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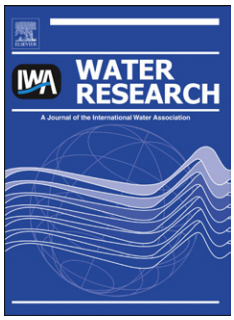

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The effect of anoxia and anaerobia on ciliate community in biological nutrient removal systems using laboratory-scale sequencing batch reactors (SBRs)

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Abstract

Little is known about the effect of anaerobic and anoxic stages on the protozoan community in the activated sludge process and how this subsequently affects performance. Using a laboratory-scale BNR system the effect of different periods of anoxia on both the protozoan community and performance efficiency have been examined. Four SBRs were operated at two cycles per day using a range of combined anoxic/anaerobic periods (0, 60, 120 to 200 min). Effluent quality (TOC, BOD, TP, TN, NH_4-N, NO_3-N and NO_2-N), sludge settleability and ciliate community (species diversity and abundance) were analyzed over a periods of up to 24 days of operation. The species richness and total abundance of ciliates were found to decrease with longer anoxic/anaerobic periods. Both, positive and negative significant correlations between the abundance of certain species and the period of anoxia was observed (e.g. *Opercularia microdiscum*, *Epicarchesium granulatum*), although other species (i.e. *Acineria uncinata*, *Epistylis* sp.) were unaffected by exposure to anoxia. In the laboratory-scale units, the 60 minute anoxic/anaerobic period resulted in good process performance (TOC and BOD removal of 97 to 98% respectively), nitrification (80-90%), denitrification (52%) but poor levels of biological P removal (12%); with the protozoan community moderately affected but still diverse with high abundances. Increasing the length of anoxia to up to 200 min did not enhance denitrification although P removal rates increased to between 22-33%; however, ciliate species richness and total abundance both decreased and sludge settleability became poorer. The study shows that activated sludge ciliate protozoa display a range of tolerances to anoxia that result in altered ciliate communities depending on the length of combined anoxic/anaerobic periods within the treatment process. It is recommended that anoxic/anaerobic periods should be optimized to sustain the protozoan community while achieving maximum performance and nutrient removal.

Keywords: protozoa, protists, wastewater treatment, activated sludge, phosphorus removal, process performance

1. Introduction

Nutrients in wastewater such as phosphates and nitrogen compounds lead to accelerated eutrophication in natural water bodies such as rivers, lakes, estuarines and coastal waters. Biological nutrient removal (BNR) from domestic and industrial wastewaters is a key factor in preventing eutrophication in receiving waters being one of the most economical and efficient methods of nutrient control (Akpor *et al.*, 2008). This is reflected in the rapid increase in the use of BNR systems since the introduction of the EU Urban Wastewater Treatment Directive (91/271/EEC) which specifies nitrogen and phosphorus limits for effluents discharged to sensitive areas. To integrate biological nutrient removal (BNR) into the activated sludge process anaerobic, anoxic and aerobic cycles are needed. This has lead to a rising importance of sequencing batch reactors (SBRs) which provide a better operation management of the mixed liquor with excellent control over oxygen and redox conditions, employing separate aerobic, anoxic and anaerobic cycles (Carucci *et al.*, 1994; Gray, 2004; Hu *et al.*, 2005; Obaja *et al.*, 2005; Spagni *et al.*, 2007). Therefore, unlike conventional activated sludge systems, the biota in BNR systems experience unique kinetic and metabolic stresses arising from redox shifts. Apart from the operational steps required for the nutrient removal, anoxia can also occur during sludge separation, storage, return, under-aeration and overloading (Gray, 2004).

Comparative studies of the protozoan community in wastewater treatment plants operating under a wide variety of conditions have concluded that certain species display higher tolerances to low dissolved oxygen (Esteban *et al.*, 1991a; Madoni *et al.*, 1993; Lee *et al.*, 2004). However, few studies have specifically examined the effect of anoxia/anaerobia on protozoan communities or which species can endure the complete absence of dissolved oxygen. Maurines-Carboneill *et al.* (1998) found that protozoa and metazoa in activated sludge disappeared completely after three days of anaerobiosis. Toman and Rejic (1988), using a laboratory scale reactor, found that exposure to either zero or very low oxygen concentrations induced by intermittent 24 hour interruptions in the

aeration neither adversely affected performance nor the activated sludge biocenosis. Little is known about the long term effect of the stress caused by the repeated exposure of shorter periods of anoxia/anaerobia, as it occurs in BNR systems, on the development or maintenance of protozoan species. Due to the important role of protozoan in the purification process (Curds *et al.*, 1968; Curds and Fey, 1969), it would be detrimental to SBR and BNR operational performance if the alternating oxidation-reduction potential (ORP) adversely affect the protozoan community. Enabling protozoan community structure to be predicted in relation to anoxia will permit more effective process management resulting in optimum treatment capability.

Thus the aim of this study was to determine the effect of anoxia/anaerobia on both the protozoan community and performance efficiency in BNR activated sludge systems. Information concerning the ability of ciliates to tolerate anoxia was also obtained and tolerant and sensitive species identified.

2. Material and methods

2.1. Laboratory-scale Sequencing Batch Reactors (SBRs)

Four identical 3.4 L volume laboratory scale SBRs were constructed as outlined in Fig 1. A magnetic stirrer (SB161, Stuart Scientific, UK) ensured homogeneous mixing during the reaction periods. Aeration was supplied by an aquarium air pump through a diffuser, obtaining dissolved oxygen concentrations between 1 and 2 mg L^{-1} in the aeration phase. The reactors were operated at two cycles per day using different combined anoxic/anaerobic periods increasing from 0, 60, 120 to 200 min in reactor 1, 2, 3 and 4 respectively. Detailed cycle time configurations for the laboratory-scale SBRs can be found in Table 1. During each cycle, 1.7 L effluent was decanted and replaced with synthetic sewage (i.e., 50% volumetric exchange ratio) giving a HRT of 1 day (Ndon, 2007). OECD synthetic sewage (Christofi *et al.*, 2003; Gendig *et al.*, 2003) was used as the feed for the lab-scale plant. The 100-fold concentrated stock solution was stored at -

18˚C, thawed when required and diluted to the necessary concentration to refill the sewage reservoir and to provide the desired sludge loading of 0.1 g BOD_5 g^{-1} MLSS d^{-1}. To avoided a decrease in the reactor pH during nitrification, as slightly acidic conditions are known to adversely affect the ciliate community (Cybis and Horan, 1997), $NaHCO_3$ was added to the synthetic sewage at a final concentration of 0.6 g L^{-1} (Christofi *et al.*, 2003). The storage containers were kept cooled at an approximate temperature of 4-10˚C to reduce bacterial growth and prevent degradation of the sewage. Each reactor was fitted with two peristaltic pumps (iProcess, USA), for feeding and for drawing off effluent and excess sludge, respectively. The reactor MLSS was maintained at between 3000 and 3400 mg L^{-1} by wasting excess sludge on a batch basis before the start of the settling period. The SBR operation cycles (Table 1) were automatically controlled via a computer and programmable external timer power control units (IP Power 9258, Audon Electronics, UK). To monitor the operating conditions, each reactor was equipped with an ORP (platinum-rod electrode ORP-31C, single junction Ag/AgCl Gel reference, Nico2000 Ltd., UK) and a pH electrode (ELIT P11, AgCl reference, Nico2000 Ltd., UK). The electrodes were connected to the computer through an analyser (8 Channel Analyser ELIT 9808, Nico2000 Ltd., UK) and readings were recorded every 5 minutes.

Figure 1: Configuration of the laboratory-scale SBRs

Table 1: Cycle time configurations for the laboratory-scale SBRs

The reactors were operated under identical conditions each time using mixed liquor from different full-scale WWTP as a seed. During the first experiment the reactors were operated for 16 days which was extended to 24 days in the second experiment to determine whether there were significant changes in the developments of protozoan communities were observable over a longer period.

2.2. SBR inoculums

The sludge used to seed the reactors for the first experiment was obtained from Leixlip Wastewater Treatment Plant (WWTP), a medium sized (45 000 p.e.) conventional plant with completely mixed aeration tanks treating mainly domestic (80%) wastewater. For the second run mixed liquor was sourced from Swords WWTP (60 000 p.e.). This plant is an extended aeration BNR-system, incorporating both anoxic and anaerobic periods, which treats mainly domestic (95%) wastewater.

2.3. Microscopic analysis of protozoan community

Microscopic analyses of the mixed liquor were carried out at the start, after 8, 16 days and also in experiment 2 after 24 days. Ciliate enumeration was performed using phase contrast microscopy at 100x magnification with species differentiation and identification at higher magnifications up to 400x depending on the size of species using the keys of Foissner *et al.* (1991; 1992; 1994; 1995) Curds (1969) and Curds *et al.* (2008). The estimation of ciliate population densities were based on enumerations using four 25 µL sub-sample replicates. With an average process time of 20-30 min per replicate, depending on species abundances, the total number of replicates that could be counted at one day was limited to four. According to Dubber and Gray (2009) this ensures a species recovery of approximately 75% of all except the rarest species (i.e. those comprising <1% of the total protozoan abundance).

