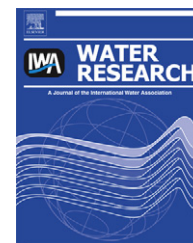


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Aerobic treatment of dairy wastewater in an industrial three-reactor plant: Effect of aeration regime on performances and on protozoan and bacterial communities

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ABSTRACT

An industrial three-reactor plant treating $45 \text{ m}^3 \text{ d}^{-1}$ of dairy wastewater was monitored to investigate the effect of different aeration regimes on performance efficiency and to find relationships with bacterial and protozoan communities in the activated sludge. During the study, the plant was maintained at six different “on/off” cycles of the blower (45/15, 15/15, 15/45, 30/30, 30/45 and 30/60 min), providing between 30.2 and 90.6 $\text{kg O}_2 \text{ d}^{-1}$, and the main chemical/biochemical parameters (COD, BOD, NH_4^+ , NO_2^- , NO_3^- , PO_4^{3-} , etc.) were determined. When at least 45.4 $\text{kg O}_2 \text{ d}^{-1}$ (30/45) were provided, COD removal efficiencies were always in the range 88–94% but decreased to about 70% under aeration regimes 15/45 and 30/60. Ammonium ion degradation performance was compromised only in the lowest aeration regime (15/45). Total number of protozoa and their species richness, and bacterial viable counts and denaturing gradient gel electrophoresis (DGGE) profiles were used to characterize the microbiota of the activated sludge. Cell abundances and community structures of protozoa and bacteria were very similar in the three aerated reactors but changed with the aeration regimes. In particular, the 15/45 and 30/60 regimes led to low protozoan diversity with prevalence of flagellates of the genus *Trepomonas* at the expense of the mobile and sessile forms and, thus, to a less efficient activated sludge as indicated by Sludge Biotic Index values (3 and 4.5 for the two regimes, respectively). The structure of the bacterial community strongly changed when the aeration regimes varied, as indicated by the low similarity values between the DGGE profiles. On the contrary, number of viable bacteria and values of the biodiversity index remained stable throughout the whole experimentation. Taken together, the results of the present study clearly indicate that aeration regime variations strongly influence the structure of both protozoan and bacterial communities and, above all, that a high biodiversity among protozoan populations in the activated sludge is prerequisite for high performances in dairy wastewater treatment.

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1. Introduction

Nowadays, the dairy industry is considered the largest source of food processing wastewater. The effluent, that includes wasted milk and water from cleaning, sanitization, heating, cooling and floor washing, but excludes whey that is generally separated and differently treated or upgraded, is characterized by variable volumes, flow rates and organic matter content, which ranges approx. from 0.8 to 7.0 g/l COD (Britz et al., 2006). Dairy wastewaters require, therefore, specialized treatments (EU Directive 2000/60/EC) to meet effluent discharge standards and to reduce the risk of environmental problems such as eutrophication in rivers, lakes and coastal waters.

Conventional dairy wastewater treatment plants (WTPs) are mainly based on activated sludge processes that involve the aerobic microbial metabolism of fats, lactose and proteins. The anaerobic treatment, which is often inhibited by the presence of fats causing poor nutrient removal (Vidal et al., 2000), is generally considered more suitable for high organic loads (e.g., effluents that include whey) (Britz et al., 2006; Kushwaha et al., 2011). Treatment based on intermittently aerated reactors consisting of alternate anoxic/anaerobic and aerobic phases has been proved to be the best way to achieve carbon, as well as nitrogen and phosphorus removals (Gutierrez et al., 2007; Kushwaha et al., 2011). In this respect, the control of the aeration regimes represents a key issue since anaerobic under aeration may lead to partially treated effluent, while over-aeration results in higher than necessary oxygen that may cause destabilization of the sludge and, definitely, in higher electricity and maintenance costs (Britz et al., 2006).

Activated sludge systems consist of a complex mixture of bacteria and protozoa that remove organic substances and nutrient contaminants from wastewaters. Thus, a better understanding of the microbial communities of activated sludge, and particularly of the correlation between microbial diversity and ecosystem function, is necessary to rapidly monitor and assess process performances and to optimize the biological processes occurring in wastewater treatment plants (Sanz and Kochling, 2007).

Protozoa populations play a major role in the microbial food webs during the biological treatment in WTPs and their abundance and diversity are commonly used as an indicator of activated sludge plant performance (Seviour and Nielsen, 2010). In this respect, Madoni (1994) has introduced an objective index, the Sludge Biotic Index (SBI), based on the presence and abundance of certain key protozoan groups, that provides a numerical value that enables the operator to monitor the prevalent plant operating conditions and performances on a daily basis. In the last decade, several studies have been aimed at demonstrating the applicability of the SBI as a useful monitoring tool to assess the activated sludge health by using different WTP typologies and/or wastewaters added of possible toxic substances (e.g., chromium VI, copper, phenol and cyanide) (Papadimitriou et al., 2007; Drzewicki and Kulikowska, 2011). Although the majority of the studies have reported direct correlations between high SBIs and good plant treatment performances, the index does not appear to be

always reliable (Arevalo et al., 2009; Drzewicki and Kulikowska, 2011).

Further, and in spite of their importance in the activated sludge, the information on the ecological role of the bacterial populations in wastewater treatment systems is quite limited. Conventional microbiological techniques based on cultivation-dependent approaches have, in fact, proven inadequate since cultivable bacteria represent only a minor fraction of the whole community of such complex ecosystems. On the contrary, molecular methods based on polymerase chain reaction (PCR) amplification of 16S ribosomal RNA (rRNA) genes allow the profiling of complex bacterial communities on the basis of sequence diversity, thus avoiding the biases associated with laboratory culturing. Among the genetic fingerprinting methods, denaturing gradient gel electrophoresis (DGGE) analysis of 16S rRNA genes permits direct visualization and rapid comparison of the structure of bacterial communities, thus showing useful in investigating the microbial ecology of the activated sludge (Liu et al., 2007; Sanz and Kochling, 2007).

In this context it is worth noting that, to the best of our knowledge, no studies have been reported on the combined monitoring of both bacterial and protozoan populations of the activated sludge in order to assess possible relationships between microbial communities and treatment performances. With these points in mind, objective of the present study was to assess the effect of six different aeration regimes on both biotreatment performances and activated sludge microbiota in a dairy WTP. To this end, an industrial plant characterized by three aerated reactors working in series (namely, R1, R2 and R3) was operated at six different “on/off” cycles of the blower with consequent various extents of aerobiosis and anoxia. Under these aeration regimes, relationships between removal efficiency of the main chemical/biochemical parameters and the structures of both protozoan population and bacterial community have also been investigated.

2. Materials and methods

2.1. Dairy wastewater

The “Buona Tavola Sini” dairy (Monterosi, Viterbo, Italy) processes 15,000–20,000 L of milk per day and produces about 45 m³ d⁻¹ of wastewater. The dairy mostly treats sheep milk and, in much lesser amount, bovine milk. The wastewater mainly comes from the cleaning of the equipment in contact with milk or milk derivatives; whey is disposed of separately. The wastewater characteristics are reported in Table 1.

2.2. Wastewater treatment plant (WTP) and operative conditions

The WTP, designed and manufactured by Manzi Aurelio Srl (Montefiascone, Italy), is constituted of a primary section for sedimentation of 200 m³, three aerated reactors (R1, R2 and R3) connected in series and of 18 m³ capacity each and a secondary section for sedimentation of 18 m³. Also, there is

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