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Temperature Effects on the Activity of Denitrifying Phosphate Accumulating Microorganisms and Sulphate Reducing Bacteria in Anaerobic Side-Stream Reactor

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Abstract

Four different temperatures, 5, 10, 15 and 20°C were tested in batch assays to investigate temperature effects on denitrifying phosphate accumulating organisms (DPAOs) and sulphate reducing bacteria (SRBs), showed to play a main role in the biological sludge reduction process. The effect of temperature on aerobic PAOs was also investigated for reason of completeness. Tests were performed using a selected biomass from a laboratory scale anaerobic side-stream reactor (ASSR) process coupled in a sequencing batch reactor (SBR) for nutrient removal.

Results showed that the phosphate release and uptake kinetics of PAOs and DPAOs were influenced by the variation of the temperature, while temperature did not influence significantly the anaerobic and the anoxic stoichiometry of the process.

In general, decreasing the temperature, a decreasing in the P-uptake and P-release rates was observed. The temperature had a moderate effect on SRBs activity.

Arrhenius temperature coefficients, θ, for anaerobic, aerobic and anoxic PAOs metabolism were found to be 1.114, 1.121 and 1.165, respectively, while θ for SRBs metabolism was 1.087. Those results indicated that DPAO activity was more affected by lower temperatures than aerobic PAOs and SRBs activities, representing the limiting step of the biological sludge reduction process at low temperature.

Keywords: Temperature; PAO; DPAO; SRB; ASSR

Abbreviations: PAO: Phosphate Accumulating Organisms; DPAO: Denitrifying Phosphate Accumulating Organisms; SRB: Sulphate Reducing Bacteria; ASSR: Anaerobic Side-Stream Reactor

Introduction

Temperature changes could strongly affect microbial activity. This dependence is linked to the kind of bacteria used. Any species' response to temperature is characterized by upper and lower limits of temperature for growth [1]. When the temperature increases the rate of denaturation of particular cell components increases as well, with the consequent disruption of cellular function. On the contrary, when the temperature is too low a loss of efficiency of transport proteins embedded in the membrane, and thus a loss of affinity for substrates, occurs [2]. Temperatures below the optimum typically have a more significant effect on growth rate than temperatures above the optimum [3].

Fermenting bacteria, able to release extracellular polymeric substances and hydrolyze organic matter, and slow growing bacteria, involved in nutrient removal, could have an important role in the biological sludge reduction process, where an anaerobic side-stream reactor (ASSR) is implemented in an activated sludge system [4,5]. The selection of sulphate reducing bacteria (SRBs) and denitrifying phosphate accumulating organisms (DPAOs) has been detected in the ASSR coupled in a sequencing batch reactor (SBR) for nutrient removal [4]. Most of the literature studies regarding the implementation of an ASSR in a activated sludge system were performed at controlled temperature ranging between 18-20°C. However, the effects of temperature on the ASSR performance have not yet investigated in literature [6]. Thus, one of the main questions that may arise is how the temperature could affect the activity of two important bacteria selected in the ASSR: SRBs and DPAOs.

SRBs are anaerobic microorganisms that are widespread in anaerobic habitats. They use sulphate as a terminal electron acceptor for the degradation of organic compounds, producing sulphide. Although limited studies have been performed so far, the presence and the activity of SRBs in urban wastewater treatment plants has been clearly observed [7, 8]. Further, they are of increasing importance in wastewater treatment because of their ability to remove COD in the presence of sulphate with low excess sludge production (0.3 gVSS/gCOD) [9]. The effect of the temperature on SRBs has been little studied, with contrasting results. Sahinkaya et al. [10] reported an unsuccessful reactor operating at 8°C with ethanol as electron donor for SRBs. On the contrary, Ingvorsen et al. [11] reported that at 5°C SRBs in activated sludge still show an exponential growth profile, although the rate was lower than that at 20°C.

Phosphate accumulating organisms (PAOs) have been identified in the biological phosphorus removal (BPR) process. Aerobic PAOs are capable to release phosphate under anaerobic conditions, while consuming it only under aerobic conditions [12]. Further, Jørgensen and Pauli [13] demonstrated that, a part from aerobic conditions, also under anoxic conditions, with nitrate as the electron acceptor, denitrifying phosphate accumulating organisms (DPAOs) are capable

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of polyphosphate accumulation. Thus, the following PAOs metabolic pathways have been identified [14]: (i) under anaerobic conditions, acetate or other low molecular weight organic compounds are converted to polyhydroxyalkanoates (PHAs), while intracellular stored polyphosphate (poly-P) and glycogen are degraded, and phosphate, generated from Poly-P degradation, is released into the bulk liquid; (ii) under aerobic or anoxic conditions, the stored PHAs are converted to glycogen, phosphate is assimilated, and poly-P is intracellular produced using oxygen and nitrate/nitrite as electron acceptor, respectively for aerobic or anoxic environment. Thus, bacterial growth and phosphate uptake are regulated by the energy released from the breakdown of PHAs.

Up to now, the temperature effect on PAOs activity had been investigated under anaerobic conditions (phosphate release process) and aerobic conditions (phosphate uptake process). On the contrary, studies on the effect of temperature on the anoxic DPAOs activity are missing in literature.

