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Accelerated anaerobic hydrolysis rates under a combination of intermittent aeration and anaerobic conditions

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Keywords: Activated return sludge process (ARP), anaerobic hydrolysis, intermittent aeration, wastewater treatment

Abstract: Anaerobic hydrolysis in activated return sludge was investigated in laboratory scale experiments to find if intermittent aeration would accelerate anaerobic hydrolysis rates compared to anaerobic hydrolysis rates under strict anaerobic conditions. The intermittent reactors were set up in a 240 h experiment with intermittent aeration (3h:3h) in a period of 24 h followed by a subsequent anaerobic period of 24 h in a cycle of 48 hours which was repeated 5 times during the experiment. The anaerobic reactors were kept under strict anaerobic conditions in the same period (240 h). Two methods for calculating hydrolysis rates based on soluble COD were compared. Two-way ANOVA with the Bonferroni post-test was performed in order to register any significant difference between reactors with intermittent aeration and strictly anaerobic conditions respectively. The experiment demonstrated a statistically significant difference in favor of the reactors with intermittent aeration showing a tendency towards accelerated anaerobic hydrolysis rates due to application of intermittent aeration. The conclusion of the work is thus that intermittent aeration applied in the activated return sludge process (ARP) can improve the treatment capacity further in full scale applications.

Introduction

Optimization of biological processes at wastewater treatment plants (WWTPs)[1] is important for decreasing operational costs. Disposal of excess sludge and wastewater taxes comprise some of the most prominent expenses in wastewater treatment. According to Horan (1990) up to 60 % of the total operational costs at WWTPs are related to disposal of excess sludge depending on disposal costs. Furthermore the wastewater taxes based on effluent concentrations of nutrients and organic matter have been raised with 50 % in Denmark in 2014 (Danish Tax ministry, 2014).

The incentive for optimizing biological processes for sludge minimization, phosphorous removal and higher treatment efficiencies is therefore imminent. Hydrolysis is the rate limiting step for degradation of organic matter (Henze et al., 2002) and it is thus a key factor in biological processes in municipal wastewater treatment. In anaerobic hydrolysis, extracellular enzymes excreted by hydrolytic microorganisms are degrading and solubilizing particulate organic matter converting these constituents into more easily biodegradable compounds, which during the next step (fermentation) are converted by fermentative bacteria

[1] **Abbreviations used**
Activated return sludge process (ARP),
Analysis of variance (ANOVA),
Chemical oxygen demand (COD),
Enhanced biological phosphorous removal (EBPR).
Return activated sludge (RAS),
Side stream hydrolysis (SSH),
Wastewater treatment plant (WWTP),
Volatile fatty acid (VFA).

into soluble chemical oxygen demand (COD), e.g. volatile fatty acids (VFAs). VFA is utilized by microorganisms to facilitate enhanced biological phosphorous removal (EBPR). A large part of the soluble COD is furthermore utilized for denitrification.

Several studies have been looking into anaerobic hydrolysis rates. In Ucisik & Henze (2008) anaerobic hydrolysis has been investigated in regard to sludge type and production and composition of VFA. In Vollertsen et al., (2005) the improvement of the EBPR process by hydrolysis was investigated in full scale. Zhu & Chen (2005) explored the impact of hydrolysis on minimization of activated sludge production in a system with simultaneous sludge digestion and wastewater treatment. In anaerobic tanks organic matter was solubilized by hydrolysis and following recirculated to an aeration tank for decomposition.

In recent studies improvement of anaerobic hydrolysis rates by utilizing intermittent aeration (or microaeration) as a pretreatment before anaerobic digestion have been investigated (Xu et al. 2014; Lim & Wang, 2013; Jönsson & La Cour, 2006). These studies showed positive results in improving the anaerobic hydrolysis processes with sufficient micro-aeration.

