

# Dairy wastewater treatment using an activated sludge–microalgae system at different light intensities

O. Tricolici, C. Bumbac, V. Patroescu and C. Postolache

## ABSTRACT

A microalgae–bacteria system was used for dairy industry wastewater treatment in sequenced batch mode in a photobioreactor. The research investigated the influence of two light intensities: 360 and 820  $\mu\text{mol m}^{-2} \text{s}^{-1}$  on treatment performances, microalgal cell recovery and dynamics of the protozoan community. Results showed that the light intensity of 360  $\mu\text{mol m}^{-2} \text{s}^{-1}$  was found to be insufficient to support photosynthetic activity after the increase of bacterial biomass leading to the decrease of organic matter and ammonium removal efficiencies from 95 to 78% and 95 to 41%, respectively. Maximum microalgal cells recovery was about 63%. Continuous modification in the protozoan community was also noticed during this test. Increasing the light intensity to 820  $\mu\text{mol m}^{-2} \text{s}^{-1}$  led to better microalgal cells recovery (up to 88%) and improved treatment performances. However, the decrease of protozoan richness to small flagellates and free-swimming ciliates was noticed. Moreover, the developed protozoan trophic network was found to be different from that identified in the conventional activated sludge system. The study emphasized that high increase of bacterial biomass promoted in nutrient- and organic matter-rich wastewater can strongly affect the treatment performances as a result of the shadow effect produced on the photoautotrophic microalgae aggregates.

**Key words** | dairy wastewater treatment, light intensity, microalgae–bacteria system, microalgae recovery, protozoan community

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## INTRODUCTION

Considering the symbiotic relationship that can be established between photoautotrophic microalgae and bacteria, a new biotechnology for wastewater treatment based on an ‘activated algae’ system has been studied since the early 1960s (McGriff & McKinney 1972). In the last decade, several reported studies on the domestic and industrial wastewater treatment using microalgae biotechnology (Su *et al.* 2011) underlined the sustainable character of this alternative (Pittman *et al.* 2011) due to the many advantages resulting from microalgal cells’ metabolism. Thereby, microalgae ability for high nutrient uptake, oxygen production through the photosynthesis process, which is required by bacteria for organic matter degradation (Subashchandra-bose *et al.* 2011), and high-valued microalgal biomass, considered as a potential biodiesel feedstock (Craggs *et al.* 2011), made the microalgae biotechnology a feasible solution for biological wastewater treatment. However, one of the application’s drawbacks is represented by the poor settling

property of microalgae cells in treated effluent, most of the applied harvesting methods being costly or unfeasible (Batten *et al.* 2013).

Reducing energy requirements and costs for wastewater treatment has been continuously studied and represents a topic of major interest nowadays (Leu *et al.* 2009). Using microalgae as an alternative biological system for wastewater treatment can promote the elimination of the aeration costs with simultaneous removal of nutrients. However, to ensure the efficiency of the proposed solution, proper growth conditions for microalgae must be provided. For instance, beside carbon dioxide ( $\text{CO}_2$ ), light intensity is a limiting factor that must be considered when high removal of nutrients is required (Rusten & Sahu 2011). The analysis of the effect of different light intensities on photosynthetic activity of microalgae was previously reported (Min *et al.* 2012; Yan *et al.* 2013) for autoclaved or synthetic wastewater treatment. However, no studies reported the influence of

light intensity on an activated algae system, thus eliminating the potential shadow effect produced by bacteria growth on phototrophic microalgae during wastewater treatment.

In conventional wastewater treatment, the diversity and dynamics of the protozoan community are well discussed, being commonly used as bioindicators for indirect evaluation of treatment performances and impact of operating conditions (Perez-Uz et al. 2010). However, such investigations conducted on biological wastewater treatment processes using a microalgae–bacteria consortium have not been reported.

In this study we analyzed the influence of two light intensities (360 and 820  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) on dairy wastewater treatment performances using an activated sludge–microalgae system. Another goal of the research was to assess the effect of light intensity on microalgal cell recovery. The dynamics of the protozoan species was also investigated considering the lack of knowledge related to the bioindicators community supported by an activated algae system during strong-wastewater treatment.

