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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)

*Donata Dubber**, *N.F. Gray*

Centre for the Environment, Trinity College Dublin, Dublin 2, Ireland

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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 and 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 analysed 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 min anoxic/anaerobic period resulted in good process performance (TOC and BOD removal of 97–98% 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 and 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 optimised to sustain the protozoan community while achieving maximum performance and nutrient removal.

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

* *Corresponding author.*
E-mail address: dubberd@tcd.ie (D. Dubber).
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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 h 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 and 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 avoid 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 min.

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