2.4. Process performance and physico-chemical analysis

To monitor process performance and nutrient removal associated with the prevalent protozoan community effluent samples have been collected after 1, 8, 16 days and also after 24 days in experiment 2. Biological oxygen demand (BOD_5), total organic carbon (TOC), total phosphorus (TP), total nitrogen (TN), ammonia, nitrate and nitrite concentrations have been measured according to Standard Methods (APHA, 2005). Because the COD analysis produces hazardous wastes, including mercury and

hexavalent chromium, estimation of process performance by COD removal was avoided. Instead, TOC together with the BOD_5 was used as a performance parameter (Dubber and Gray, 2010). The BOD_5 was measured respirometrically using the Oxitop® system (Reuschenbach *et al.*, 2003) suppressing nitrification by the addition of 0.5 mg L^{-1} allythiourea (ISO, 2003). TOC was measured by thermocatalytic oxidation with a high temperature TOC analyser (vario TOC cube, Elementar, Germany). Using the same analyser, the total bound nitrogen was determined. The phosphorus compounds were oxidised to ortho-phosphates applying the acid persulfate method, then the ascorbic acid method was used to measure total phosphorus. The ammonia was determined using an ion-selective electrode (ELIT 8051, Nico 2000) and nitrate concentrations were measured using the automated cadmium reduction method (Lachat flow injection analyser Quick Chem 8500, Lachat Instruments, US). Nitrite was measured colourmetrically using the same Lachat flow injector that was used for nitrate. The same methods were used to characterise and quantify the influent composition. To include the variation of influent composition that occurred due to storage in the refill tanks influent samples were taken from the freshly prepared dilution and after 12 hours of storage just before the start of the 2^{nd} cycle.

The mixed liquor suspended solids (MLSS) and the sludge volume index (SVI) of the mixed liquor were determined using Standard Methods (APHA, 2005). While the MLSS was monitored throughout the whole experiment the SVI was only measured at the end. However, the settleability in all reactors was observed relative to each other throughout the experiment by comparison of the sludge height after settling. Using the wasted volume of mixed liquor and its MLSS concentration the amount of solids wasted on average per day was estimated.

2.5. Statistical analysis

The effluent quality (in terms of TOC, BOD_5, TP, TN, NH_4-N, NO_3-N and NO_2-N) as well as the counted protozoan abundances have been analysed for statistical differences between the four SBRs.

In order to quantify the treatment performance of the reactors, removal rates [%] were calculated for organic matter (BOD_5, TOC) and nutrients (NH_4, TP, TN) using equation 1 with c_{in} and c_{out} being the concentrations in influent and effluent respectively.

$$R = 100 - \left(\frac{c_{out}}{c_{in}} \cdot 100 \right) \quad (1)$$

Propagation of uncertainty was used to obtain the standard deviation for the calculated removal rates. Equation 2 was applied to equation 1 with σ_{in} and σ_{out} being the standard deviations obtained from the measurements of influent and effluent concentrations respectively.

$$\sigma_R = \sqrt{\left(\frac{\partial R}{\partial c_{in}} \cdot \sigma_{in} \right)^2 + \left(\frac{\partial R}{\partial c_{out}} \cdot \sigma_{out} \right)^2} \quad (2)$$

To detect significant differences in removal rates between the reactors, 95% confidence intervals were calculated.

In order to further assess the similarity of protozoan community structure between the different reactors cluster analysis was employed using total ciliate abundances. The cluster analysis was performed with standardised variables using the single linkage (nearest neighbour) method and the squared Euclidean distance. For all statistical analyses the programmes SPSS 14 and Minitab 15 were used.

3. Results and discussion

3.1. Operational conditions

3.1.1. Sludge loading and influent characteristics

The sludge loading of the reactors was successfully maintained between 0.08 and 0.1 g BOD g^{-1} MLSS d^{-1} throughout the first experiment with an average of 0.092 ± 0.007 g $g^{-1}d^{-1}$. During the second experiment the average sludge loading was 0.098 ± 0.004 g $g^{-1}d^{-1}$. The variation in the sludge loading between the 4 reactors was 0.003 and 0.0017 g $g^{-1}d^{-1}$ in the first and second experiment, respectively. The characteristics of the synthetic sewage feed are summarised in Table 2 which shows that small changes in quality occurred during storage in the refill tanks.

Table 2: Characteristics of OECD sewage in the used concentration

3.1.2. Sludge production

The amount of solids wasted from the reactors per day to maintain the operating MLSS varied from 386 to 445.6 mg during experiment 1. The highest sludge production was observed in reactor 3 and 4. During the second experiment between 274.2 and 367.5 mg solids were wasted per day. Again more solids were produced in reactor 3 and 4 than in the other reactors.

Lee and Welander (1996) have shown that the feeding activity of rotifers have a strong influence on sludge production. During the first experiment rotifers were not quantified but in the second experiment rotifer abundance was observed to decline with increasing period of aeration disruption within the cycle which could explain the higher sludge production observed in reactor 3 and 4.

3.1.3. ORP and pH monitoring

In previous studies (Yu *et al.*, 1997; Akin and Ugurlu, 2005; Spagni *et al.*, 2007) it has been shown that with online ORP and pH measurements it is possible to obtain important information about the processes in the reactors and to identify the end of denitrification and nitrification. The ORP gives a measure of the general condition of the liquid and whether the oxidation or the reduction reactions dominate as a process (Gray, 2004). This is mainly dependent on DO concentrations but the ORP as an indicator of the DO provides much better information about the processes at low DO concentrations and in the anoxic phase (Fuerhacker *et al.*, 2000; Akin and Ugurlu, 2005; Hu *et al.*, 2005). Figure 2 shows typical ORP and pH profiles in the four reactors that were recorded during both experiments. With the substrate consumption being a reduction reaction the ORP starts decreasing at the beginning of the cycle in each reactor. However, only in the reactors 2, 3 and 4 the DO decreases to zero, reaching anoxic conditions with the ORP decreasing further to negative values. This is due to the reactors not being supplied with oxygen during their first operational step. At an ORP level of around -170 to -200 mV a sharp decrease in the ORP can be noticed that usually appears when nitrate concentrations are close to zero (Yu *et al.*, 1997). After this “nitrate knee” the ORP decreases further and with no nitrogen bound oxygen present the conditions in the reactor change from anoxic to true anaerobic (Akin and Ugurlu, 2005). Simultaneously due to OH^- production during denitrification the pH increases and reaches a maximum with a subsequent decrease due to the formation of fatty acids under anaerobic conditions. This bending point in the pH profile, which occurs at the same time as the nitrate knee, is known as the “nitrate apex” (Yu *et al.*, 1997; Spagni *et al.*, 2007). With the onset of aeration the ORP increases, nitrification occurs and the pH decreases due to H^+ release. It eventually reaches a “valley” that indicates the depletion of ammonia and is clearly visible in the recorded profiles (“ammonia valley”, Fig. 2). After an increase the pH forms a plateau with the values staying constant, indicating the end of the phosphate uptake (Akin and Ugurlu, 2005). With the end of nitrification and a decrease in bacterial respiratory the DO

increases so that a break point in the ORP profile (ORP elbow) can be observed as well (Fig. 2a) but it is not always as visible as the ammonia valley in the pH profile (Akin and Ugurlu, 2005; Spagni *et al.*, 2007).

Figure 2: Typical ORP and pH profile in the four reactors during a treatment cycle in a) experiment 1 (seed from Leixlip WWTP) and b) experiment 2 (seed from Swords WWTP); aeration disruptions of 0, 60, 120 and 200 minutes in reactor 1, 2, 3 and 4 respectively

Consulting the ORP and pH profiles throughout the experiment it shows the prevalent conditions in the reactors that result from the different cycle settings and indicates the reactions that are taking place. Although being continuously aerated, in the first experiment reactor 1 occasionally reached anoxic conditions with ORP values below 0 mV for up to 50 minutes (Table 3). This was probably due to a high respiration in response to feeding that could not be fully satisfied by the oxygen supply. However, this was not observed during experiment 2 with reactor 1 remaining aerobic throughout each cycle (Table 3). Without anoxia no denitrification could take place and therefore no nitrate knee or apex was observed (Fig. 2). In reactor 2 anoxic periods were obtained with an average length of 144-176 min during experiment 1 and 90-113 min during experiment 2 (Table 3). True anaerobic conditions were usually not reached in this reactor. The protozoan community in reactor 3 was exposed to long anoxic/anaerobic periods up to 193 and 200 min (Table 3) towards the end of which conditions frequently turned anaerobic. On average these anaerobic intervals were 60 to 79 min and 42 to 58 min long during experiment 1 and 2 respectively (Fig. 3). In the ORP profile of reactor 4 the nitrate knee was usually clearly visible (Fig. 2) so that full denitrification had been reached resulting in anaerobic periods of between 125 to 158 min and 126 to 138 min during experiment 1 and 2 respectively (Fig. 3).

Table 3: Average length of aerobic and anoxic/anaerobic time (ORP < 0mV) observed in the reactors at a) experiment 1 (seed from Leixlip WWTP) and b) experiment 2 (seed from Swords WWTP)

Figure 3: Observed length of aerobic (ORP > 0 mV) anoxic (0 mV > ORP > -190 mV) and anaerobic (ORP < -190 mV) periods in the 4 reactors depending on their cycle time settings in a) experiment 1 (seed from Leixlip WWTP) and b) experiment 2 (seed from Swords WWTP)

3.2. Process performance and effluent quality

3.2.1. Effluent BOD_5 and TOC

Throughout both experimental runs organic removal was high in all reactors with the effluent BOD_5 ranging from 2 to 12.5 mg L^{-1} (95 to 99% removal) and TOC 2.89 to 5.57 mg L^{-1} (96 to 98% removal) with no significant differences detected between the treated reactors.