Concerning PAOs, conflicting results have been achieved. Brdjanovic et al. [15, 16] suggested that the contrasting results obtained so far on the temperature effects on BPR could be explained by the use of different substances, activated sludge and measurement methods. The authors, probably for the first time, studied the temperature effects (over a range 5 - 30 °C) on stoichiometry and kinetics of aerobic PAOs. Results showed that the stoichiometry of the anaerobic process was insensitive to temperature changes while several effects on aerobic stoichiometry were observed. On the contrary, the temperature had a strong impact on the kinetics of the process under anaerobic as well as under aerobic conditions. Based on these results, the authors calculated the anaerobic and aerobic temperature coefficients θ from short-term steady state experiments that were found to be 1.055 and 1.065, respectively [16]. Temperature coefficients obtained by Brdjanovic et al. [16] were used in Meijer [17] to create a new extended model for BPR. Henze et al [18] reported that this model was successfully applied for simulation of enhanced BPR (EBPR) systems [19,20] and for developing an anaerobic and aerobic metabolic models that incorporated carbon source, temperature and pH dependences of PAOs and (glycogen-accumulating organisms) GAOs [21, 22]. The results of this study showed that, for pH of 7.5 and temperature lower than 20°C, PAOs tended to be the dominant microorganisms and, therefore, beneficial for the BPR.

In this light, the main goal of this study was to establish the short term temperature effects on anaerobic, aerobic and anoxic metabolisms of aerobic- and denitrifying- PAOs and anaerobic metabolism of SRBs. The effects of temperature on the stoichiometry and kinetics of the biological processes have been evaluated. Four different temperatures, 5, 10, 15 and 20°C were tested in batch assays using a selected biomass from a laboratory scale ASSR process performed at room temperature.

Material and methods

PAOs and SRBs enriched sludge

A sludge enriched with PAOs and SRBs was developed in a laboratory scale ASSR integrated in a nutrient removal activated sludge system as previously described [4, 23]. The experimental set-up consisted of a sequencing batch reactor (SBR) and an ASSR where the produced sludge was treated. A denitrifying side-stream reactor (DSSR) was introduced in the treatment scheme both to increase the solid concentration in the sludge to cycled back to the ASSR and to complete the nitrate removal in order to ensure a tightly anaerobic environment in the ASSR (oxidation - reduction potential (ORP = -400 mV). The SBR had a working volume of 10 liters and operated with six cycles a day in alternate denitrification/ nitrification mode to remove carbon and nutrient from real urban wastewater.

The ASSR, having a working liquid volume of 10 liters, was completely mixed and equipped with a mechanical stirrer. The anaerobic solid retention time (SRT_{ASSR}) of the ASSR was 2.5 d and the sludge interchange ratio (IR) was equal to 100%, meaning that the whole sludge in the SBR was subjected to the anaerobic conditions in the ASSR. Figure 1 shows the SBR-ASSR experimental set-up and the system mode of operation.

The lab-scale system operated at room temperature ranged between 18 and 21°C. The ORP and the pH in the ASSR were monitored and left to vary. The characteristics of the ASSR biomass are reported in Table 1.

Batch phosphate accumulating bacteria assays

The assays were performed in a double jacked laboratory reactor with a maximal operating volume of 2.0 L and a working volume of sludge of 1.5 L. The reactor was mixed with a magnetic stirrer (150 rpm). The total suspended solid (TSS) concentration in the batch test reactor was approximately 8.5 gTSS / L. Each test lasted at least 12 hours, and it was composed of three different phases: anaerobic (240 min), aerobic (at least 240 min), and anoxic (at least for 240 min). The initial pH value was equal to 7.4. The final pH value of each phase was always measured in order to check that it was maintained in the optimal range for the biological activity (7.0 - 7.5). The batch tests were sampled every 30 minutes. The samples were immediately vacuum filtered on 0.45 µm membrane filters and analyzed. A summary of operating conditions is reported in Table 2.

Anaerobic phase: The anaerobic phase was performed to evaluate the total release of phosphate by total (PAOs), both aerobic PAOs and DPAOs. Nitrogen gas was injected into the reactor at the beginning of the anaerobic experiments. DO and ORP were continually monitored. Nitrate was also monitored during the experiment to ensure that anaerobic conditions were present. Working temperatures (5, 10, 15 or 20 °C) in the batch reactor were set 1 h before the beginning of the test, in order to acclimatize the biomass. After this period, acetate was added to the batch reactor as substrate for TPAOs. In accordance to Brdjanovic et al. [15], the amount of acetate added to the batch reactor was less, but not limiting, at lower temperature (from 350 mg COD/L at 20°C to 250 mg COD/L at 5°C) in order to obtain a similar acetate uptake at each temperature without changing the duration of the anaerobic phase. At the beginning of the anaerobic phase, sulphate was further added in order to enhance the activity of SRBs. A concentration of 50 mg SO_4^{2}-S/L was ensured at the beginning of the tests. Anaerobic conditions were maintained for 240 min. For each temperature, the anaerobic phase was double performed in two reactors (test A, B).

Aerobic phase: In the Test A, the aerobic phase was performed to evaluate the aerobic phosphate uptake rate of TPAOs. Aerobic conditions were ensured by sparging air through the bulk liquid using an aquarium air stone and an air compressor (Schego M2K3350). The resulting DO concentration was about 5.5 mg O_2 / L. The aerobic phases were carried out until no further changes in concentration of PO_4-P could be observed and lasted at least for 240 min.