## METHODS

### Microalgal cell cultivation

The inoculum was obtained from a laboratory-scale sequencing batch reactor fed with dairy industry wastewater. In order to increase the microalgal biomass concentration, the inoculum was enriched over a 3 month period in 250 mL Erlenmeyer flasks containing 120 mL of nutrient-rich medium ‘*Chlorella* Broth’ (Atlas 2010) and 30 mL of dairy wastewater. Prepared flasks were incubated in an incubated shaker (INNOVA<sup>®</sup>44 R, New Brunswick Scientific, USA) at  $25 \text{ }^\circ\text{C} \pm 1 \text{ }^\circ\text{C}$  with photosynthetic light applying 12:4 hours light:dark photoperiod and 100 rotation per minute (rpm) shaking speed in order to avoid microalgae settling. After every 7 days of cultivation, the shaker was stopped for one night to allow microalgae settling and about 50% of the liquor was replaced with new prepared growth media.

### Experimental operation

The experiment was conducted in two phases and involved the use of a microalgae–bacteria consortium for dairy wastewater treatment in batch mode at different light intensities.

The first phase involved four batches, each with 96 hours of hydraulic retention time (HRT). As there was no reference for dairy wastewater treatment in the activated

algae system, the HRT was established based on daily monitoring of the system’s treatment performances. The light intensity was adjusted to 360  $\mu\text{mol m}^{-2} \text{s}^{-1}$  taking into account that for most microalgae the light intensity saturation level ranges between 200 and 400  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (Radakovits et al. 2010). Initial microalgae–bacteria inoculum was about 0.57 g dry weight  $\text{L}^{-1}$ .

The second phase was performed at 820  $\mu\text{mol m}^{-2} \text{s}^{-1}$  light intensity involving six batches, each with 72 hours HRT. The microalgae–bacteria inoculum was represented by the biological system remaining after the first phase. During the entire experiment, at the end of each batch, the developed microalgae–bacteria consortium was allowed to settle for 1 hour, the resulting effluent being replaced with 3 L of untreated and non-autoclaved dairy wastewater. The physico-chemical parameters of the influent varied as follows: pH 6.90–7.24, dissolved oxygen saturation ( $\text{O}_2$ ) <5%, chemical oxygen demand (COD) 1,214–1,716 mg  $\text{O}_2/\text{L}$ , ammonium ( $\text{NH}_4^+$ ) 21.9–72.3 mg/L, nitrite ( $\text{NO}_2^-$ ) <0.1 mg/L, nitrate ( $\text{NO}_3^-$ ) <0.1–77.1 mg/L, phosphate ( $\text{PO}_4^{3-}$ ) 2–49.8 mg/L. All batches were conducted in a stirred tank bioreactor (BIOSTAT<sup>®</sup> Bplus, Sartorius, Germany) with 5 L culture vessel at 75 rpm for a good homogenization of the microalgae–bacteria mixture. The photoperiodicity was adjusted to 15 hours light : 9 hours dark, light being provided by a cool circular lamp that surrounded the bioreactor. Both light intensities used in the experiment were lower than the average of the solar irradiance that reaches the earth surface on a sunny day (Masojidek et al. 2004). Temperature presented similar trends for all batches, with values ranging between 20 and 31  $^\circ\text{C}$  corresponding to dark and light periods.