Different full scale processes are based on anaerobic return activated sludge (RAS) hydrolysis. For instance anaerobic hydrolysis in side stream hydrolysis (SSH), which is used to promote a stable VFA production for EBPR as well as denitrification. Another full scale technology developed in recent years is the activated return sludge process (ARP) developed by EnviDan A/S (Petersen, 2005; Mikkelsen & Madsen, 2014). The purpose of the ARP technology is to increase the hydraulic and organic capacity of a WWTP without increasing the volume of the activated sludge process tanks and the secondary sedimentation tanks. In the ARP technology a side stream of RAS is fed to a separate ARP tank operated with a hydraulic retention time of approximately 20-30 hours. The volume is intermittently aerated in order to shift repeatedly between aerobic and anoxic conditions. The RAS flow ratio is typically controlled below 50 % of the influent wastewater flow. Thereby a higher concentration of suspended solids is present in the ARP volume compared with the main aeration tanks of the WWTP which drastically increase the amount of activated sludge that is maintained in the WWTP without increasing the volume of the activated sludge plant and the load on the secondary sedimentation tank.

By combining the ARP technology with a traditional anaerobic SSH process in the same volume the assumption is that the intermittent aeration in the ARP will enhance the anaerobic hydrolysis rates under the following anaerobic conditions during the SSH process.

The advantages of this process consist in a higher degree of nitrate and phosphorus removal. Furthermore the surplus sludge production from a given amount of COD in the wastewater decreases due to an increased production of easily biodegradable organic matter and a higher degree of biological phosphorus removal, which again reduces the need for chemicals for precipitating phosphate and thereby the chemical fraction comprised in the sludge production. If the processes are controlled optimally the outcome will be a lower amount of sludge for disposal as well as lower outlet concentrations of nutrients.

A prerequisite has risen up to demonstrate the validity of the assumption behind the combination of the ARP and SHH processes. The present experiment is mimicking the combination of the Activated Return sludge Process (ARP) utilizing intermittent aeration with a traditional SSH. The research intends to investigate if anaerobic hydrolysis rates can be accelerated by employment of intermittent aeration. Two methods for calculating the hydrolysis rates based on produced soluble COD will furthermore be compared.

Materials and Methods

Figure 1 displays the principle setup of the combined system of ARP/SSH as a side stream treatment at a municipal WWTP. RAS is recirculated from the secondary clarifiers to the ARP/SSH volume. The solubilized organic matter from the ARP/SSH is returned to the influent of the WWTP after a sufficient reaction period. In this experiment the focus only lies on the processes within the ARP/SSH volume.

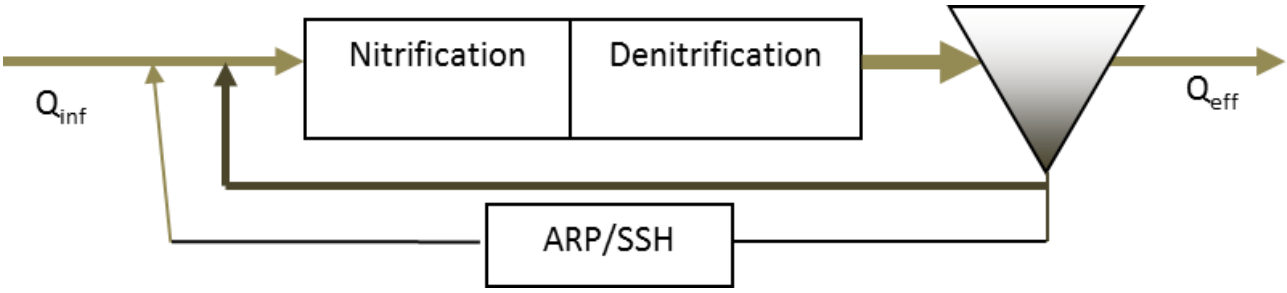

Figure 1: Principle setup of treatment processes with ARP (activated return sludge process) and side stream hydrolysis (SSH).

