors of the systems.

Mixed influent concentrations of different parameters depicted in the figures enclosed were calculated based on the content in the feed and that of the recycled sludge.

3 Results and discussion

The initial MLSS values for biomass concentrations in the systems were set to 6 g/l. The influent flow rate was maintained at 0.5 l/h and the sludge recycle rate at 0.75 l/h. Therefore, the hydraulic retention time (HRT) was 19 h in both systems, and the solids retention time (SRT) was approximately 6 days. In the anaerobic selectors the pH values usually proved to be 6.7-7.0, lower than those in the aerated bioreactors (7.8-8.2), where the average DO concentration was maintained between 3 and 4.5 mg O_2/l. The temperature was monitored but not controlled throughout the experiment; its value remained at 23.5±1.5 °C.

3.1 System1 operated at marginal N-availability

This system was set to operate on the edge of nitrogen deficiency. The consequent instable performance can easily be followed through the changes of the biomass concentration and settling characteristics in Fig. 1a. Having started the experiment, DSVI values showed a steep increase from ~80 cm^3/g to 1100 cm^3/g in just 11 days. Periodical improvement of sludge settling characteristics can be brought in connection with decreasing biomass concentration. However, DSVI values remained in the range of 300-600 cm^3/g, and unambiguous tendency for reaching the really low starting point could just be observed after P-dosing had been interrupted. Fig. 2 shows, that on the first 7 experimental days ammonia was entirely used up in the aerated reactor under low-S conditions, i.e. at low readily biodegradable COD (rbCOD) concentrations [28]. As there was no detectable nitrate or nitrite production, it can be assumed that all of the N available was built into the cells referring to marginal availability. Comparing the ~12 mgN/l ammonia consumed in the selector and the ~35 mgN/l ammonia consumed in the reactor, to the appropriate amounts of COD removed, ratios of 100 rbCOD to ~4.8 NH_4N in the selector and 100 rbCOD to ~6.14 NH_4N in the reactor can be calculated. It can be assumed that the marginal N-availability coupled with low-S conditions in the reactor where the major part of rbCOD was removed initiated the severe filamentous bulking (see also Fig. 4a) in the system.

The increasing filamentous ratio in the biomass caused compacting problems in the secondary clarifier resulting in decreasing biomass concentration in the reactors. This consequence appears as a negative peak in sludge concentration starting from operational day 5 in Figs.1a and 2b.

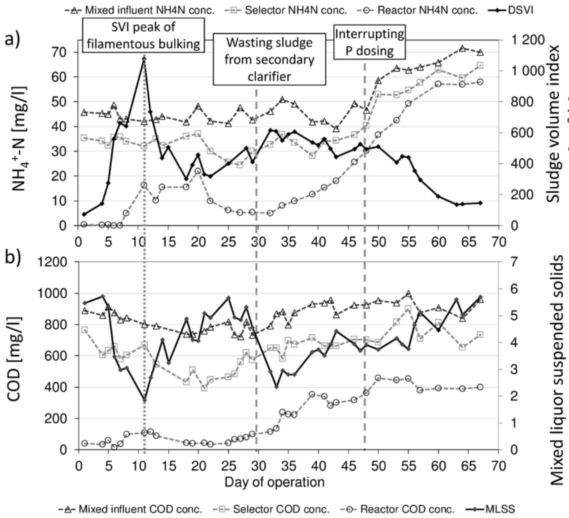

Fig. 2 (a) Ammonia concentrations and SVI values and (b) COD concentrations and MLSS values in System1

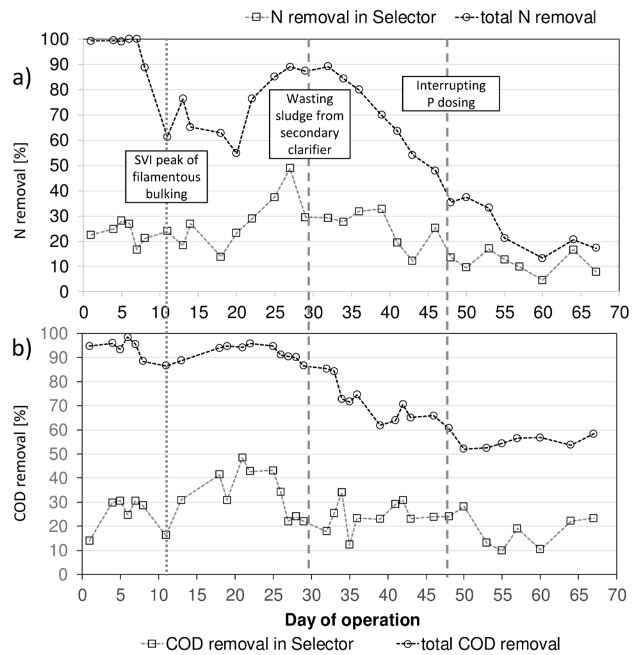

Fig. 3 (a) Nitrogen and (b) COD removals in System1

Due to the decreasing consumption rates, effluent levels of both COD and NH_4N increased leading to less favourable growth of filaments, better sludge settling and increasing MLSS concentrations. However, this again pushed down the effluent levels and caused increasing difficulties in sludge compacting (Figs.1a, 2a and 2b.).