3.2.2. Nutrient removal

The ammonia valley was usually clearly visible in all the reactors (Fig. 2) confirming that nitrification was taking place. During experiment 1, ammonia removal varied between 78 and 91% with similar removal rates from 73 up to 90% in experiment 2 (Fig. 4a). Throughout both experiments the final effluent ammonical-N concentrations in all reactors was <3 mg NH_4-N L^{-1}. While no significant differences in ammonia removal between the different reactors were detected (Fig. 4a), Lee and Oleszkiewicz (2003) consistently observed greater nitrification rates in alternating anoxic/aerobic SBRs than in solely aerobic reactors. After further investigations they excluded the possibility that under anoxic/anaerobic conditions the decreased activity of rotifers was responsible for the increased nitrification due to reduced grazing pressure on nitrifying bacteria. Instead they proposed that the difference was due to the feast/famine phenomena. If biomass is

subjected to starvation, followed by a substrate-rich environment then a higher substrate uptake rate will be observed. When exposed to a feasting period the amount of energy required for metabolic functions increases dramatically over that of a non-stressed population due to the additional energy that is necessary for the repair process (Lee and Oleszkiewicz, 2003). Although ammonia is available during the anoxic phase it can't be utilised by obligate aerobic autotrophs due to the lack of oxygen nor can they store it under anoxic conditions the way facultative heterotrophic aerobes store energy from substrate, so that a similar feast/famine phenomenon can develop (Lee and Oleszkiewicz, 2003). However, in the present study such a phenomenon was not observed.

Figure 4: Ammonia removal (a), total nitrogen (b) and total phosphorus (c) removal in the SBRs with different length of aeration disruption throughout experiment 1 and 2 (Error bars represent 95% confidence interval)

Similar final effluent total nitrogen (TN) concentrations of 40 to 70 mg L^{-1} and 30 to 60 mg L^{-1} were observed in the reactors during experiment 1 and 2, respectively. During experiment 1 maximum nitrogen removal rates were achieved in reactor 4 (56% ± 1.4) while during the second experiment maximum removal occurred in reactor 3 (66% ± 4). In the first experiment the TN-removal in reactors 2, 3 and 4, comprising anoxic periods, was always significantly higher than in reactor 1 (Fig. 4b) being on average 32 - 36% higher. Likewise in experiment 2 the aerobic reactor (reactor 1) had the lowest nitrogen removal efficiency (30-39%) with those with anoxic periods (reactors 2 to 4) achieving 46 to 66% TN-removal with the latter two occasionally performing significantly better (Fig. 4b). This suggests that the anoxic periods in reactor 2 were not always long enough to ensure complete denitrification. On average the difference between reactor 2 and reactors 3 and 4 was 8 and 10% respectively.

The highest final effluent nitrate concentrations were recorded in reactor 1 at 61 to 64 mg NO_3-N L^{-1} and 58 to 66 mg NO_3-N L^{-1} during experiment 1 and 2 respectively. During experiment 1 no significant difference was detected between the final nitrate concentrations of reactors 2 and 3 (30 - 39 mg NO_3-N L^{-1}) with reactor 4 achieving significantly lower concentrations at 28 to 33 mg NO_3-N L^{-1} corresponding to the longest aeration stop in its cycle. A similar trend was visible in experiment 2 where effluent nitrate concentrations decreased when the length of the aeration disruption in the treatment cycles of the reactors increased.

Throughout both experiments the nitrite concentrations in the effluent showed no particular pattern or trend between the reactors. Nitrite levels ranged from 19 to 66 µg NO_2-N L^{-1} and 44 to 85 µg NO_2-N L^{-1} during experiments 1 and 2 respectively over the first 16 days of operation. This rose to 110 and 1813 µg NO_2-N L^{-1} in reactor 1 and 4 respectively after 24 days during the extended experiment 2. These high concentrations are due to incomplete nitrification or denitrification processes. Since the factors that would affect nitrification, such as water temperature, toxic compounds in the influent, pH, BOD removal, sludge retention time (SRT) and MLSS were maintained at the same level between all reactors, the high nitrite concentrations in reactor 4 must be due to low DO concentrations causing incomplete nitrification during the aeration period. Since reactor 4 has the longest anoxic/anaerobic period it consequently has a very short aeration period that could cause difficulties in supplying the oxygen that is needed to ensure complete nitrification. The observed ammonia valley in the pH profile only indicates the depletion of ammonia and its conversion to nitrite so that with its occurrence complete nitrification can't be assumed. However, the ORP elbow occurs with the decrease in bacterial respiratory due to complete nitrification (Akin and Ugurlu, 2005; Spagni *et al.*, 2007). The oxygen consumption is reduced and the DO increases. This break point was always visible in the recorded profiles. It usually appeared up to 200 min before the end of the aeration period reaching a constant redox potential similar to the one observed in the other reactors. This

suggests that there could be another process responsible for the high nitrite concentration in reactor 4. A nitrite build-up can also occur as a result of the predominant presence of incomplete denitrifiers within denitrifying bacterial communities (Drysdale *et al.*, 2001). Drysdale *et al.* (2001) isolated and characterised the ordinary heterotrophic organisms in a NDBEPR (Nitrification Denitrification Biological Enhanced Phosphorus Removal) system according to their ability to reduce nitrates and/or nitrites under anoxic conditions. They found that 95.6% of the total denitrifying heterotrophic bacteria they isolated were capable of nitrate reduction compared to only 35.8% that were able to reduce nitrites. The other 64.2% of the isolates were missing the required nitrite reductase enzymes (Drysdale *et al.*, 2001). Ekama and Wentzel (1999) who determined denitrification kinetics for NDBEPR processes noticed an initial nitrite build-up as the nitrite reduction rate was found to be only approximately $1/10^{th}$ of the nitrate reduction rate. The fact that in the present study a significant nitrite build-up was only observed after 24 days in reactor 4 could mean that the frequent exposure to prolonged anaerobic conditions in the long run may have affected the denitrifying bacterial community. Further investigation would be needed to clarify this. However, since other operational factors that affect denitrification, such as water temperature, mixing conditions, SRT and HRT were constant throughout the duration of the run and identical between all reactors they can't be responsible for a possible incomplete denitrification.

Final effluent total phosphorous (TP) concentrations ranged from 6 to 8 mg L^{-1} and 6 to 10 mg L^{-1} during experiment 1 and 2 respectively. In experiment 1 a significant increase in TP removal with longer aeration interruptions was clearly discernible (Fig. 4c). In reactors 3 and 4, where anaerobic conditions occurred, a maximum TP removal rate of between 20 and 33% was observed respectively. During experiment 2 an undefined pattern over the first 12 days was observed with negative TP removal rates at times. These values suggest that P-release into the final effluent occurred due to the sludge becoming anaerobic during the settling phase. However, another possibility is that due to a lack of volatile fatty acids

(VFAs) secondary phosphorus release occurred during the anaerobic stage. Unlike the phosphorus released in the presence of VFAs (primary release) the phosphorus being released in the absence of VFAs will not be removed by the phosphorus accumulating organisms (PAOs) in the subsequent aerobic stage (Danesh and Oleszkiewicz, 1997). After 16 days however TP-removal rates had become stable and reached maximum values of up to 24%. Contrary to the results from the first experiment no significant differences were detected between reactors with different periods of anoxia and anaerobia (Fig. 4c).

Maximum removal rates could probably be improved by using a synthetic feed of a different composition. While the OECD synthetic sewage is based solely on peptone as an organic substrate it is known that PAOs prefer acetate and glucose as a carbon source (Kargi and Uygur, 2003). Acetate is essential for the poly-hydroxy butyrate (PHB) synthesis under anaerobic and anoxic conditions while glucose or similar carbohydrates are required for the energy generation to be used in PHB or poly-phosphate synthesis. The presence of those carbon sources might have resulted in improved P-removal (Kargi and Uygur, 2003). However, real sewage doesn't directly provide these compounds either so that the used OECD sewage formula reflects the real scenario better than synthetic sewages that artificially enhance nutrient removal. Also Carucci *et al.* (1994) have shown that there is a competition for organic substrate between PAOs and denitrifying bacteria so that nitrates inhibit EBPR (Enhanced Biological Phosphorus Removal). In lab scale SBRs they observed an increase in P-removal efficiency when nitrate concentrations in the feed were reduced or nitrification failed (Carucci *et al.*, 1994). In this study high denitrification rates were obtained so that a large amount of COD might have been used up by denitrifying bacteria thereby limiting the activity of PAOs.

3.3. Settleability

The settleability in all reactors was very poor with values for the SVI ranging from 107.3 to 193.3 mL g^{-1} in experiment 1 and 119.4 to 271.6 ml g^{-1} in experiment 2, with poorest settleability in the two reactors exposed to the longest periods of anoxia and anaerobia. A few days after the start and throughout each experiment the trend was noticeable that the settleability was best in the aerobic control reactor and worsened in the reactors with anaerobic periods. In contrast, granular sludges with good settling characteristics have been reported in SBRs with alternating anaerobic/anoxic/aerobic periods (Danesh and Oleszkiewicz, 1997; Hu *et al.*, 2005). For similar anoxic periods (60 and 120 min) and ratios of aerobic/anoxic cycle times (1 and 3) as used in the present study (Table 1), Hu *et al.* (2005) found significantly improved settling properties (SVI = 98 ml g^{-1}) compared to their aerobic control reactor (SVI = 239 ml g^{-1}). They consider the aggregation to be a protecting mechanism for the microorganisms from toxic compounds or stressful environments such as anoxic/anaerobic conditions, resulting in better settleability. Danesh and Oleszkiewicz (1997) observed better settling characteristics for sludges with higher P-removal capabilities and explained this with the higher relative abundance of PAOs in the microbial community. The P-accumulating bacteria are thought to be good floc formers and due to their accumulated phosphorus they are heavier and thus settle more rapidly (Danesh and Oleszkiewicz, 1997). A possible reason why the formation of a well settling and granular sludge during these experiments failed could be that the long settling period of 60 min did not select for rapid settling flocs (Hu *et al.*, 2005). Furthermore the use of synthetic sewage could also be the reason for the general poor settleability. With real influent colloids and particles would normally be introduced into the reactors. The adsorption of suspended and colloidal matter onto the flocs promotes the creation of denser flocs ensuring a better settleability (Gray, 1990).