Set up of batch reactors

Samples of RAS were collected at Lundtofte Wastewater treatment plant, which is a plant with a low degree of chemical phosphorus removal. The samples were distributed into 6 times 2 L reactors with installed mixer devices. 3 of the reactors (reactor 1-3) were set up for intermittent conditions with intermittent aeration (3h:3h) in a period of 24 h followed by a subsequent anaerobic period of 24 h in a cycle of 48 hours which was repeated 5 times during the experiment. The remaining 3 reactors (reactor 4-6) were kept under strict anaerobic conditions during the same period. The reactors with intermittent conditions were thus set up to mimic the combined ARP/SSH process. The aeration time of 3 h is a purely empirical setting selected to be within the interval of aeration time that is typically chosen in full scale application at WWTPs. The aeration period selected for the experiment is in the higher end of this interval to secure a period with sufficient length to accommodate proper statistical evaluation. The principle behind this is that the aeration period should be long enough to build up measurable levels of ammonia removal and nitrate increases in the aerobic periods and measurable ammonia increase and nitrate decrease and most important phosphorus release in the anoxic/anaerobic periods. The cycles of intermittent aeration and anaerobic periods were repeated five times. The aeration was supplied by aquarium pumps and distributed evenly by the mixer pallets.

Measurements

Oxygen in the intermittent reactors was measured with an oxygen probe at 15 °C. Levels of pH were measured for each sample. The temperature was kept constant in the room (15 °C).

During the anaerobic periods measurements were carried out on the parameters; soluble COD, NH_4-N and PO_4-P in both the strictly anaerobic reactors and the intermittent reactors. A triple determination on each parameter as well as statistical analysis were performed.

Samples were centrifuged for 12 min.at 3000 rpm and at a temperature of 20°, the supernatant collected and filtered. NH_4-N were measured with Merk kits using a MerkNova 60 spectrofotometer at 690 nm (equivalent to EPA 350.1). PO_4 levels were as well measured on a MerkNova 60 spectrofotometre at 690 nm. COD was measured with Hach Lange Kits 314 (15-150 mg/l) and 414 (5-60 mg/l). Samples were heated in Hach Lange HT 200s and after cooling analyzed on a Hach Lange spectrophotometer Dr 2800 at 440 nm.

TSS and VSS were measured using standard methods.

Calculation of anaerobic hydrolysis rates

In this experiment two methods have been applied to calculate the anaerobic hydrolysis rates in the reactors. The methods are empirical and are indirect means of calculating the anaerobic hydrolysis rates based on the total production of soluble COD (COD (S)) during anaerobic hydrolysis. The two methods are displayed below in equation 1 and 2. All concentrations are in mg/L of each element:

$\Delta COD_1 = 11.4 \cdot \Delta NH_4\text{-}N$ [eq.1]

$\Delta COD_2 = 2.5 \cdot \Delta PO_4\text{-}P + \Delta sCOD_1$ [eq.2]

Principle of method 1 (equation 1)

In method 1 the amount of NH_4-N released during anaerobic conditions is a measure of calculating the hydrolysis rate. Equation 1 is solely an expression for protein based hydrolysis where soluble COD and NH_4-N are released. Equation 1 is excluding hydrolysis of lipids and carbonhydrates as these compounds have limited content of N and are only degraded into COD(S).

During hydrolysis, proteins ($C_5H_7NO_3$) are utilized as substrate by microorganisms resulting in release of ammonium (NH_4). Proteins practically represent the source of produced COD(S) for the hydrolysis processes. The NH_4-N concentration multiplied by the COD/N relation will thereby be a measure for the quantity of COD (S) produced during the hydrolysis period. As 2 moles of NH_4-N are produced by degradation of 1 mole of protein the following COD/N relation can be achieved:

$$\frac{COD}{N} = \frac{M_{(protein)}}{M_{(2*NH_4-N)}} = \frac{\sim 320\ g/mol}{\sim 28\ g/mol} = 11{,}4\ \frac{g\ COD\ (S) produced}{g\ N\ released} \quad \text{[Eq. 3]}$$

Principle of method 2 (equation 2)

The ratio presented in method 2 is derived from a common fermentation reaction where a typical biomass composition is converted to acetate as presented below in equation 4 and 5. VFA is the main product after sludge fermentation.