Anoxic phase: In the Test B, the anoxic phase was performed in order to evaluate the denitrifying phosphate uptake rate of DPAOs. Anoxic conditions were obtained by adding nitrate to the bulk solution

Table 1: Characteristics of the biomass used as culture

Parameter	ASSR
Soluble COD	43 mg sCOD/ L
Ammonium Nitrogen	30 mg NH_4-N /L
Nitrate	0.1 mg NO_3-N/L
Soluble phosphate	32 mg PO_4-P/L
Sulphate	13 mg SO_4^{2-}-S/L
Total suspended solids (TSS)	8.5 g TSS / L
pH	7.4
ORP	-400 mV

Table 2: Batch phosphate accumulating bacteria assays: operative parameters

Test	Phases	5°C	10°C	15°C	20°C
Test A	*Anaerobic*	250 mg^{s}COD / L*	300 mg^{s}COD / L*	325 mg^{s}COD /L*	350 mg^{s}COD / L*
		50 mg SO_4^{2-}-S /L*	50 mg SO_4^{2-}-S / L*	50 mg SO_4^{2-}-S /L*	50 mg SO_4^{2-}-S /L*
(TPAO)	*Aerobic*	5.5 mg O_2/ L	5.5 mg O_2/ L	5.5 mg O_2/ L	5.5 mg O_2/ L
Test B	*Anaerobic*	250 mg^{s}COD / L*	300 mg^{s}COD / L*	325 mg^{s}COD / L*	350 mg^{s}COD / L*
		50 mg SO_4^{2-}-S / L*	50 mg SO_4^{2-}-S / L*	50 mg SO_4^{2-}-S / L*	50 mg SO_4^{2-}-S / L*
(DPAOs)	*Anoxic*	30 mg NO_3^--N / L	30 mg NO_3^--N / L	30 mg NO_3^--N / L	30 mg NO_3^--N / L

*Concentrations measured after working temperature was achieved in each batch test (after 60 min from the beginning of batch tests)

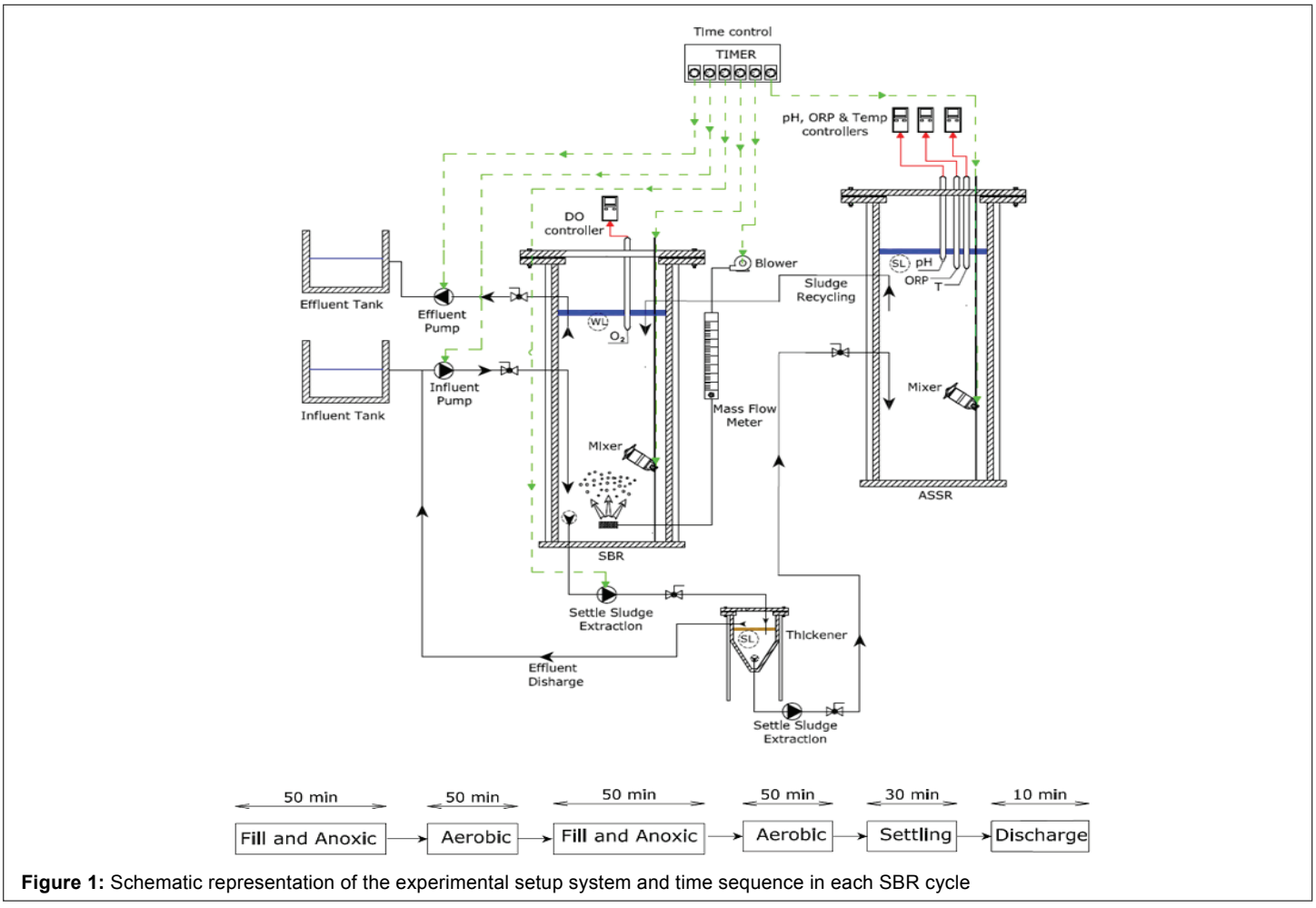

Figure 1: Schematic representation of the experimental setup system and time sequence in each SBR cycle

at a concentration of 30 mg NO_3^--N / L. DO and ORP values were continuously monitored during the entire experimental test. The anoxic phase was carried out until no further changes in concentration of PO_4-P could be observed and lasted at least for 240 min (Table 2)