3.4. Protozoan community

The protozoan species recorded throughout both experiments are listed in Table 4. Compared to the seeding sludge originally used in the experiment all the reactors

demonstrated a discernible change in community structure during the experimental period. This was expected since the bacterial community acclimatises to the synthetic sewage which consequently affects the protozoan community. While the system had not reached steady-state conditions during the experimental period, the effects of anoxia and anaerobiosis on the evolution of the community structure can be seen by direct comparison with the aerobic control reactor.

3.4.1. Community complexity

The most complex protozoan community was observed in reactor 2 with an aeration disruption of 60 min and anoxic periods of between 100 to 160 min. Compared to the number of species recorded in the inoculated sludge the reduction in community complexity ranged from 0 to 20% in reactor 2, while in reactor 1, 3 and 4 a reduction of 9 - 40%, 9 - 60% and 50 - 73% of was observed, respectively (Table 5). This suggests that short periods of anoxia do not reduce but enhance protozoan community complexity in activated sludge by providing an additional niche. This is confirmed by Perez-Uz *et al.* (2010) who studied three N-removal full scale plants and found a higher diverse protozoan communities in these systems incorporating anoxic stages compared to conventional systems. However, with the introduction of anaerobic periods in the treatment cycle a significant number of species couldn't be detected anymore, increasing as the anaerobic period increases (Table 5). The more protozoan species that are present in a system the higher the degree of stability in biological functions resulting in a higher resistance to disturbances (Liu *et al.*, 2008). Thus, the length of anaerobia should be kept as short as possible to minimize the reduction in community complexity. However, a compromise between effective P-removal and sustaining protozoan diversity has to be made.

Table 4: Protozoan species and their occurrence throughout a) experiment 1 (seeding sludge from Leixlip WWTP) and b) experiment 2 (seeding sludge from Swords WWTP); aeration disruptions 0, 60, 120 and 200 min in reactor 1, 2, 3 and 4 respectively

Table 5: Reduction in community complexity [%] compared to recorded species richness in sludge from Leixlip WWTP in experiment 1 and Swords WWTP in experiment 2 used for seeding the reactors

3.4.2. Species tolerances

Looking at species abundances of the different treatments clear trends are visible that support the hypothesis that protozoa have different tolerance levels to anoxia and anaerobia (Fig. 5). *Chilodonella uncinata* was only observed in the continuously aerated control (reactor 1), which suggest that this species is intolerant to anoxic conditions (Fig. 5). Further sensitive species include *Epicarchesium granulatum* and *Cinetochilium margaritaceum*, both becoming undetectable as soon as conditions within the treatment cycle turn anaerobic as in reactor 3 and 4 with an aeration disruption of 120 and 200 min, respectively (Fig. 5a, 5b). In both experimental runs the abundance of *E. granulatum* gradually decreased with increasing length of aeration interruptions from reactor 1 till 4. Similar trends were detected in experiment 2 for *Epistylis entzii* and *D. revoluta* (Fig. 5b, 5c).

Figure 5: Total abundance of selected species after 16 days in the reactors of a) experiment 1 (seed from Leixlip WWTP), b) after 16 days in experiment 2 (seed from Swords WWTP) and c) after 24 days of experiment 2 (Error bars represent 95% confidence interval)

At all times *Trochilia minuta* was almost exclusively observed in reactor 2 suggesting that the short times of anoxic conditions favour this species and provide a niche for it to compete with other species. Throughout the second experiment *Vorticella convallaria* endured short times of anoxia as created by an aeration disruption of 60 min in reactor 2 but decreased in numbers till the species became undetectable under prolonged anaerobic conditions (Figure 5b, 5c).

Species, which were able to survive in sufficient numbers over 16 or up to 24 days under frequent exposure to prolonged anoxic/anaerobic periods, were: *Acineria uncinata*, *Opercularia microdiscum*, *Epistylis cambari* and *Epistylis coronata* (Table 4). *Vorticella microdiscum* was observed throughout the second experiment and tolerated a repeated exposure to anaerobic periods.

No significant differences in abundances for *A. uncinata* were observed in reactor 1, 2 and 3 but although still detected in reactor 4 and therefore being able to survive prolonged anaerobic conditions numbers of this species were significantly reduced (Fig. 5a).

A species that increased in numbers with increasing length of anoxic/anaerobic conditions was *O. microdiscum* (Fig. 5a). In previous studies *O. microdiscum* was found to show a high resistance to extreme environmental conditions such as presence of toxicity, extreme temperature, high organic matter and low DO (Esteban *et al.*, 1991b; Madoni *et al.*, 1993; Lee *et al.*, 2004). With its tolerance and adaptation to changes *O. microdiscum* in this experiment survives the anoxic and anaerobic conditions better than other protozoans (Esteban *et al.*, 1991b; Madoni *et al.*, 1993) resulting in densities increasing with longer anoxic/anaerobic periods due to the decreasing competition by other species. Further explanation for the better resistance shown by this species is given by Esteban *et al.* (1991a) who suggest that being a colonial organism might help the species to endure certain conditions. However, this study shows that such a general explanation might not be suitable as with increasing length of aeration interruptions other colonial species like *E. granulatum* and *Epistylis entzii* gradually decreased in numbers.

Vorticella microstoma was detected during the second experiment but only in the reactors exposed to prolonged anoxic and anaerobic conditions with highest abundances in reactor 4 (Fig. 5b, 5c). This is in close agreement with findings in previous studies where this species was found to be related with low DO levels showing a high resistance to the influence of anoxia (Toman and Rejic, 1988; Madoni *et al.*, 1993; Madoni, 1994; Lee *et al.*, 2004). The fact that the abundances of *O. microdiscum* and *V. microstoma* also increased

over time within the anaerobic reactors suggests that they must either reproduce within an anoxic/anaerobic environment or at least only need a short time to recover sufficiently from the exposure to those conditions. To clarify this, further investigations would be required.

Although *V. microstoma* was identified as a polysaprobic species with a saprobic value of 3.5 (Foissner *et al.*, 1992) and both species, *O. microdiscum* and *V. microstoma*, were previously found to be related with high effluent BOD_5 (Poole, 1984; Madoni *et al.*, 1993; Salvado *et al.*, 1995), in this study no evidence has been found that effluent quality declined in the presence of those species. Reactors with ciliate community clearly dominated or even consisting only of *O. microdiscum* and *V. microstoma* still delivered effluents of very good quality with BOD_5 values between 3.5 and 8.5 mg L^{-1}. However, since previous observations (Poole, 1984; Madoni *et al.*, 1993; Salvado *et al.*, 1995) have been based on full scale plant studies and thus on the use of real sewage the reason for not detecting such a relationship could be connected to the characteristics of the synthetic sewage.

V. microstoma is frequently present in the plant during the first phase of colonisation but is substituted by *V. convallaria* which become dominant during stable conditions. When there is a extreme reduction in the dissolved oxygen concentration in the mixed liquor, an alternation of the two species can be observed, due to their different degree of tolerance to the lack of oxygen (Madoni, 1994). This behaviour was also observed during experiment 2 (Fig. 5b, 5c).

3.4.3. Total protozoan abundance

Total ciliate abundance was reduced by length of anoxic/anaerobic exposure (Fig. 6). In experiment 1 the number of ciliates counted in reactor 4 ranged from 400 to 800 ind mg^{-1} and was always significantly lower than in the other reactors where abundances varied

between 1000 and 2700 ind mg^{-1}. In the second experiment ciliate numbers dropped dramatically from nearly 4000 down to only 230 ind mg^{-1} within the first 8 days in the same reactor. The reduced ciliate abundance resulted in the effluent becoming very cloudy with an elevated BOD_5. While the BOD_5 of the other three reactors effluents ranged from 5 to 9 mg L^{-1}, the effluent in reactor 4 had a BOD_5 of 12.5 mg L^{-1}. These observations agree with earlier findings (Curds *et al.*, 1968; Esteban *et al.*, 1991a; Salvado *et al.*, 1995). Curds *et al.* (1968) found that in a protozoan free lab scale treatment plant very turbid effluent of inferior quality (high BOD_5, organic carbon and non-settleable suspended solids) was produced. With the inoculation of ciliate seed the clarity as well as BOD_5 and suspended solid concentrations of the effluent greatly improved. They were able to demonstrate that the effluent turbidity is related to the number of free swimming bacteria and that the presence of ciliates with their ability to feed upon those bacteria and suspended solids is responsible for a clear, high quality effluent. Esteban *et al.* (1991a) also observed a significant decrease of effluent COD and colour following an increase in ciliate concentrations. However, in the present study the low ciliate abundances observed in reactor 4 recovered again after 16 days and reached average numbers comparable to the reactors exposed to shorter anoxic/anaerobic periods. This is due to the establishment of fewer tolerant species that reach high densities of > 1000 ind mg^{-1} and start dominating the community (Fig. 5b, 5c). Simultaneously the clarity and the BOD_5 of the reactors effluent greatly improved.