$C_6H_{12}O_6 \rightarrow 3\ CH_3COOH$ [eq.4]

$C_5H_7NO_3 + 3H_2O \rightarrow 2.5\ CH_3COOH + NH_3$ [eq.5]

In method 2 the hydrolysis rate is based on PO_4-P release from phosphate accumulating organisms (PAOs), measured COD(S) and NO_3-N. During anaerobic conditions a ratio of 2.5 g of produced soluble COD is taken up by the PAOs per 1 g of PO_4-P released as shown in equation 6. It is assumed that all COD(S) consists of VFA.

0.4 g PP-P + 1 g VFA-COD$\rightarrow$ 0.4 PO_4-P + 1 g PHA-COD + 0.04 g H^+ [eq. 6] (Henze et al., 2000)

COD(S) consumption as a consequence of denitrification is not taken into account in method 2.

NO_3-N is consumed as electron acceptor during the initial denitrification (previous to strict anaerobic conditions). This appears with simultaneous consumption of produced soluble COD in the ratio of up to 3.5 g of soluble COD per 1 g of NO_3-N consumed. Applying the consumption of soluble COD during denitrification to equation 2 would give following formula: $\Delta COD_2 = 2.5 \cdot \Delta PO_4\text{-}P + \Delta sCOD + 3.5 \cdot NO_3\text{-}N$.

Statistical analysis

The statistical analyses were performed using the program Prism 5 to perform two-way Analysis of variance (ANOVA) with the Bonferroni post-test in order to register any significant difference between the mean of the different reactors for each day. The data treatment provides the opportunity to determine how the response in this experiment is affected by two factors; (1) the difference in redox conditions between the reactors and (2) time.

Results and Discussion

Initial conditions

The initial conditions of intermittent reactors (1-3) and anaerobic reactors (4-6) were 0.25±0.08, 0.4 0± 0.08, 24.75 ± 0.95 and 7.24 ± 0.02 for the parameters NH_4-N (mg/l), PO_4-P (mg/l), soluble COD (mg/l) and pH, respectively. Due to an experimental shortcoming the nitrate concentrations were not measured initially and at the end of the each of the aerobic periods. The volatile suspended sludge concentrations in the reactors were in the range 5.7 ± 0.5 g/l. The temperature was kept constant at 15 °C by performing the experiment in a temperature controlled room.

Variations of pH and dissolved oxygen during the experiment

No significant difference between the initial and final pH were detected during the experiment. However, in the anaerobic reactors (4-6) some variations occurred along the experiments. These variations are likely to be related to the VFA production, the stripping of CO_2 or methane formation during the experiment. When the production of VFA is higher than the CO_2 stripping the pH decrease. When the opposite occurs there is an increase in pH. It is also possible that the formation of methane results in an increase in pH. In the intermittent reactors, pH increases during aeration due to CO_2 stripping and decreases under anaerobic conditions due to VFA production. The level of dissolved oxygen during the experiment increase in the intermittent reactors during the first 20 minutes of aeration until a maximum level is reached.

Release and uptake rates during the experiment

In the experiment the release and uptake rates were followed with measurements in the start and at the end of each intermittent or anaerobic period. Profiles of measured soluble COD, NH_4-N and PO_4-P in intermittent reactors 1-3 and anaerobic reactors 4-6 along the experiment are displayed in figure 2 and compared in columns with respective standard deviations shown in figure 3.