### Analytical methods

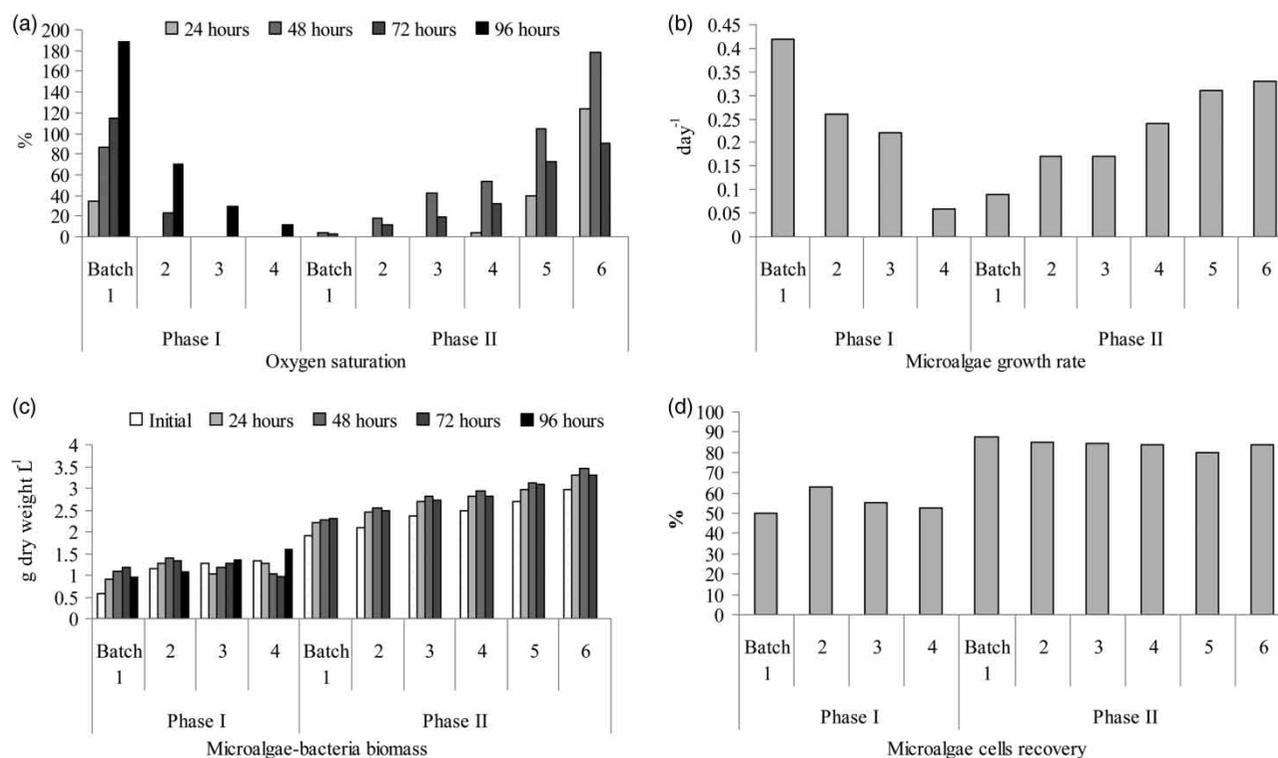
pH, oxygen and temperature were monitored continuously by a built-in sensor and electrodes of the bioreactor. After every 24 hours of treatment, 30 mL of liquor was sampled from the same sampling port of the bioreactor without stopping stirring. The sample was centrifuged at 5,000 rpm (Model U-320, Boeco, Germany) for 20 min at room temperature. The supernatant was analyzed for COD,  $\text{NH}_4^+$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$  and  $\text{PO}_4^{3-}$ . COD was analyzed volumetrically based on the potassium dichromate method according to the ISO standard (SR ISO 6060:1996) and using a heating mantle (model KI16, Gerhardt, Germany).  $\text{NH}_4^+$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$  and  $\text{PO}_4^{3-}$  were determined according to the SR EN ISO 14911:2003 and SR EN ISO 10304/1:2009 standards

(for the last three indicators), respectively, using the ion chromatography system ICS-3000 (Dionex, USA). The microalgae–bacteria pellet resulting after centrifugation was resuspended in distilled water and a part was analyzed spectrophotometrically at 680 nm wavelength (UV/VIS spectrophotometer DR/5000™, Hach Lange, Germany) for microalgae growth rate determination according to Wang *et al.* (2010). The remaining pellet was analyzed gravimetrically for microalgae–bacteria dry weight biomass determination according to Yun *et al.* (1997). The procedure involved the filtration of resuspended pellet through a dried and weighed glass microfibre filter (934-AH™) at vacuum/pressure pump (WP6122050, Millipore, USA) and sample maintenance at 90 °C for 24 hours. Microalgal cell recovery was analyzed according to Salim *et al.* (2011) by sampling 50 mL of mixed liquor from the bioreactor in a 50 mL vessel and leaving it to settle for 30 min. Five millilitres of sample was collected from the liquid surface initially and after 30 min, and analyzed spectrophotometrically at 680 nm. Identification of microalgae species and the protozoan community was performed using a trinocular light microscope (B1, Optech, Germany) and identification guides.