Figure 6: Total ciliate abundance in the SBRs with different length of aeration disruption throughout experiment 1 and 2 (Error bars represent 95% confidence interval)

3.4.4. Community similarities

Cluster analysis revealed that the protozoan communities that had evolved in the reactors under exposure to different anoxic and anaerobic periods were quite dissimilar. After 16 days the communities showed similarities of less than 51% and 60% in experiment 1 and

2 respectively and less than 40% after 24 days in experiment 2. Considering that all other parameters including sludge loading, SRT and HRT were maintained at identical rates in all treatments, these findings suggest that the aeration conditions play a major role in how protozoan communities develop.

4. Conclusions

- Aeration conditions play a major role in how protozoan communities develop.
- Activated sludge ciliate protozoa display a range of tolerances to anoxia and anaerobia. Most sensitive were *Chilodonella uncinata*, *Epicarchesium granulatum* and *Cinetochilium margaritaceum*. Species that survive longer times of anoxic and anaerobic conditions are *Opercularium microdiscum*, *Vorticella microstoma*, *Epistylis coronata* and *Acineria uncinata*.
- By creating a new niche short times of anoxia (up to 60 min) enhance protozoan community complexity with abundances only being moderately affected. Under these conditions denitrification is improved but P-removal is still poor.
- Increasing the time of anoxia and introducing anaerobic conditions (time of aeration interruption >60 min) protozoan community complexity decreases. Species abundances can increase over time with the establishment of fewer tolerant species. A radical decrease in protozoan abundance can lead to a cloudy final effluent with increased BOD_5.
- As P-removal only occurs when anaerobic conditions are present within the cycle a compromise has to be made between effective P-removal and sustaining protozoan diversity by keeping the length of anaerobia as short as possible.

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615 **References**

Akin, B.S., Ugurlu, A., 2005. Monitoring and control of biological nutrient removal in a Sequencing Batch Reactor. Process Biochem. 40, 2873-2878.

Akpor, O.B., Momba, M.N.B., Okonkwo, J.O., Coetzee, M.A., 2008. Nutrient removal from activated sludge mixed liquor by wastewater protozoa in a laboratory scale batch reactor.
620 Int. J. Environ. Sci. Technol. 5, 463-470.

APHA, 2005. Standard Methods for the Examination of Water and Wastewater, 21th ed. American Public Health Association, AWWA, WEF, Baltimore.

Carucci, A., Majone, M., Ramadori, R., Rossetti, S., 1994. Dynamics of phosphorus and organic substrates in anaerobic and aerobic phases of a sequencing batch reactor. Water
625 Sci. Technol. 30, 237-246.

Christofi, N., Aspichueta, E., Dalzell, D., De la Sota, A., Etxebarria, J., Fernandes, T., Gutierrez, M., Morton, J., Obst, U., Schmellenkamp, P., 2003. Congruence in the performance of model nitrifying activated sludge plants located in Germany, Scotland and Spain. Water Res. 37, 177-187.

630 Curds, C.R., 1969. An illustrated key to the British Freshwater Ciliated Protozoa commonly found in activated sludge, Technical Paper No.12. Water Pollution Research Laboratory, London.

Curds, C.R., Cockburn, A., Vandyke, J.M., 1968. An experimental study of the role of the ciliated protozoa in the activated-sludge process. Wat. Pollut. Control 67, 312-329.

635 Curds, C.R., Fey, G.J., 1969. Effect of ciliated protozoa on fate of Escherichia coli in activated-sludge process. Water Res. 3, 853-867.

Curds, C.R., Warren, A., Salvadó Cabré, H., Roberts, D., 2008. An atlas of Ciliated Protozoa commonly found in aerobic sewage-treatment processes. An aid to monitor treatment -plant performance. Natural History Museum London.

640 Cybis, L.F.D., Horan, N.J., 1997. Protozoan and metazoan populations in sequencing batch reactors operated for nitrification and/or denitrification. Water Sci. Technol. 35, 81-86.

Danesh, S., Oleszkiewicz, J.A., 1997. Use of a new anaerobic-aerobic sequencing batch reactor system to enhance biological phosphorus removal. Water Sci. Technol. 35, 137-
645 144.

Drysdale, G.D., Kasan, H.C., Bux, F., 2001. Assessment of denitrification by the ordinary heterotrophic organisms in an NDBEPR activated sludge sytem. Water Sci. Technol. 43, 147-154.

Dubber, D., Gray, N.F., 2009. Enumeration of protozoan ciliates in activated sludge: Determination of replicate number using probability. Water Res. 43, 3443-3452.

Dubber, D., Gray, N.F., 2010. Replacement of chemical oxygen demand (COD) with total organic carbon (TOC) for monitoring wastewater treatment performance to minimize disposal of toxic analytical waste. J. Environ. Sci. Health Part A Toxic-Hazard. Subst. Environ. Eng. 45, 1595-1600.

Ekama, G.A., Wentzel, M.C., 1999. Denitrification kinetics in biological N and P removal activated sludge systems treating municipal wastewaters. Water Sci. Technol. 39, 69-77.

Esteban, G., Tellez, C., Bautista, L.M., 1991a. Dynamics of ciliated protozoa communities in activated-sludge process. Water Res. 25, 967-972.

Esteban, G., Tellez, C., Bautista, L.M., 1991b. Effects of habitat quality on ciliated protozoa communities in sewage-treatment plants. Environ. Technol. 12, 381-386.

Foissner, W., Berger, H., Kohmann, F., 1992. Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems, Band II: Peritrichia, Heterotrichia, Odontostomatida. Landesamt für Wasserwirtschaft, München.

Foissner, W., Berger, H., Kohmann, F., 1994. Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems, Band III: Hymenostomata, Prostomatida, Nasulida. Landesamt für Wasserwirtschaft, München.

Foissner, W., Blatterer, H., Berger, H., Kohmann, F., 1991. Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems, Band I: Cyrtophorida, Oligotrichida, Hypotrichida, Colpode. Landesamt für Wasserwirtschaft München.

Foissner, W., Blatterer, H., Berger, H., Kohmann, F., 1995. Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems, Band IV: Gymnostomatea, Loxodes, Suctoria. Landesamt für Wasserwirtschaft, München.

Fuerhacker, M., Bauer, H., Ellinger, R., Sree, U., Schmid, H., Zibuschka, F., Puxbaum, H., 2000. Approach for a novel control strategy for simultaneous nitrification/denitrification in activated sludge reactors. Water Res. 34, 2499-2506.

Gendig, C., Domogala, G., Agnoli, F., Pagga, U., Strotmann, U.J., 2003. Evaluation and further development of the activated sludge respiration inhibition test. Chemosphere 52, 143-149.

Gray, N.F., 1990. Activated Sludge - Theory and Practice. Oxford University Press, Oxford, New York, Tokyo.

Gray, N.F., 2004. Biology of Wastewater Treatment, 2nd ed. Imperial College Press, London.

Hu, Z.Q., Ferraina, R.A., Ericson, J.F., MacKay, A.A., Smets, B.F., 2005. Biomass characteristics in three sequencing batch reactors treating a wastewater containing synthetic organic chemicals. Water Res. 39, 710-720.

ISO, 2003. Water quality: Determination of biochemical oxygen demand after n days (BODn), Part 1: Dilution and seeding method with allylthiourea addition. ISO 5815-1:2003. International Organization for Standardization, Geneva.

Kargi, F., Uygur, A., 2003. Effect of carbon source on biological nutrient removal in a sequencing batch reactor. Bioresour. Technol. 89, 89-93.

Lee, N.M., Welander, T., 1996. Use of protozoa and metazoa for decreasing sludge production in aerobic wastewater treatment. Biotechnol. Lett. 18, 429-434.

Lee, S., Basu, S., Tyler, C.W., Wei, I.W., 2004. Ciliate populations as bio-indicators at Deer Island Treatment Plant. Adv. Environ. Res. 8, 371-378.

Lee, Y., Oleszkiewicz, J.A., 2003. Effects of predation and ORP conditions on the performance of nitrifiers in activated sludge systems. Water Res. 37, 4202-4210.

Liu, J., Yang, M., Qi, R., An, W., Zhou, J., 2008. Comparative study of protozoan communities in full-scale MWTPs in Beijing related to treatment processes. Water Res. 42, 1907-1918.

Madoni, P., 1994. A sludge biotic index (SBI) for the evaluation of the biological performance of activated-sludge plants based on the microfauna analysis. Water Res. 28, 67-75.

Madoni, P., Davoli, D., Chierici, E., 1993. Comparative-analysis of the activated-sludge microfauna in several sewage-treatment works. Water Res. 27, 1485-1491.

Maurines-Carboneill, C., Morin, L., Pernelle, J.J., Derlet, N., Sachon, G., Leblon, G., 1998. Reviviscence of aerobic chemoheterotrophic bacteria in an activated sludge pilot plant after a prolonged absence of oxygen. Water Res. 32, 2211-2219.

Ndon, U.J., 2007. Analysis and kinetics of the sequencing batch reactors. Environ. Eng. Sci. 24, 505-514.

Obaja, D., Mace, S., Mata-Alvarez, J., 2005. Biological nutrient removal by a sequencing batch reactor (SBR) using an internal organic carbon source in digested piggery wastewater. Bioresour. Technol. 96, 7-14.

Pérez-Uz, B., Arregui, L., Calvo, P., Salvadó, H., Fernández, N., Rodríguez, E., Zornoza, A., Serrano, S., 2010. Assessment of plausible bioindicators for plant performance in advanced wastewater treatment systems. Water Res. 44, 5059-5069.

Poole, J.E.P., 1984. A study of the relationship between the mixed liquor fauna and plant performance for a variety of activated sludge sewage treatment works. Water Res. 18, 281-287.