In the secondary clarifier the cumulated sludge started to decay, which was confirmed by grab samples that showed higher ammonia concentration in this unit compared to the values measured in the reactor (data not shown). Therefore, on the 30th day of the experiment a part of the sludge was removed from the system and the rest was diluted by the effluent. Although by this intervention about the same MLSS concentration could be achieved as the one measured on the 10th experimental day, recovering proved to be slower in sludge structure indicated by less steeply decreasing DSVI and increasing MLSS concentration. Relatively low removal rates of both COD and ammonia being probably also affected by anaerobic sludge decay caused increasing effluent concentrations (see Figs. 2 and 3).

The most lucrative effect of interrupting P-dosing is the drastic improvement of sludge settling characteristics (see Fig. 1a), and thereby the quick stabilization of the performance. However, under these conditions just about 60% of the influent COD could be removed (see Fig. 3b), using up NH_4N at a ratio of 100:1.7. It is important to emphasize that the bulk of N-source was externally dosed to the influent during the experiment. Thus, the remaining high concentration of effluent NH_4-N may just refer to the possibility of nutrient overdosing to a nutrient deficient wastewater. In this undesired case, nitrification and denitrification should be applied in order to remove excess N-concentration for meeting the effluent requirement.

As it is illustrated in picture Fig. 4c, microscopic observations support that the highly improved settling characteristics may be attributed to the enhanced growth of tetrad-shaped floc-forming organisms. Fig. 1c shows accordingly the results of intra- and extracellular carbohydrate content of the biomass throughout the 68 experimental days. The starting values of 15-20% intracellular carbohydrate contents correspond well to a 1:1 mixture of domestic and GAO-based activated sludges [18, 19]. In System1, at marginal N-availability this value basically decreases until pronounced P-deficiency is created. Then the intracellular polysaccharide content increases gradually up to 40-45% having been found to be characteristic of a GAO-dominated activated sludge treating winery wastewater under nutrient deficiency [18, 19].

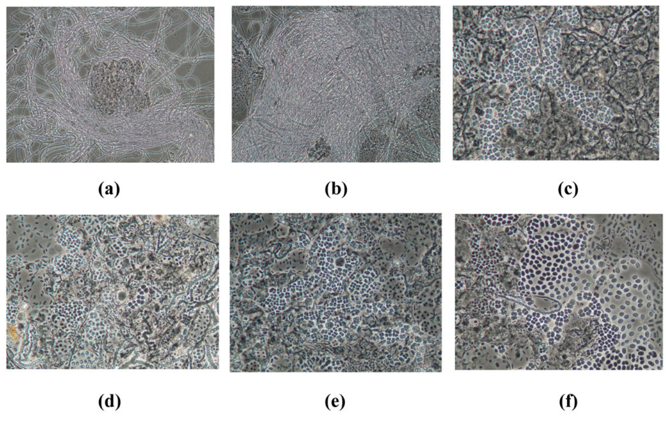

Fig. 4 Activated sludge floc structure of System1 (a) filamentous bulking on the 11th day, 200x, (b) filamentous bulking on the 29th day 200x, (c) Tetrad-shaped floc forming organisms on the 62th day, 1000x, Phase contrast; and GAO proliferation in the biomass of System2 (d) GAOs from seeding sludge on 4th day (e) large number of TFO type GAOs on the 32nd day (f) Tetrad-shaped and oval floc forming organisms on the 67th day, 1000x, Phase contrast

3.2 System2 without any external N-dosing

In this system at TN ~2 mg/l coming from the wine, impact of sole deficiency of nitrogen combined with P-deficiency in the last period of the experiment was investigated. As it is illustrated in Fig. 1b, System2 showed a highly increased stability regarding both sludge concentration and settling characteristics compared to System1 (see Fig. 1a) operated with marginal N-availability. There could be no steep changes observed, even the transient to and through the P-deficiency proved to be smooth preserving the relatively good biomass settling abilities. Microscopic observations showed that abundance of filamentous bacteria initially present in the seed was decreasing, while floc forming microorganisms had an enhanced growth (see pictures in Figs. 4d and e).

Experimental data refer to the competition of two kinds of floc-formers: phosphorus accumulating microorganisms (PAOs) and GAOs. As it is shown in Fig. 5a, at the beginning of the experiment significant phosphorus release (~12 mgP/l) could be detected in the non-aerated selector. However, this showed a gradual decrease practically from the beginning of the experiment, just like the TP content of the biomass (originally 1.3-1.5% of DS). The decreasing P-release could very well be brought in connection with the increasing intracellular carbohydrate content of the biomass (see Fig. 5b). This verifies that under the given conditions, i.e. in severe deficiency of N, GAOs may outcompete PAOs.