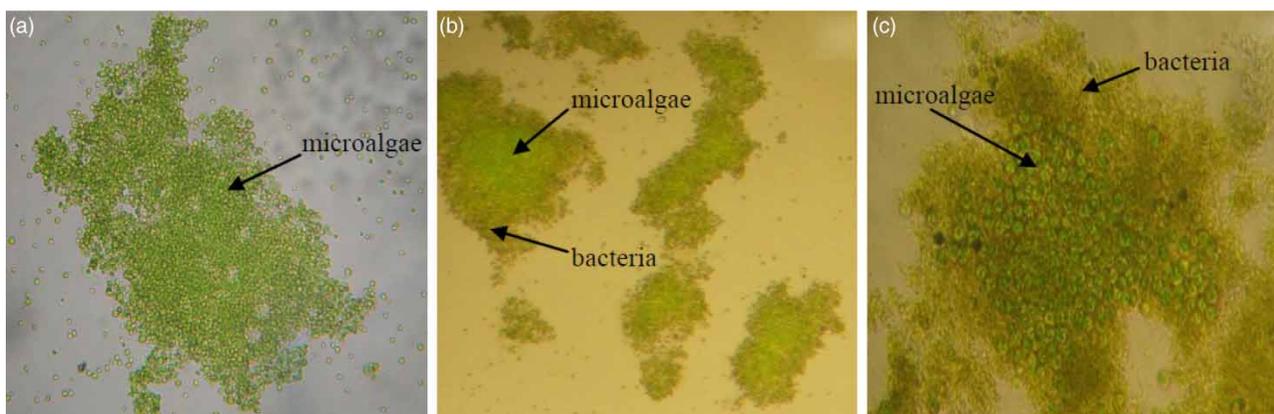
## RESULTS AND DISCUSSION

### Dynamics of the oxygen saturation and microalgae growth rate

Variations of oxygen saturation level and of microalgae growth rate are shown in Figures 1(a) and 1(b), respectively. During the first phase of the experiment a sharp decrease of oxygen saturation was recorded with the increase of solids retention time. The decline of oxygen supply was directly correlated with the decrease of microalgae photosynthetic activity, as was proved by the high decrease of microalgae growth rate. According to the results of the microscopic investigation, a high increase of bacterial biomass was noticed surrounding the microalgal aggregates, thus acting as a shield for photon fluxes (Figures 2(a) and 2(b)) and, implicitly, as a hindering factor for the photosynthesis process due to the shadow effect produced on microalgal cells. The increase, in the first phase, of microalgae–bacteria biomass from initial 0.57 g dry weight L<sup>-1</sup> to maximum 1.40 g dry weight L<sup>-1</sup> (Figure 1(c)) could also contribute to the decrease of the light uptake efficiency but, considering the continuous stirring in the bioreactor, the last mentioned



**Figure 1** | Variation of maximum oxygen saturation (%) (a), microalgae growth rate (day<sup>-1</sup>) (b), microalgae–bacteria biomass (g dry weight L<sup>-1</sup>) (c), and microalgal cell recovery (%) (d) at 360  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (Phase I) and 820  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (Phase II) light intensity.



**Figure 2** | Light microscopy images of initial microalgae inoculum ( $\times 100$ ) (a), microalgae–bacteria system developed in the fourth batch treatment at  $360 \mu\text{mol m}^{-2} \text{s}^{-1}$  light intensity ( $\times 40$ ), where bacteria surrounded the microalgae aggregates (b), and microalgae–bacteria inoculum used at  $820 \mu\text{mol m}^{-2} \text{s}^{-1}$  light intensity ( $\times 200$ ) (c).

pressure factor could not be responsible for such reduction of photosynthetic activity. To overcome this drawback, in the second phase of the experiment, the light intensity was increased up to  $820 \mu\text{mol m}^{-2} \text{s}^{-1}$ .