Sulphate reducing bacteria assays

The SRB assay was performed simultaneously with the evaluation of the total release of phosphate by TPAOs in the anaerobic phase. Tests were conducted at 5, 10, 15 and 20°C. At the beginning of the phase a concentration of 50 mg/L of sulphate was added in order to enhance the activity of SRBs. Anaerobic conditions were maintained for 240 min.

Calculations

Specific uptake rate: The uptake and release rates were determined as the maximum slope of the substrates profile through linear regression. The specific uptake and production rates were calculated as the ratios of uptake and release rates and the biomass concentration as TSS.

Temperature coefficients: The temperature coefficient θ was calculated for each reaction rate using the simplified Arrhenius equation for the temperature dependency (Equation 1):

$$r_T = r_{20} \cdot \theta^{(T-T_{20})} \qquad (1)$$

where T is the temperature in °C, r_T is the kinetic parameter at temperature T, T_{20} is the reference temperature (20°C), r_{20} is the kinetic parameter at a temperature equal to 20°C, and θ is the Arrhenius temperature coefficient. This expression was used to describe the effect of the temperature both on phosphorus release and on sulphate consumption in anaerobic tests, and on phosphorus uptake in aerobic

and anoxic tests.

Chemical analyses

TSS was measured according to the Standard Methods [24]. The samples were filtered through 0.45 µm membrane filters. The filtrate was analyzed for soluble COD (sCOD), ammonium nitrogen (NH_4^+-N), nitrate as nitrogen (NO_3^- -N), soluble orthophosphate (PO_4-P) and sulphate as sulphur (SO_4^{2-}-S). NH_4^+-N, NO_3^--N and PO_4-P concentrations were determinate according to the Standard Methods [24]. Sulphates were analyzed by an ion chromatograph (DIONEX ICS-100) equipped with an AS9-HC column. COD in the form of sodium acetate (CH_3COONa), nitrate in the form of sodium nitrate ($NaNO_3$) and sulphate in the form of sulphuric acid (H_2SO_4) were added at the required final concentration.

Results

Effect of temperature on P release by total PAOs

Figure 2 shows the distribution of PO_4-P, NH_4^+-N, SO_4^{2-}-S and sCOD during the anaerobic period for all culture series. Nitrate concentration was monitored during the entire experimental period and its value was always equal to zero. The orthophosphates profiles in the anaerobic phase have been related to the anaerobic metabolism of total PAOs, as they store acetic acids as PHB through the cleavage of poly-P with the associated release of phosphate in the bulk solution [25].

The graphs clearly indicated that, both the P- release and the sCOD uptake changed largely with a different temperature. In each assay, the added acetate was partially consumed, corresponding to sCOD removal efficiencies of 2%, 3%, 9% and 10% at 5, 10, 15 and 20°C, respectively (Figure 2).

At 5°C (Figure 2a) and 10°C (Figure 2b) the specific P- release rates were very low and equal to 0.06 mg PO_4-P / (g TSS h) and 0.08 mg PO_4-P / (g TSS h), respectively. At 15°C (Figure 2c) the specific P release rate increased up to 0.20 mg PO_4-P / (g TSS h), reaching the maximum value of 0.30 mg PO_4-P / (g TSS h) at 20°C. Thus, data showed that the P release rate at 15, 10 and 5°C was 33, 73 and 81% lower than that measured at 20°C, respectively.

Similarly, the rate of sCOD uptake was very low both at 5 and 10°C, accounting for 0.23 mg sCOD / (gTSS h) and 0.27 mg sCOD / (gTSS h), respectively. The rate of sCOD uptake increased at 15 and 20°C, reaching values of 0.89 and 1.15 mg sCOD / (gTSS h). The sCOD uptake rate at 15, 10 and 5°C was 23, 77 and 80% lower than that measured at 20°C, respectively.

The stoichiometric ratios of the PO_4-P released and the sCOD consumed at each experimental temperature were listed in Table 3.

Smolders et al. [26] obtained similar results from a MBR sludge, where for 1 C-mol of acetate 0.5 P-mol phosphate was released in anaerobic conditions, obtaining a PO_4-P /acetate ratios of about 0.25. Brdjanovic et al. [16] showed that under anaerobic conditions, P release activity increased from 5°C to 20°C, reaching a maximum at 20°C.