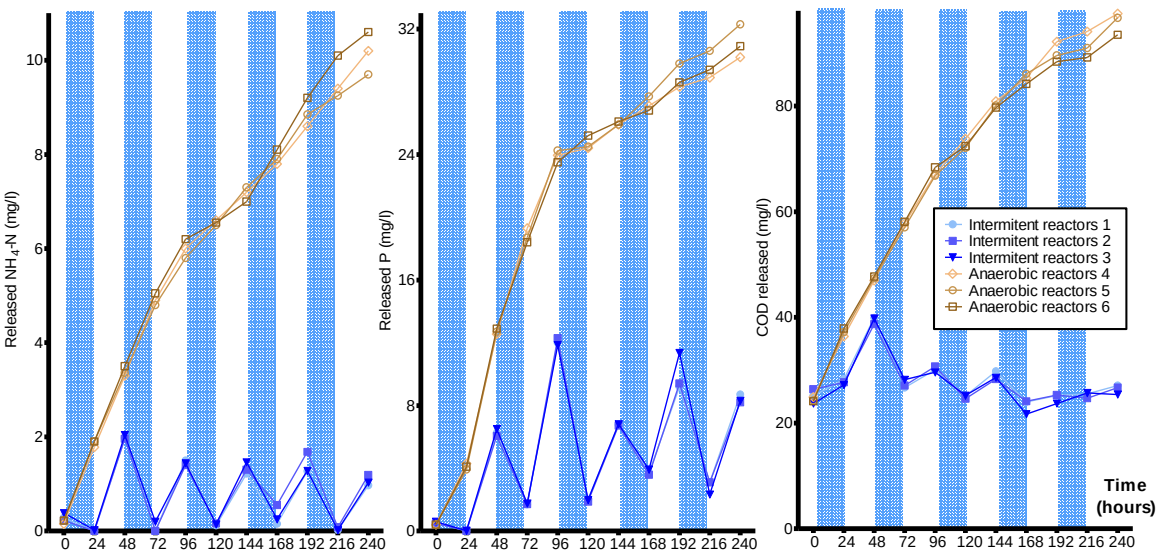

Figure 2: Profiles of measured soluble COD, released ammonium and phosphate for reactor 1-3 with intermittent aeration was evaluated in batch reactors 1-3 in the experiment. For comparison with a control of (closed symbols) and reactor 4-6 with strict anaerobic conditions

batch reactors 4-6 were employed (open symbols).

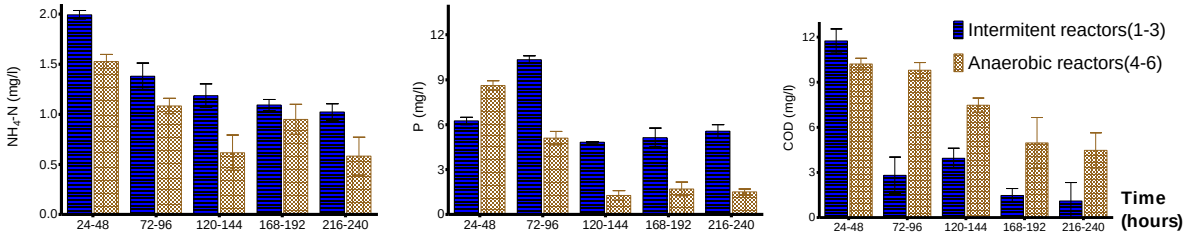

Figure 3: Comparison of released ammonium, soluble COD and phosphate for reactor 1-3 with intermittent conditions and reactor 4-6 with strict anaerobic conditions. Calculations have been performed in 24 h anaerobic periods for both type of reactors. T-bars indicate the standard deviation of average results for each reactor.

Release and uptake of ammonium during the experiment

As displayed in figure 2 NH_4-N levels decreased in the intermittent aeration periods in the intermittent reactors (1-3) due to uptake by nitrifiers and increased during the anaerobic periods due to protein hydrolysis/fermentation. Release rates during anaerobic hydrolysis in the intermittent reactors decreased slightly along the experiment. The uptake and release rates of NH_4-N are displayed in table 1. According to the table the release rates in the anaerobic reactors seems to be constant until 96 hours with an average of app. 0.011±0.0004 mg NH_4-N/g VSS·h. In the rest of the experiment (96-240 h) the release rate decreases to an average of 0.0051±0.003 mg NH_4-N/gVSS·h. Through the experiment the release rates of NH_4-N were found higher in the intermittent reactors as a significant difference between the intermittent and anaerobic reactors was established (see figure 3). This means that the introduction of intermittent aeration in reactors 1-3 has improved the hydrolysis rate (release rate) of NH_4-N under anaerobic hydrolysis compared to release rates under strict anaerobic conditions in reactors 4-6.