Reuschenbach, P., Pagga, U., Strotmann, U., 2003. A critical comparison of respirometric biodegradation tests based on OECD 301 and related test methods. Water Res. 37, 1571-1582.

Salvado, H., Gracia, M.P., Amigo, J.M., 1995. Capability of ciliated protozoa as indicators of effluent quality in activated-sludge plants. Water Res. 29, 1041-1050.

Spagni, A., Lavagnolo, M.C., Scarpa, C., Vendrame, P., Rizzo, A., Luccarini, L., 2007. Nitrogen removal optimization in a sequencing batch reactor treating sanitary landfill leachate. J. Environ. Sci. Health Part A Toxic-Hazard. Subst. Environ. Eng. 42, 757-765.

Toman, M., Rejic, M., 1988. The effects of low concentrations and short-period lack of dissolved-oxygen upon the organisms of the concomitant biocenosis in activated-sludge. Z. Wasser Abwass. Forsch. 21, 189-193.

Yu, R.F., Liaw, S.L., Chang, C.N., Lu, H.J., Cheng, W.Y., 1997. Monitoring and control using on-line ORP on the continuous-flow activated sludge batch reactor system. Water Sci. Technol. 35, 57-66.

Table 1: Cycle time configurations for the laboratory-scale SBRs

Reactor	Cycle time [min]	Number of cycles/day	Aeration off [min]	Aeration on [min]	Settle [min]	Draw and fill [min]	Ratio aerobic/ anaerobic time
1	720	2		610	60	50	
2	720	2	60	550	60	50	9.17
3	720	2	120	490	60	50	4.08
4	720	2	200	410	60	50	2.05

Table 3: Average length of aerobic and anoxic/anaerobic time (ORP < 0mV) observed in the reactors at a) experiment 1 (seed from Leixlip WWTP) and b) experiment 2 (seed from Swords WWTP)

a)	1-16 cycles			17-32 cycles		
	time ORP<0mV [min]	aerobic time [min]	ratio*	time ORP<0mV [min]	aerobic time [min]	ratio*
Reactor 1	17	592	34.8	27	582	21.4
Reactor 2	184	425	2.3	131	478	3.7
Reactor 3	173	436	2.5	194	415	2.1
Reactor 4	244	365	1.5	263	346	1.3

b)	1-16 cycles			17-32 cycles			33-48 cycles		
	time ORP<0mV [min]	aerobic time [min]	ratio*	time ORP<0mV [min]	aerobic time [min]	ratio*	time ORP<0mV [min]	aerobic time [min]	ratio*
Reactor 1	0	609		0	609		0	609	
Reactor 2	91	518	5.7	90	519	5.7	113	496	4.4
Reactor 3	172	437	2.5	183	427	2.3	200	409	2.0
Reactor 4	274	335	1.2	271	338	1.2	253	356	1.4

* ratio = aerobic time / anaerobic time

Table 4: Protozoan species and their occurrence throughout a) experiment 1 (seeding sludge from Leixlip WWTP) and b) experiment 2 (seeding sludge from Swords WWTP); aeration disruptions 0, 60, 120 and 200 min in reactor 1, 2, 3 and 4 respectively

a)		8 days				16 days			
	start	R 1	R 2	R 3	R 4	R 1	R 2	R 3	R 4
Vorticella sp.	x	x	x	x	x	x	x		x
Epistylis entzii	x		x				x		
Epistylis coronata	x	x	x	x	x	x	x	x	x
Epistylis cambari	x	x	x	x	x	x	x	x	x
Epicarchesium granulatum	x	x	x			x	x		
Opercularia microdiscum				x	x	x	x	x	x
Thuricola kellicottiana	x								
Trochilia minuta	x		x				x		
Chilodonella uncinata	x					x			
Acinera uncinata	x	x	x	x	x	x	x	x	x
Cinetochilum margaritaceum	x	x	x			x	x		

b)		8 days				16 days				24 days			
	start	R 1	R 2	R 3	R 4	R 1	R 2	R 3	R 4	R 1	R 2	R 3	R 4
Vorticella convallaria		x	x	x	x	x	x	x		x	x	x	
Vorticella sp.	x	x	x			x	x	x	x	x	x	x	
Vorticella microstoma									x	x		x	x
Epistylis entzii	x	x	x	x		x	x	x		x	x		
Epistylis coronata	x	x	x	x		x	x	x		x	x		x
Epicarchesium granulatum	x	x	x	x		x	x	x			x		
Opercularia microdiscum	x	x	x	x	x	x	x	x	x	x	x	x	x
Thuricola kellicottiana	x	x	x	x		x	x						
Aspidisca lynceus	x												
Euplotes affinis	x												
Trochilia minuta							x	x			x		
Chilodonella uncinata	x												
Acinera uncinata	x	x	x	x	x	x	x	x	x	x	x	x	x
Litonotus sp.	x						x	x					
Drepanomonas revoluta		x				x	x	x	x	x	x	x	

Table 5: Reduction in community complexity [%] compared to recorded species richness in sludge from Leixlip WWTP in experiment 1 and Swords WWTP in experiment 2 used for seeding the reactors

Reduction in community complexity [%]	Experiment 1		Experiment 2		
	8 days	16 days	8 days	16 days	24 days
Reactor 1 continuously aerated	40	20	27	9	27
Reactor 2 60 min aeration stop	20	10	18	0	18
Reactor 3 120 min aeration stop	50	60	36	9	45
Reactor 4 200 min aeration stop	50	60	73	55	64

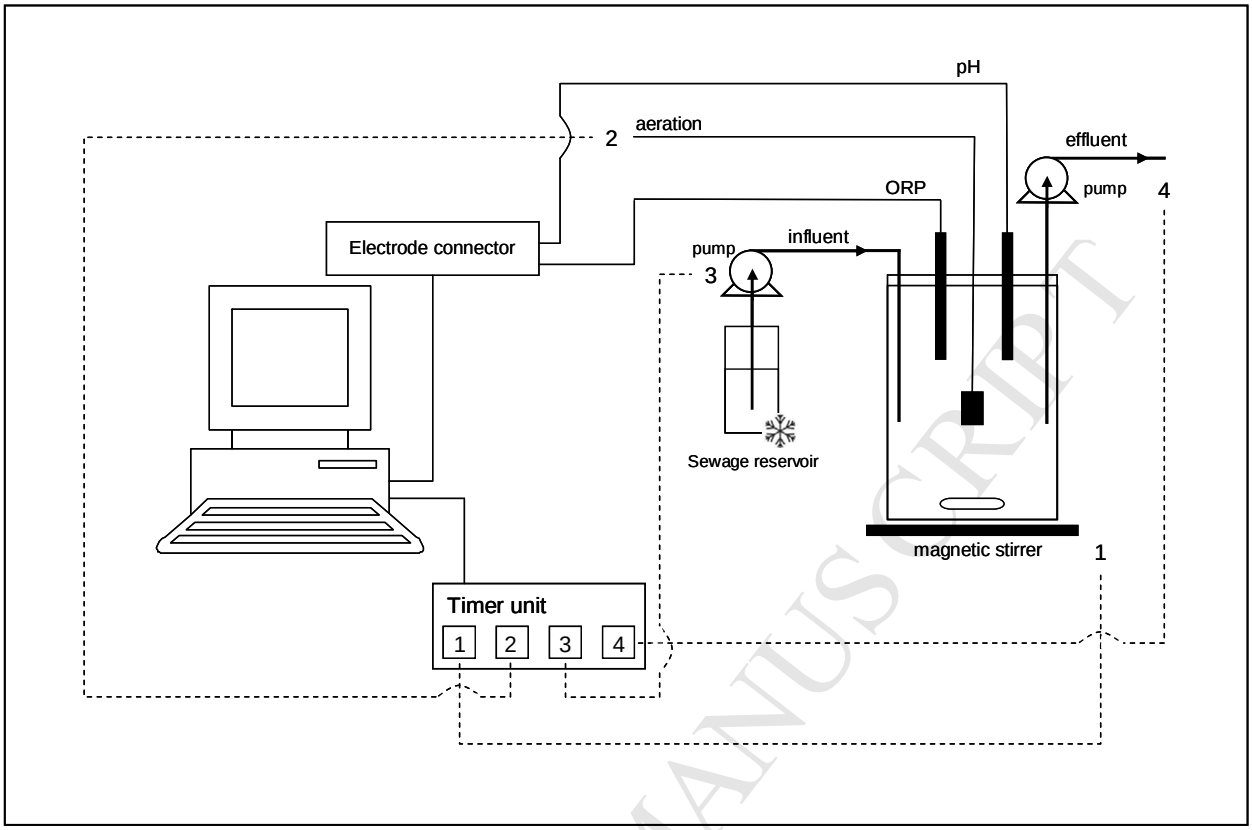
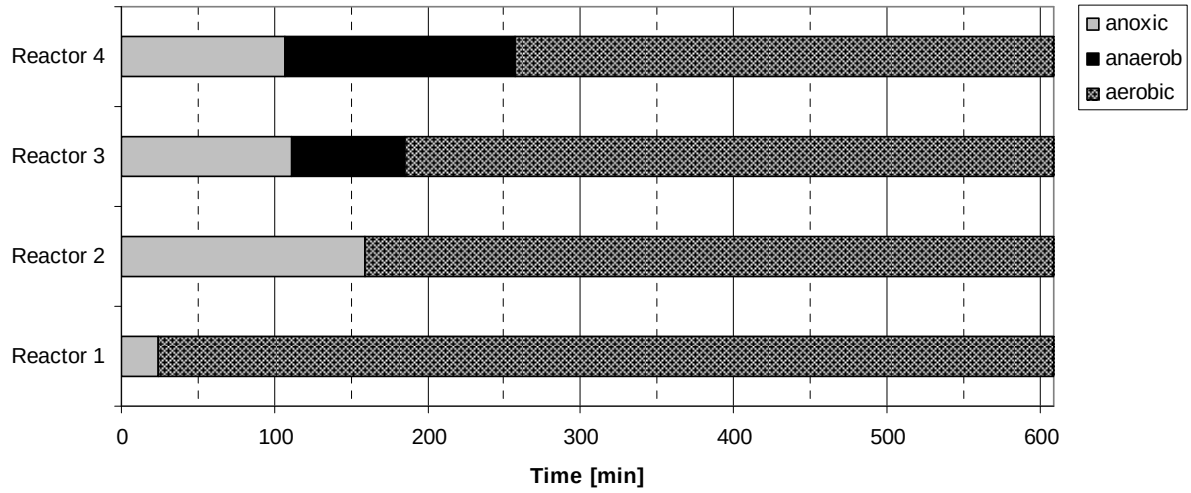