In this study, results from short-term tests confirmed that the stoichiometry of the anaerobic phase was relatively insensitive to temperature changes. However, the stoichiometric ratios measured in this study were lower than the values reported by Brdjanovic et al. [16]

Table 3: Stoichiometric PO_4 -P release/s COD uptake(anaerobic conditions) and PO_4 -P uptake / NO_3^--N consumed(anoxic conditions) ratios

Temperature [°C]	5	10	15	20
PO_4^--P release/s COD uptake	0.26	0.29	0.22	0.26
PO_4 -P uptake/ NO_3^--N consumed	0.07	0.10	0.19	0.29

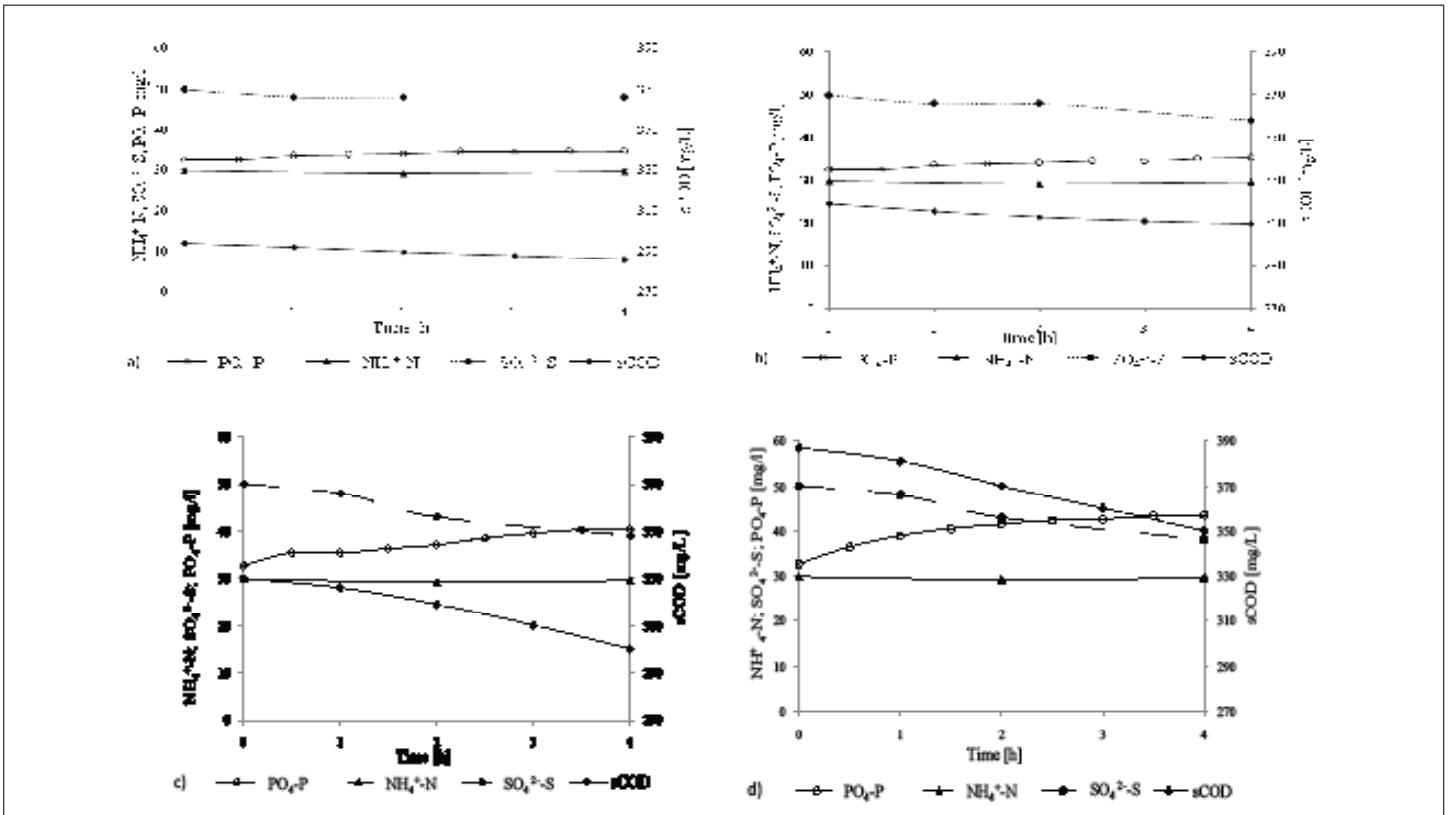

Figure 2: Temperature effects in anaerobic phase of batch phosphate accumulating bacteria assays: a) 5°C; b) 10°C; c) 15°C; d) 20°C

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who measured PO_4-P / acetate ratios of about 0.4. The lower ratios measured in this study have been related to the simultaneous SRBs and total PAOs metabolic activity in the tests, that cause a higher consumption of the sCOD.SRBs consumed sulphate for the degradation of organic compounds (Figure 2). The additional sCOD consumption by SRBs during the anaerobic conditions caused a lower PO_4-P / acetate ratio.

At the end the tests the TSS concentrations at 5, 10, 15 or 20°C were 8.5, 8.4, 8.5 and 8.4 g / L, respectively. These data showed that bacteria did not grow during the batch tests. Further, ammonia concentration was monitored and was always constant during the anaerobic phase in all batch assays. These results further indicated that the sludge decay and extracellular polymeric substances (EPS) destructuration processes were negligible during the short term experiments.