Table 1: Release and uptake rates of ammonium, phosphate and soluble COD during the experiment. Estimated hydrolysis rates of soluble COD calculated by method 1 and Method 2.

		Intermittent reactors		Anaerobic reactors	
	Period (Hours)	Mean uptake rates (intermittent aeration)	Mean release rates (anaerobic hydrolysis)	Mean release rates (anaerobic hydrolysis)	
NH_4^+, mg-N/(g-VSS·h)	0-24	0.0021 ± 0.0005		0.011 ± 0.0004 *(0-96 h)*	
	24-48		0.015 ± 0.0002		
	48-72	0.014 ± 0.0006			
	72-96		0.010 ± 0.001		
	96-120	0.0096 ± 0.0005		0.0051 ± 0.003 *(96-240 h)*	
	120-144		0.0087 ± 0.0009		
	144-168	0.0074 ± 0.002			
	168-192		0.0080 ± 0.0004		
	192-216	0.010 ± 0.001			
	216-240		0.0075 ± 0.0006		
PO_4^{3-}, mg-P/(g-VSS·h)	0-24	0.0038 ± 0.0004		0.043 ± 0.0007 *(0-96 h)*	
	24-48		0.046 ± 0.002		
	48-72	0.033 ± 0.001			
	72-96		0.076 ± 0.002		
	96-120	0.074 ± 0.0015		0.0088 ± 0.001 *(96-240 h)*	
	120-144		0.035 ± 0.0003		
	144-168	0.022 ± 0.0006			
	168-192		0.046 ± 0.008		
	192-216	0.052 ± 0.0081			
	216-240		0.041 ± 0.003		
Soluble COD, mg-O_2/(g-VSS·h)	0-24	-0.020 ± 0.009		0.079 ± 0.002 *(0-96 h)*	
	24-48		0.086 ± 0.006		
	48-72	0.089 ± 0.007			
	72-96		0.021 ± 0.008		
	96-120	0.038 ± 0.006		0.035 ± 0.004 *(96-240 h)*	
	120-144		0.029 ± 0.005		
	144-168	0.041 ± 0.010			
	168-192		0.011 ± 0.003		
	192-216	0.0041 ± 0.0100			
	216-240		0.0080 ± 0.0080		
		Method 1	Method 2	Method 1	Method 2
Released COD ΔCOD, mg-O_2/(g-VSS·h)	24-48	0.17	0.20	0.13	0.23
	72-96	0.12	0.21	0.090	0.16
	120-144	0.11	0.12	0.050	0.077
	168-192	0.099	0.12	0.079	0.067
	216-240	0.086	0.11	0.049	0.060

In regard to the uptake rate in the intermittent reactors the first aeration period (0-24 h) is low due to the initial limited availability of NH_4-N (see figure 2). The uptake is highest in the second period (48-72 h) with 0.014±0.0006 mg NH_4-N/gVSS·h as the production level of NH_4-N is at the highest point in the preceding first period with anaerobic hydrolysis (24-48 h; see table 1). After this a decreasing tendency is observed during the next two aeration periods. In the last period the uptake rate is higher. This is due to accumulation of NH_4-N in the intermittent reactors as the uptake of NH_4-N is not complete in the second and third aeration period.

