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ScienceDirect

European Journal of Protistology 51 (2015) 470–479

European Journal of PROTISTOLOGY

www.elsevier.com/locate/ejop

Effect of high levels of the rotifer *Lecane inermis* on the ciliate community in laboratory-scale sequencing batch bioreactors (SBRs)

Janusz Fyda[a,*], Roman Babko[b], Edyta Fiałkowska[a], Agnieszka Pajdak-Stós[a], Wioleta Kocerba-Soroka[a], Mateusz Sobczyk[a], Łukasz Sobczyk[a]

[a]*Institute of Environmental Sciences, Jagiellonian University in Kraków, Gronostajowa 7, 30-387 Kraków, Poland*
[b]*Schmalhausen Institute of Zoology of the National Academy of Sciences of Ukraine, Vul. B. Khmelnytskogo, 15, 01601 Kyiv, Ukraine*

Received 26 May 2015; received in revised form 24 August 2015; accepted 19 September 2015
Available online 28 September 2015

Abstract

Due to its ability to feed on filamentous bacteria, the rotifer *Lecane inermis* has already been recognized as a potential control agent of activated sludge bulking, which is usually caused by the excessive growth of filamentous microorganisms. However, their effectiveness depends, in part, on their abundance. We studied the influence of high densities of *L. inermis* on the protozoan community in activated sludge from a wastewater treatment plant (WWTP) in 4 laboratory-scale sequencing batch bioreactors (SBRs). Two treatments and two controls were subjected to nutrient removal system in process similar to that used in a WWTP. The experiment lasted 9 days and was repeated in 24-h cycles, including phases of agitation with feeding, aeration and agitation and sedimentation with decantation at the end of the cycle. In total, 32 taxa were identified, among which 25 were ciliated protozoa, 4 were amoebae, 2 were flagellates, and one was a nematode. Rotifers were then introduced to 2 bioreactors at a final concentration of 500 ind. mL^{-1}, and the taxonomic composition and abundance of the activated sludge microfauna were assessed 2, 5 and 8 days thereafter. The mean density of ciliates on the first day of experiment was 12,610 ind. mL^{-1} and diminished to 4868 ± 432 ind. mL- $\pm$ 432 ind. mL^{-1} in the control and 5496 ± 638 ind. mL^{-1} in the rotifer-treated group on the last day. Thus, even extremely high densities of artificially introduced rotifers did not negatively affect the protozoan community. On the contrary, the protozoan community was more diverse in the treatment group than in the control.

Keywords: Activated sludge; Ciliates; *Lecane inermis*; Testate amoebae; Rotifers

Introduction

The activated sludge bioreactors utilized in wastewater treatment plants are no longer considered a “black box” but now are known to be full of life supporting a well developed biocenosis to purify sewage in aerobic processes. Although activated sludge biocenoses consist of many different types of microorganisms, the most extensively studied of these organisms are bacteria and ciliated protozoa (Chen et al. 2004; Curds 1982a,b; Madoni 2011; Martin-Cereceda et al. 1996; Wilen et al. 2008; Zhou et al. 2006). These studies have emphasized the important role of ciliated protozoa, especially sessile and creeping ciliates, in the removal of vast amounts of sewage bacteria. However, some of the interactions among different species of ciliated protozoans, ciliates and metazoans in activated sludge are typical

*Corresponding author. Tel.: +48 126645181.
E-mail address: janusz.fyda@uj.edu.pl (J. Fyda).

http://dx.doi.org/10.1016/j.ejop.2015.09.001
0932-4739/

prey-predator relationships and are less studied. Predatory protozoans and metazoans can change the abundance and composition of bacteriophagous protists in sludge and consequently affect biological treatment processes. The abundance of ciliated protozoa highly correlates with biological treatment processes and effluent quality (Hu et al. 2013; Zhou et al. 2006). Specifically, decreases in ciliate abundance and diversity in activated sludge result in low treatment effectiveness and hinder the removal of biological agents (Chen et al. 2004). Moreover, activated sludge biocenoses plays an important role in flock formation, which aids in flocculation and sedimentation processes (Hu et al. 2013). A well-developed activated sludge biocenosis is more resistant to changes in influent composition, which ensures the stability and proper operation of biological treatment processes. Conversely, decreases in ciliated protozoa diversity and/or the dominance of some ciliate species may signal community dysfunction, toxins and/or problems in the bioreactor of the wastewater treatment plant (Curds and Cockburn 1970).