Effect of temperature on P uptake in aerobic phase by total PAOs

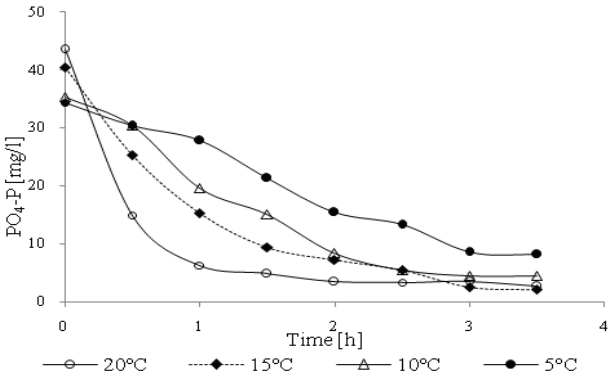

Figure 3: Temperature effects on P -uptake in aerobic phase of batch phosphate accumulating bacteria assays at 5, 10, 15 and 20°C

Figure 3 shows the aerobic phosphate uptake at 5, 10, 15 and 20°C, which has been related to the aerobic metabolism of TPAOs (Figure 3).

The phosphate uptake at 20, 15 and 10°C had an exponential trend. On the contrary, at 5°C the phosphate uptake had a fairly linear trend. The maximum specific P uptake rate was 4.53 mg PO_4-P / (g TSS h) at 20°C. At 15°C, the P uptake rate decreased down to 2.41 mg PO_4-P / (g TSS h). At 10°C and 5°C, the P uptake was 1.47 mg PO_4-P / (g TSS h) and 0.95 mg PO_4-P / (g TSS h), respectively. In aerobic conditions, TPAOs activity was influenced by temperature as well as in anaerobic conditions, but in a less significant way at 10 and 5°C. Indeed, a reduction of 47%, 67% and 79% was observed at 15, 10 and 5°C, respectively. After 3.5 h the same PO_4-P concentration of 3.0 mg PO_4-P /L was reached both at 20 and 15°C. On the contrary, at 10°C and 5°C, the final PO_4-P concentrations were higher, and equal to 5.0 and 10.0 mg PO_4-P /L, respectively. These findings confirmed the results of Brdjanovic et al. [16] who reported that at 5 and 10°C an incomplete P-uptake was observed in the aerobic phase, while a complete P-uptake at 20 and 30°C was measured.

Effect of temperature on P uptake in anoxic phase

Figure 4 shows the anoxic phosphate uptake at 5, 10, 15 and 20°C, linked to the anoxic metabolism of DPAOs. The added nitrate was totally consumed only at 20°C, while it was partially consumed up to 44%, 68% and 84% at 5, 10 and 15°C, respectively (Figure 4).

Results showed that the anoxic P- uptake kinetic was influenced by the temperature. It had an exponential trend at 20 and 15°C, which became linear at 10 and 5°C. At 5 and 10°C the specific P- uptake rate was equal to 0.24 mg PO_4 -P / (g TSS h) (Figure 4a) and 0.53 mg PO_4 -P / (g TSS h) (Figure 4b), respectively. At 15°C specific P -uptake rate was 1.55 mg PO_4 -P / (g TSS h) (Figure 4c), increasing up to 3.01 mg PO_4 -P / (g TSS h) at 20°C. Thus anoxic P -uptake rate was 48, 82 and 92% lower than the value measured at 20°C. The trends of the phosphorus uptake at different temperatures showed that the anoxic DPAOs metabolism could be negatively affected by the low temperature, more than the aerobic PAOs metabolism.

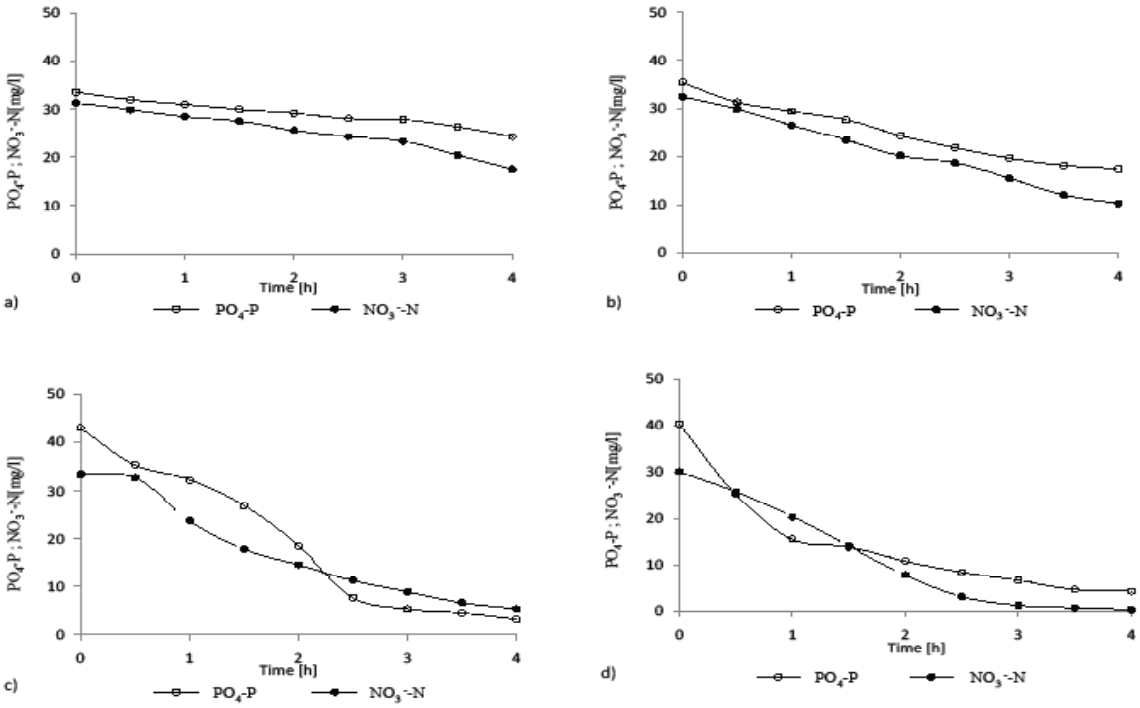

Figure 4: Temperature effects on P and nitrate uptake in anoxic phase of batch phosphate accumulating bacteria assays: a) 5°C; b) 10°C; c) 15°C; d) 20°C

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The stoichiometric ratios of the PO_4 -P uptake and the NO_3^--N consumed at each experimental temperature were listed in Table 3.