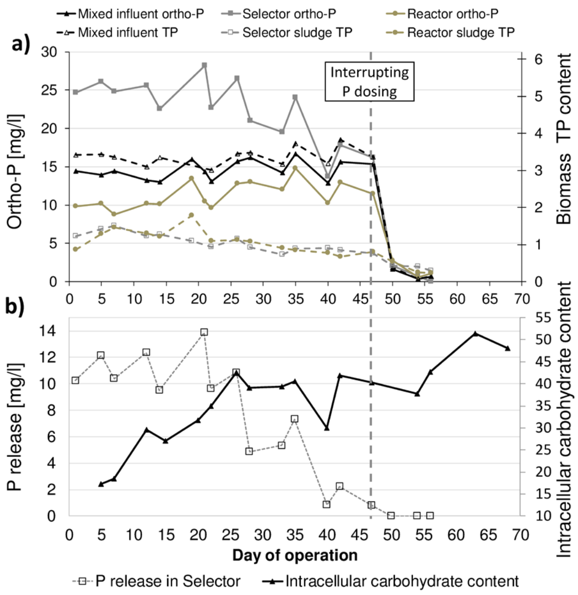

Fig. 5 (a) Ortho-phosphate concentrations and (b) P-release and biomass intracellular carbohydrate concentration values in System 2

Despite the unambiguous changes in the biomass structure, no drastic changes could be observed in COD-removal (see Fig. 6). The total COD elimination was relatively high (90% in average) compared to System1. In average 30% of COD removed was taken up in the anaerobic selector. This rate did not considerably decrease, rather increased, with decreasing PAO activity in the system. Moreover, effluent COD values it stayed stable even after the external phosphorus dosing was interrupted.

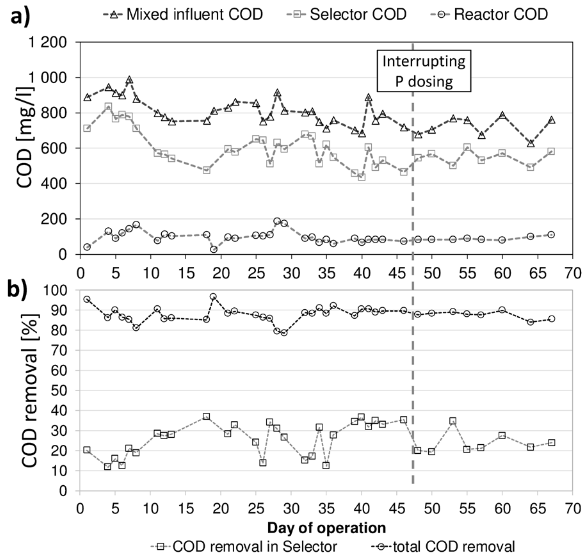

Fig. 6 (a) COD concentrations and (b) COD removal in System2

The COD consumption corresponding to the P release in the non-aerated selector (see Table 2) was calculated according to Schuler and Jenkins (2003) using the coefficient of 0.08 mol P/C-mol which corresponds to a GAO dominated media according to measurements carried out earlier in a non-aerated selector under similar conditions. While the $P_{release}$/acetate uptake ratios were calculated (Table 2) from the measured P release and COD consumption data converted into acetate C-mol to facilitate the comparison with previously reported values.

Table 2 Evolution of the $P_{release}$/acetate uptake ratio in the non-aerated selector

Day	Measured P release	Calculated P release relatable Acetate uptake in GAO dominated media*	Measured COD consumption	Anaerobic $P_{release}$/COD uptake
	mmol P	mg COD/l	mg COD/l	mol/C-mol
5	0.39	157	147	0.08
7	0.34	135	210	0.05
12	0.40	160	229	0.05
21	0.45	180	234	0.05
22	0.31	***125***	***282***	0.02
28	0.16	***63***	***285***	0.02
40	0.03	***11***	***251***	0.03
42	0.07	***29***	***263***	0.01
50	0.01	***5***	***137***	0.003

*calculated by using 0.08 mol/C-mol ratio

Results summarized in Table 2 show that following the 22nd day the measured COD consumption in the non-aerated selector proved to be considerably higher than the COD uptake relatable to PAO activity. This supports the dominance of GAOs from that time. The decrease of the calculated $P_{release}$/acetate uptake ratios in the non-aerated selector refers to the same tendency.

Interrupting external P-dosing did not lead to the complete lack of phosphorus in the influent. Moreover, its value decreased to 0.6 mg P/l, that immediately cut phosphorous removal, just like in EBPR systems where chemicals for reducing phosphorus content are overdosed [25].

4 Conclusions

Experimental results verified that

- combined marginal N and P availability created by the common practice of external nutrient dosing may cause filamentous bulking and thereby difficulties in an instable operation of an activated sludge system
- sole pronounced deficiency of either N or P source lets GAOs grow and outcompete PAOs from the biomass. These causes have to be considered in operating EBPR systems, e.g. at overdosing chemicals for P-removal.
- Joint severe deficiency of both N and P ensured stable operation and good performance through enhanced proliferation of GAOs.

Acknowledgement

The authors wish to express their special thanks to József Simon for his valuable technical support.

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