In addition to the intra- and inter-specific interactions among the bacteria and ciliated protozoa in activated sludge biocenosis, comparable attention has been paid to the relationships among metazoans that reside in activated sludge (Ratsak 2001; Ratsak and Verkuijlen 2006; Salvadó et al. 2004; Weisse and Frahm 2002). For example, high levels of bdelloid rotifers may reduce biomass production and improve effluent clarity (Lapinski and Tunnacliffe 2003). Similarly, high levels of *L. inermis* may reduce the flock area from 60 to 20 mm^2 without affecting the ability of the sludge to settle, as described by Puigagut et al. (2007). Furthermore, the oligochaetes *Tubifex* sp. and *Nais elinguis*, which belong to families with specific ecological niches, can reduce sludge production (Ratsak 2001; Rensink and Rulkens 1997). Kocerba-Soroka et al. (2013b) showed that *L. inermis* grazes at densities exceeding 2200 ind. mL^{-1}, which improved the settling properties of flocs and did not affect the main parameters of the sewage treatment process, such as the chemical oxygen demand (COD), $N–NH_4$, $N–NH_3$, $P–PO_4$ and pH.

However, even well-operated wastewater treatment plants (WWTPs) occasionally experience operational problems related to the excessive growth of filamentous bacteria, especially in the early spring. This phenomenon is called sludge bulking and is limited in treatment plants with the help of chemical compounds that cause filaments sedimentation. However, these compounds also destroy activated sludge biocenosis. Alternatively, high levels of filaments can be eliminated by natural consumers of filamentous bacteria. Inamori et al. (1991) and Drzewicki and Hul (1997) used the cyrtophoride ciliate *Trithigmostoma cucullulus* to consume filamentous bacteria and reported promising results. Recently, Fiałkowska and Pajdak-Stós (2008) also found that the rotifer *Lecane inermis* is an effective consumer of filamentous sludge bacteria. *L. inermis* is a loricate monogonont rotifer that is 92–154 μm in length and commonly found in fresh and wastewater samples worldwide (Miller 1931). Studies of these rotifers have consistently shown that they can control the growth and abundance of at least some types of filamentous bacteria, such as *Microthrix parvicella* (Fiałkowska and Pajdak-Stós 2008), Type 021N (Kocerba-Soroka et al. 2013a), Type 0092 (Drzewicki et al. 2015) and *Haliscomenobacter hydrossis* (Kowalska et al. 2015). However, these studies also showed that these positive effects require rotifer numbers much higher than those usually observed in activated sludge. Although rotifers, nematodes or oligochaete worms exert positive effects by facilitating the formation of flocs formation, reducing excess biomass production, dispersing bacteria consumption and nutrient cycling in activated sludge (Chen et al. 2004; Lapinski and Tunnacliffe 2003), the effects of high, artificially increased number of rotifers on other components of the activated sludge biocenosis are poorly understood. Because *Lecane* rotifers can feed on filamentous and free-living dispersed bacteria, they may compete with ciliates for food in high numbers and consequently influence the ciliate community.

Using a laboratory-scale SBR system that simulates basic operational WWTP processes, we studied the effect of artificially increased numbers of the rotifer *L. inermis* on the composition and abundance of the activated sludge microfauna. Specifically, this study focused on the effect of rotifers on sessile and creeping ciliates, testate and naked amoebae and the composition and abundance of flagellate species. Overall, this study aimed to identify how the artificial introduction of rotifers affects the protozoan community in activated sludge.

Material and Methods

Rotifers mass cultures

A clonal population of the monogonont rotifer *Lecane inermis* was isolated from a biological WWTP located in southern Poland. The strains were obtained from single individuals transferred with a micropipette from a sludge sample to separate wells in tissue culture test plates, which were filled with Żywiec brand spring water. An oat grain previously sterilized in boiling water was added to each well. The rotifers then fed on bacteria proliferating on the oat grains. Subsequently, the rotifers were transferred to 5 cm diameter Petri dishes and maintained in the same manner. Specifically, the rotifer cultures were kept in the dark at temperature of 20 ± 1 °C in a Sanyo Versatile Environmental Test Chamber MLR-350. Every week, the rotifer cultures were checked and transferred to fresh medium. The best-growing strain was selected for the experiment.

Design of experiment

The wastewater treatment process was simultaneously simulated in 4 fully automatically run bioreactors (BioFlo