On the contrary of the anaerobic phase, stoichiometry of the anoxic metabolism was strongly influenced by temperature changes. In particular, the PO_4 -P uptake/ NO_3^--N consumed ratio became greater as the temperature increases.

Effect of temperature on S uptake by SRBs

Sulphate concentration was monitored during each batch test as a marker of the SRBs activity. Results from the short term experiments showed that low temperatures affected SRBs activity. The specific sulphate uptake rate was 0.05, 0.17, 0.33 and 0.38 mg SO_4^{2-}-S/ (gTSS h) at 5, 10, 15 and 20°C. For temperature equal to 20 and 15°C, the sulphate uptake differed only for 13%. However, decreasing the temperature to 10 and 5°C, the percentage increased significantly, accounting for 55% and 87%, respectively.

Comparing these results with those directly measured in the laboratory scale ASSR [23], a higher value (0.74 mg SO_4^{2-}-S / (gTSS h)) was found in the ASSR conducted at room temperature than the value obtained in the present study at 20°C. This result could be explained considering that in the laboratory scale ASSR more than one type of electron donor for SRBs could be present, obtaining higher rates than batch tests feed with a single electron donor [27]. Indeed, SRBs can be divided into two main groups: those that degrade organic compounds incompletely to acetate and those that degrade organic compounds completely to carbon dioxide, which commonly also use acetate as a growth substrate [28].

In this study, acetate was selected as a carbonaceous substrate because readily biodegradable by most heterotrophic populations, among them PAOs. Nevertheless, Ferrentino et al. [23] found in a laboratory scale ASSR the presence of bacteria able to hydrolyze complex organic matter producing propionic acid in anaerobic environments. The expected variety of volatile fatty acids (VFAs) in the laboratory ASSR, of acetate and propionate for instance, therefore, is beneficial for sulphate reduction rates.

Temperature coefficients

From short term experiments, the temperature coefficients for the anaerobic, aerobic and anoxic metabolisms of PAOs were equal to 1.114, 1.121 and 1.165, respectively. Brdjanovic et al. [15, 16] for short-term steady state experiments, showed that the temperature had a moderate impact on the anaerobic P-release process rate (θ=1.071) and on the aerobic P-uptake process rate (θ=1.032). In this study, a stronger temperature effect has been evaluated on both the anaerobic P-release process rate (θ=1.114) and on the aerobic P-uptake process rate (θ=1.121). However, similarly to Brdjanovic et al. [15, 16], the aerobic metabolisms of PAOs was higher influenced by temperature as compared to the anaerobic metabolism of total PAOs.

Concerning the anoxic metabolism of DPAOs, data showed that the DPAOs were more sensitive to the temperature than aerobic PAOs.

Further, the temperature had a moderate effect on SRBs activity. The temperature coefficient for the sulphate reduction process in the anaerobic phase was 1.087.

Temperature coefficients of DPAOs (θ=1.165) were higher than those of others bacteria found to be essential in the biological sludge reduction process [4] such as fermenting bacteria (θ=1.070, [29]) and SRBs (θ=1.087, this study). Thus, it can be hypothesized that the biological phosphorus removal process is the limiting step of the biological sludge reduction process at low temperature.

Conclusions

Results achieved showed that the stoichiometry of the anoxic metabolism of DPAOs was strongly sensitive to the temperature; on the contrary temperature changes seem to have no negative effects on the anaerobic metabolism of total PAOs. Concerning the kinetic aspects, anaerobic, aerobic and anoxic metabolisms of PAOs seem to be significantly affected by low temperatures. Above all, the anoxic metabolism of DPAOs highlighted the highest sensitivity to the lowering temperature. Further, results showed that the temperature had a moderate effect on SRBs activity.

Given these results, the decrease in temperatures mainly compromised the activities of DPAOs, and supposedly the efficiencies of the reduction of sludge in an SBR-ASSR process. However, future studies directly performed running an SBR -ASSR at low temperatures are necessary to evaluate the long term effect of temperature on microbial activity, EPS destructuration and cell lysis, and thus on the whole sludge reduction process.