Figure 1: Configuration of the laboratory-scale SBRs

a)



b)

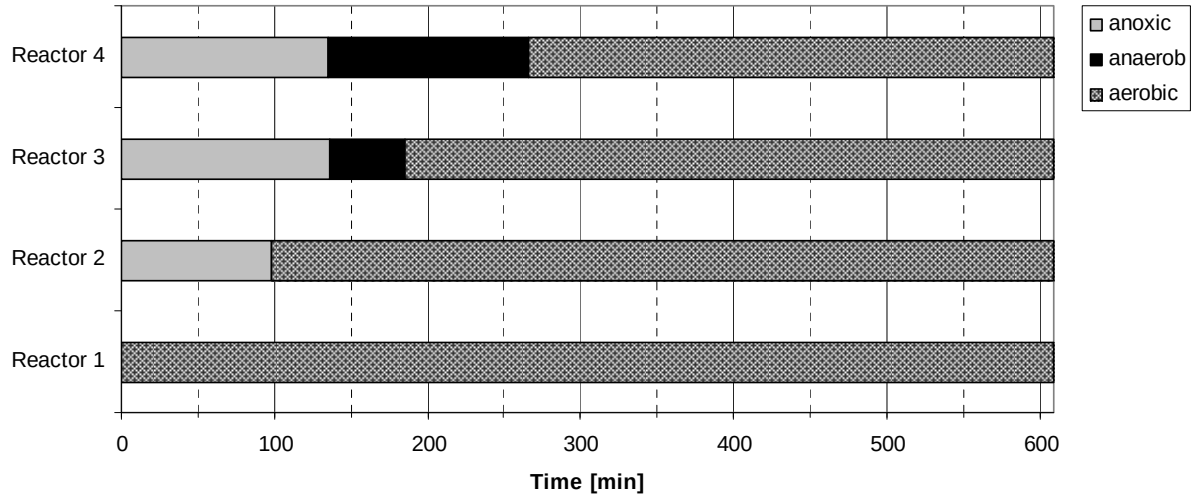


Figure 3: Observed length of aerobic (ORP > 0 mV) anoxic (0 mV > ORP > -190 mV) and anaerobic (ORP < -190 mV) periods in the 4 reactors depending on their cycle time settings in a) experiment 1 (seed from Leixlip WWTP) and b) experiment 2 (seed from Swords WWTP)

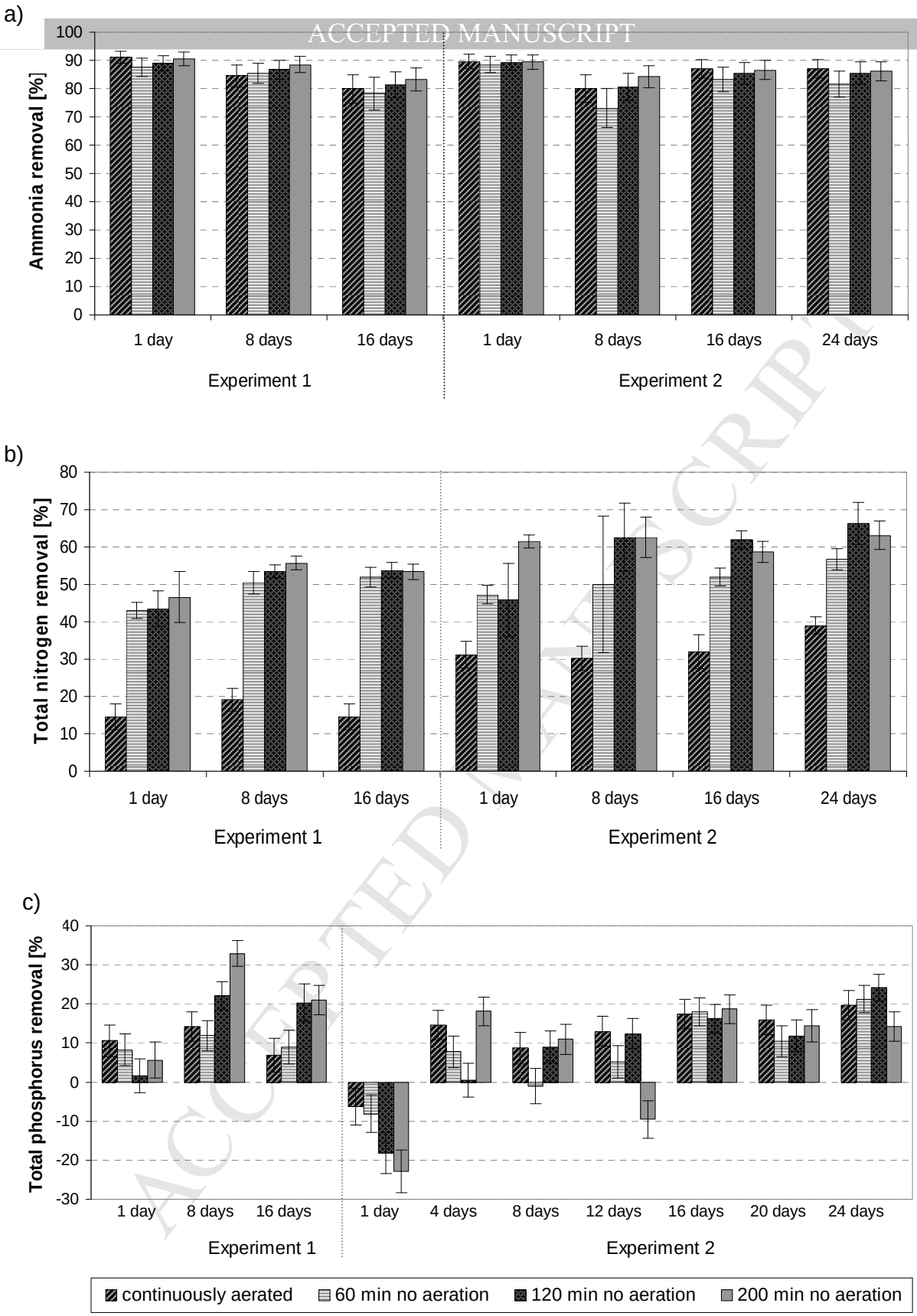
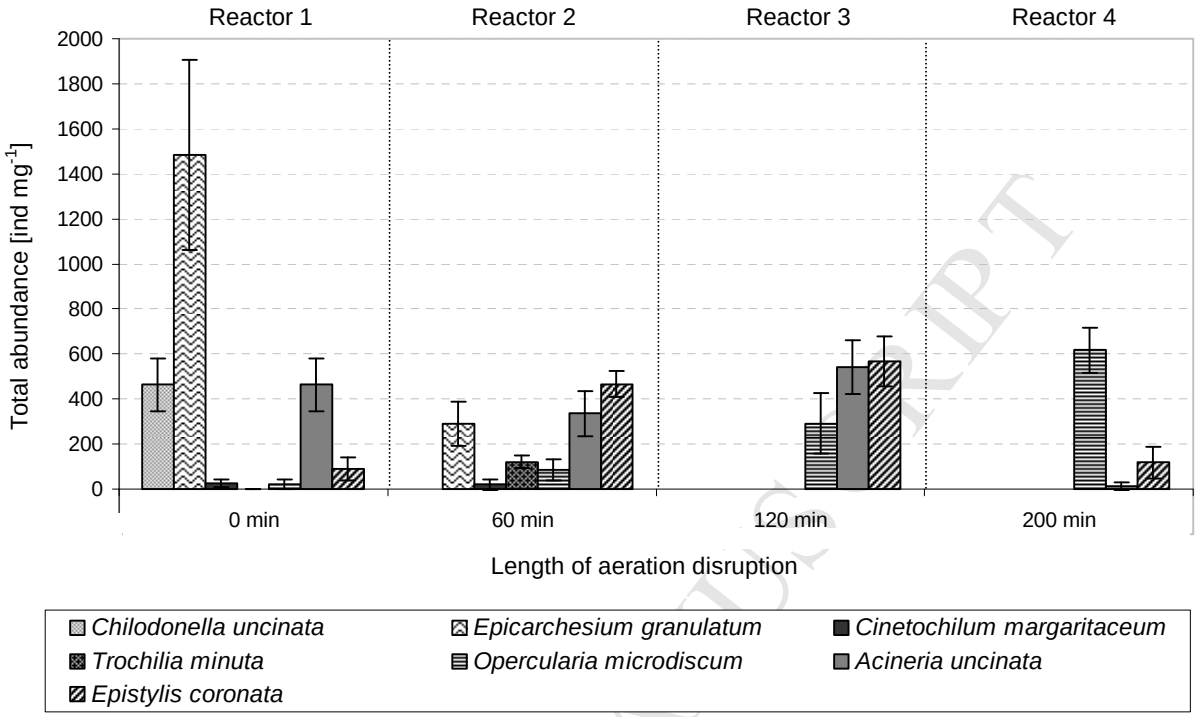
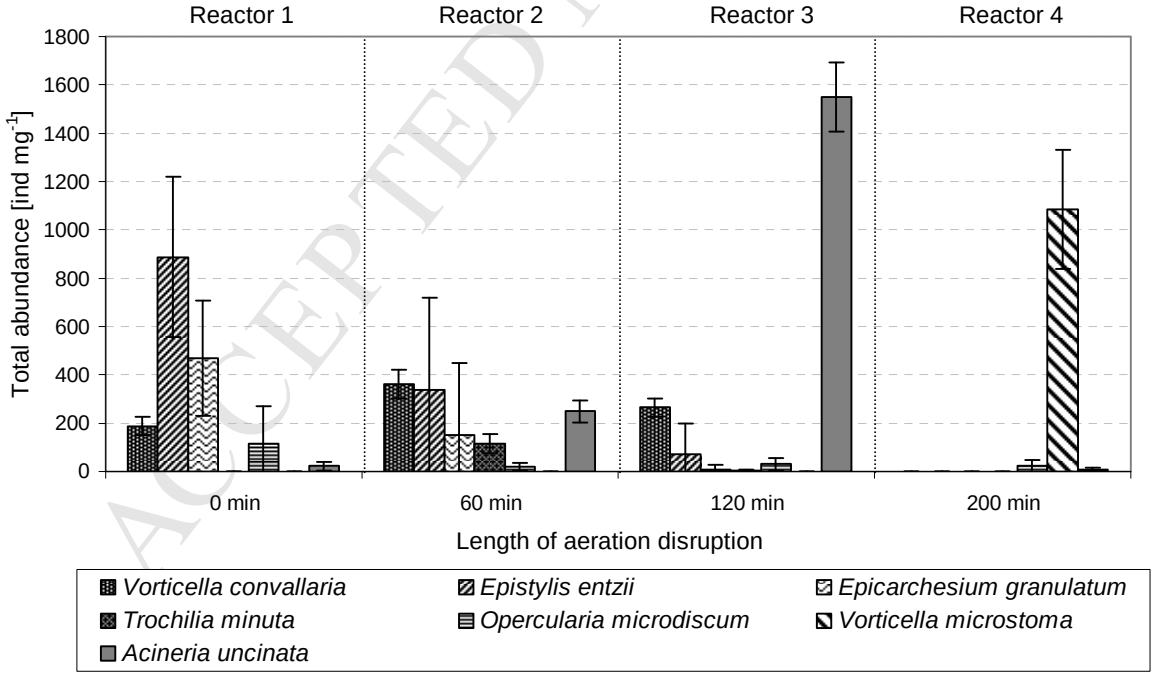