Applying in the second phase  $820 \mu\text{mol m}^{-2} \text{s}^{-1}$  light intensity led to the continuous increase of microalgae growth rate and, implicitly, of oxygen saturation level in the liquor even at high bacterial biomass concentration noticed in the biological system (Figure 2(c)). Moreover, during the dark period the oxygen saturation in the first phase dropped periodically to 0%, a result that was not recorded during the second phase, in the last batches the minimum oxygen saturation level being about 33%. The results indicated that  $820 \mu\text{mol m}^{-2} \text{s}^{-1}$  light intensity had a positive effect on the biological system's development; thus the number of batches in the second phase was increased in order to sustain the treatment performances of the activated algae system and avoid a short-term effect.

### Microalgae–bacteria biomass and microalgal cell recovery

Compared to the first phase of the experiment, during the second phase a clear tendency of microalgae–bacteria biomass concentration increase was noticed (Figure 1(c)). The low oxygen saturation level recorded in the first phase produced a negative pressure on the biological system, especially in the fourth batch treatment, where continuous decrease of biomass concentration was recorded. During the experiment, a slow decrease of biomass concentration in the last hours of every batch was also noticed, especially during the second phase. Taking into account that no negative growth rate was recorded during the second phase for

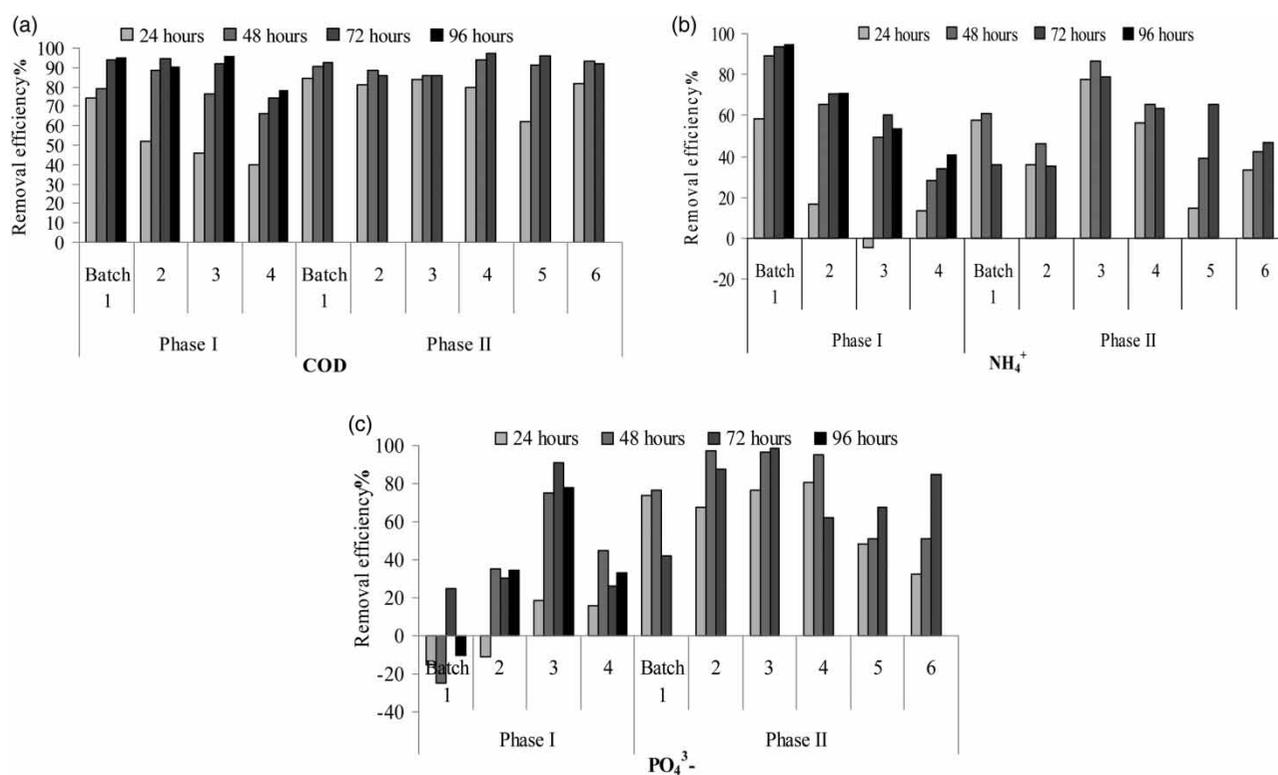
microalgal cells, it was deduced that the decrease of biomass concentration was a result of bacteria decay possibly due to the prolonged HRT and low biodegradable organic matter substrate remaining at the end of the batch treatment (as will be seen further).