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References

1. Morita RY (1975) Psychrophilic Bacteria. Bacteriol Rev 39: 144-167.
2. Nedwell DD (1999) Effect of low temperature on microbial growth: lowered affinity for substrates limits growth at low temperature. FEMS Microbiol Ecol 30: 101-111.
3. Mulkerrins D, Dobson ADW, Colleran E (2004) Parameters affecting biological phosphate removal from wastewaters. Environ Int 30: 249-259.
4. Ferrentino R, Langone M, Gandolfi I, Bertolini V, Franzetti A, et al. (2016) Shift in microbial community structure of anaerobic side-stream reactor in response to changes to anaerobic solid retention time and sludge interchange ratio. Bioresour Technol 221:588-597.
5. Zhou Z, Qiao W, Xing C, An Y, Shen X, et al. (2015) Microbial community structure of anoxic–oxic-settling-anaerobic sludge reduction process revealed by 454-pyrosequencing. Chem Eng J 266: 249-257.
6. Ferrentino R, Langone M, Merzari F, Tramonte L, Andreottola G (2016) A review of Anaerobic Side-Stream Reactor for excess sludge reduction: configurations, mechanisms and efficiency. Crit Rev Environ Sci Technol 46: 382-405.
7. Manz W, Eisenbrecher M, Neu TR, Szewzyk U (1998) Abundance and spatial organization of Gram-negative sulfate-reducing bacteria in activated sludge investigated by in situ probing with specific 16S rRNA targeted oligonucleotides. FEMS Microbiol Ecol 25: 43-61.
8. Nielsen JL, Nielsen PH (2002) Quantification of functional groups in activated sludge by microautoradiography. Water Sci Technol 46: 389-395.
9. Wang J, Lu H, Chen G, Laua GN, Tsang WL, et al. (2009) A novel sulfate reduction, autotrophic denitrification, nitrification integrated (SANI) process for saline wastewater treatment. Water Res 43: 2363-2372.
10. Sahinkaya E, Ozkaya B, Kaksonen AH, et al. (2007) Sulfidogenic fluidized-bed treatment of metal-containing wastewater at 8 and 65 degrees C temperatures is limited by acetate oxidation. Water Res 41: 2706-2714.
11. Ingvorsen K, Yde Nielsen M, Joulian C (2003) Kinetics of bacterial sulfate reduction in an activated sludge plant. FEMS Microbiol Ecol 46: 129-137.

12. Zhao J, Wang D, Li X, Yang Q, Chenet H, al. (2015) An efficient process for wastewater treatment to mitigate free nitrous acid generation and its inhibition on biological phosphorus removal Sci Rep 5: 8602.

13. Jørgensen KS, Pauli AS (1995) Polyphosphate accumulation among denitrifying bacteria in activated sludge. Anaerobe 1: 161-168.

14. Mino T, Van Loosdrecht MCM, Heijnen JJ (1998) Microbiology and biochemistry of the enhanced http://ascelibrary.org/author/Brdjanovic%2C+Damirbiological phosphate removal process. Water Re 32: 3193-3207.

15. Brdjanovic D, van Loosdrecht MCM, Hooijmans CM, Alaerts GJ (1997) Temperature effects on physiology of biological phosphorus removal. J Environ Eng 123: 144-153.

16. Brdjanovic D, Logemann S, Van Loosdrecht MCM, Hooijmansa CM, Alaertsa GJ, et al. (1998) Influence of temperature on biological phosphorus removal: Process and molecular ecological studies. Water Res 32: 1035-1048.

17. Meijer SCF (2004) Theoretical and practical aspects of modelling activated sludge processes. Delft Univ Technol Netherlands.

18. Henze M, van Loosdrecht MCM, Ekama GA, Brdjanovic D (2008) Biological wastewater treatment: principles modelling and design. London: IWA Publishing.

19. Brdjanovic D, Mithaiwala M, Moussa MS, Amy G, van Loosdrecht MC (2007) Use of modelling for optimization and upgrade of a tropical wastewater treatment plant in a developing country. Water Sci Technol 56: 21-31.

20. Pinzon A, Brdjanovic D, Moussa M, López-Vázquez CM, Meijer SC, et al. (2007) Modeling of an Oil Rafinery Wastewater Treatment Plant. Environ Technol 28: 1273-1284.

21. Lopez-Vazquez CM, Hooijmans CM, Brdjanovic D, Gijzen HJ, van Loosdrecht MC (2009) Temperature effects on glycogen accumulating organisms. Water Res 43: 2852-2864.

22. Lopez-Vazquez CM, Oehmen A, Hooijmans CM, Brdjanovic D, Gijzen HJ, et al. (2009) Modeling the PAO–GAO competition: Effects of carbon source, pH and temperature. Water Res 43: 450-462.

23. Ferrentino R., Langone M., Villa R., Andreottola G. (2017). Strict anaerobic side-stream reactor: effect of the sludge interchange ratio on sludge reduction in a biological nutrient removal process. Environ. Sci. Pollut. Res. DOI. 10.1007/s11356-017-0448-6

24. APHA, AWWA, WEF (2012) Standard methods for the examination of water and wastewater. 21st ed. Am Public Heal Assoc.

25. Grady CPL, Daigger GT, Lim HC (1999) Biological Wastewater Treatment. Second Edi. PhD Propos. Marcel Dekker Inc, New York.

26. Smolders GJF, van der Meij J, van Loosdrecht MCM, Heijnen JJ (1994) Stoichiometric model of the aerobic metabolism of the biological phosphorus removal process. Biotechnol Bioeng 44: 837-848.

27. Waybrant KR, Blowes DW, Ptacek CJ (1998) Selection of reactive mixtures for use in permeable reactive walls for treatment of mine drainage. Environ Sci Technol 32: 1972-1979.

28. Muyzer G, Stams AJM (2008) The ecology and biotechnology of sulphate-reducing bacteria. Nat Rev Microbiol 6: 441-454.

29. Henze M, Gujer W, Mino T, van Loosdrecht M (2000) Activated Sludge Models ASM1, ASM2, ASM2d and ASM3. IWA Publications 121.