Figure 4: Ammonia removal (a), total nitrogen (b) and total phosphorus (c) removal in the SBRs with different length of aeration disruption throughout experiment 1 and 2 (Error bars represent 95% confidence interval)

a)


Reactor 1
Reactor 2
Reactor 3
Reactor 4
Total abundance [ind mg-1]
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0 min
60 min
120 min
200 min
Length of aeration disruption
Chilodonella uncinata
Epicarchesium granulatum
Cinetochilum margaritaceum
Trochilia minuta
Opercularia microdiscum
Acineria uncinata
Epistylis coronata

b)


Reactor 1
Reactor 2
Reactor 3
Reactor 4
Total abundance [ind mg-1]
1800
1600
1400
1200
1000
800
600
400
200
0
0 min
60 min
120 min
200 min
Length of aeration disruption
Vorticella convallaria
Epistylis entzii
Epicarchesium granulatum
Trochilia minuta
Opercularia microdiscum
Vorticella microstoma
Acineria uncinata

c)

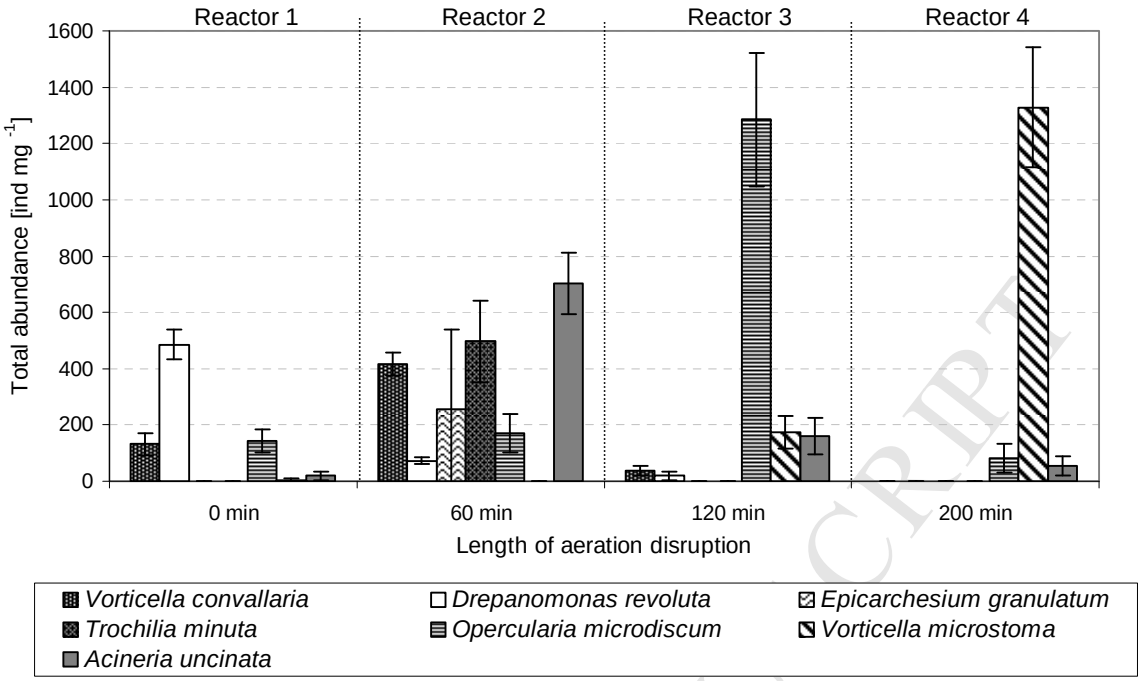


Figure 5: Total abundance of selected species after 16 days in the reactors of a) experiment 1 (seed from Leixlip WWTP), b) after 16 days in experiment 2 (seed from Swords WWTP) and c) after 24 days of experiment 2 (Error bars represent 95% confidence interval)

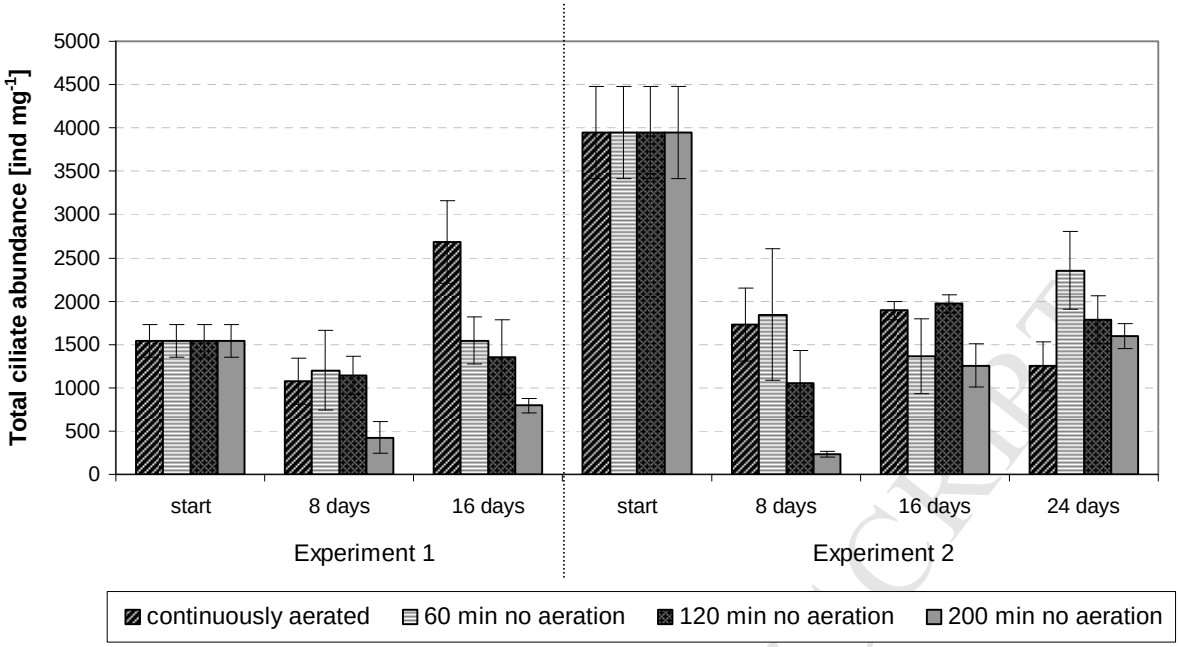


Figure 6: Total ciliate abundance in the SBRs with different length of aeration disruption throughout experiment 1 and 2 (Error bars represent 95% confidence interval)