Release and uptake of phosphate

A release of PO_4-P appears in the anaerobic reactors during most of the experiment, but decreases after 96 hours (see figure 2). In the intermittent aerated reactors PO_4-P is released during the anaerobic periods and taken up during the intermittent aeration periods. The release rates are also decreasing after 96 hours. Release and uptake rates are displayed in table 1. The PO_4-P is released by phosphate accumulating organisms (PAOs) in the anaerobic periods as the PAOs take up soluble COD as VFA for promoting growth. In the aeration periods the PAOs take up PO_4-P as an energy source for storage. The release of phosphate was higher in the intermittent reactors through the experiment, which is also displayed in table 1. However the first day was higher under anaerobic conditions than under intermittent conditions. Through the

experiment the hydrolysis rates (release rates of PO_4-P) were higher in the intermittent reactors as a significant difference between the intermittent and anaerobic reactors was established (see figure 3).

Release and uptake of COD(S) during the experiment

The release of COD(S) decreased slightly along the experiment in the intermittent reactors, which is illustrated in figure 2 and shown through the release rates in table 1. Most of the COD(S) produced by anaerobic hydrolysis was likely taken up by PAOs in the anaerobic periods. During the intermittent aeration periods COD(S) is oxidized by heterotrophic bacteria. In the anaerobic reactors COD(S) has a constant increase along the experiment as the uptake of COD(S) is low during anaerobic conditions compared with aerobic conditions. The hydrolysis rate (release rate) of COD (S) was significantly higher in the reactors with anaerobic conditions than in the intermittently aerated reactors along the experiment (see figure 3). However there was not a significant difference at the first day of comparison. The higher detected release rate of COD(S) in the anaerobic reactors under strict anaerobic conditions is due to the low uptake by microorganisms compared to the intermittent reactors where the periods with intermittent aeration favors uptake of COD(s).

In order to evaluate the actual COD(S) released it is necessary to employ Method 1 or 2 for a rough estimation of the total COD(S).

Comparison of anaerobic hydrolysis rates (Method 1 and Method 2)

The total COD(S) release was higher in the intermittent reactors as significant difference in the intermittent and anaerobic reactors was detected using method 1 ($P<0.001$) (see figure 4). There was similarly a significantly difference in everyday of comparison in the favor of the intermittent reactors. The significant difference comparison for each day was calculated with the Bonferroni post-test and the P values are shown at the top of the bars in the graphs. However, during the first day the release was higher in the anaerobic reactors. The total COD(S) released using method 2 was also significantly higher in intermittent reactors than in anaerobic reactors along the experiment ($P<0.001$). There was also a significant difference in favor of the intermittent reactors found using Bonferroni test for everyday of comparison. Hereby both methods indicate statistically significant higher hydrolysis rates in the intermittent reactors.

The hydrolysis rates calculated in Method 1 and 2 are shown in table 1 for comparison. The hydrolysis rates found by calculation in Method 1 and 2 were significantly higher in reactors with intermittent reactors compared to reactors with strict anaerobic conditions. Furthermore, the hydrolysis rate is higher for method 2, when comparing the intermittent reactors in the two methods. The same is observed in regard to the hydrolysis rate in the anaerobic reactors.

However, a range of uncertainties appear when evaluating the calculations carried out in the two methods.

In Method 1 it is assumed that all the hydrolysis products (aminoacids, carbohydrates) are fermented during the anaerobic period. This method is considering a protein based hydrolysis where all protein/aminoacids are degraded and converted into soluble COD.

Method 1 has some limitations in regard to calculation of the total soluble COD produced in the system during anaerobic hydrolysis. The organic matter degraded is also comprised of carbohydrates and minor fractions of lipids. Carbohydrates are degraded into sugars after hydrolysis/fermentation. In some cases, the total COD release could be two times higher depending on the carbohydrates fraction of the organic matter. When the composition of the carbohydrates is around 30 % of the organic matter the soluble COD could

probably be twice as high as the amount calculated from method 1. This is based on the discovery of hydrolysis rates from carbohydrates were found twice as fast as the proteins hydrolysis rates (Christ et al., 2000). However, method 1 is useful when comparing two different anaerobic hydrolysis rates as in this experiment.

In Method 2 the hydrolysis rates are based on measured soluble COD and uptake of COD(S) during phosphorous release. A fraction of the COD(s) is comprised of VFA that is taken up by PAOs under anaerobic conditions. In Jönsson & Jansen (2006) both VFA and soluble COD were measured during anaerobic hydrolysis of RAS. The RAS from a WWTP with presedimentation and long SRT, which is comparable with RAS from Lundtofte WWTP, showed a VFA fraction down to 5 % of the soluble COD. The maximum soluble COD yield was reached after 3 h. The low concentration is explained by the rapid consumption of VFA by PAOs.

The main limitation of the calculation in Method 2 is the lack of NO_3-N measurements. In order to achieve a more correct estimation of COD(S) in Method 2 it is necessary to take the consumption of COD(S) during denitrification into account as it is estimated that around 50 % of soluble COD could be utilized for denitrification. Another limitation is the presence of other microorganisms in the wastewater like GAOs that are able to take up COD(S) under anaerobic conditions without releasing PO_4-P. However, at a pH level around 7 as kept during the experiment the uptake of COD(S) by GAOs will be minor. Furthermore the room temperature was kept at 15 °C during the experiment, which is known to be a temperature that favors PAO uptake.

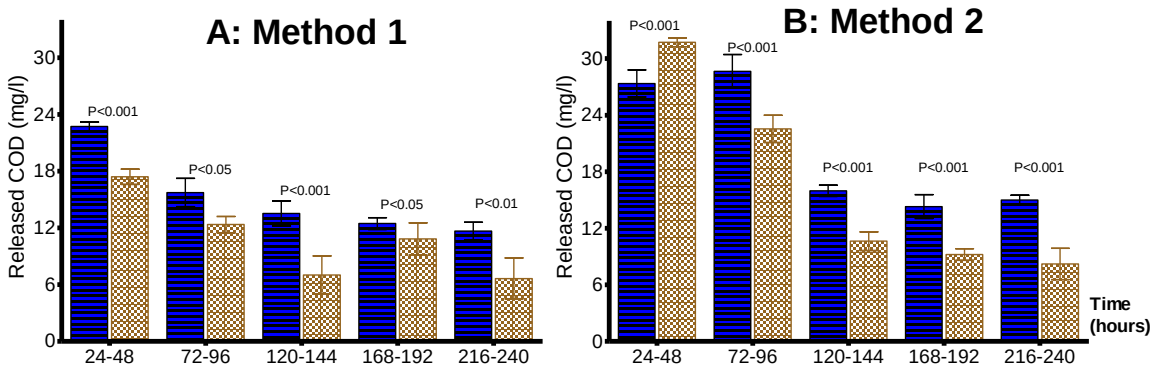

Figure 4: Comparison of hydrolysis rates based on method 1 (left) and method 2 (right) for reactor 1-3 with intermittent conditions (lines pattern) and reactor 4-6 with strict anaerobic conditions (checkered pattern). Calculations have been performed in 24 h anaerobic periods for both types of reactors. T-bars indicate the standard deviation of the average of results for each treatment.

The ARP/SSH combination

The results of the experiment indicate a positive effect on anaerobic hydrolysis rates with the introduction of intermittent aeration.

Conclusion

The experimental findings show that intermittent aeration significantly accelerates the hydrolysis rates of COD(S) (based on Method 1 and 2) as well as released NH_4-N and PO_4-P compared to conditions with strict anaerobic hydrolysis. The hydrolysis rates found by calculation in both Method 1 and 2 were significantly higher in reactors with intermittent reactors compared to reactors with strict anaerobic conditions. Furthermore, it was found that the hydrolysis rate calculated by method 2 was higher in the separate evaluation of intermittent reactors and anaerobic reactors respectively. However, a range of uncertainties

appear when evaluating the calculations carried out in the two methods. In Method 2 the lack of NO_3-N measurements is the main limitation in the calculation.

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