The lowest microalgae settleability was recorded in the first batch (Figure 1(d)) while the maximum microalgal cell recovery of about 88% was obtained during the second phase of the experiment. The improvement of microalgal cell settling in the second phase seems to be a result of biomass increase and microalgae–bacteria floc development which was promoted by higher light intensity.

### COD and nutrient removal

#### $360 \mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity

COD,  $\text{NH}_4^+$  and  $\text{PO}_4^{3-}$  removal efficiencies are presented in Figures 3(a)–3(c), respectively. Relative similar removal efficiencies were recorded after 72 and 96 hours of treatment for all mentioned indicators. Moreover, the results underlined that with the increase of solids retention time COD removal efficiency decreased after the first 24 hours of treatment in every batch from 74 to 40%. A more obvious downward trend of the removal efficiency was noticed in the case of  $\text{NH}_4^+$  decreasing in time from about 95 to 41%. Increase of  $\text{NH}_4^+$  concentration at the beginning of the third batch treatment can be a result of the bacteria decay and the decrease of the cell requirements for this nutrient. We assume that the decrease of the treatment performances represents an indirect consequence of photosynthesis process limitation due to the insufficient light intensity needed



**Figure 3** | Removal efficiencies of COD (a),  $\text{NH}_4^+$  (b), and  $\text{PO}_4^{3-}$  (c) at  $360 \mu\text{mol m}^{-2} \text{s}^{-1}$  (Phase I) and  $820 \mu\text{mol m}^{-2} \text{s}^{-1}$  (Phase II) light intensity.

to support the photoautotrophic growth of the microalgae at high bacterial biomass concentration.

During the experiment, no clear evolution was noticed for  $\text{PO}_4^{3-}$  concentration. Moreover, in the first batch treatment an increase of  $\text{PO}_4^{3-}$  concentration in the liquor was recorded that can be explained by the release of  $\text{PO}_4^{3-}$  ions from microalgae and bacteria cells as a result of high initial nutrient supply provided by the 'Chlorella Broth' medium. Taking into account the increase of microalgae–bacteria biomass and the growth rate of microalgae in the first two batches, and according also to the results reported by Powell et al. (2011), it can be assumed that, in the first two batches, the microalgae were mainly specialized in  $\text{PO}_4^{3-}$  assimilation in cellular constituents rather than phosphorus uptake from the liquor, a hypothesis which can explain the initial low  $\text{PO}_4^{3-}$  removal efficiency. In the third batch,  $\text{PO}_4^{3-}$  removal efficiency increased by about 56% compared to the previous batch, probably due to the reduction of cell phosphorus supply and high biomass concentration. Decay of the microalgae–bacteria system recorded in the last batch treatment could explain the sharp decrease of phosphorus removal efficiency.

High levels of  $\text{NO}_3^-$  concentration were recorded in the influent of the second and third batch treatment: 69.7 and 77.1 mg  $\text{NO}_3^-/\text{L}$ , respectively, which were consumed

almost entirely (about 99% removal efficiencies) in the first 24 hours of treatment. This underlines the preference of cultured microalgal cells (mainly *Chlorella* sp.) for  $\text{NO}_3^-$ , as it was used as nitrogen source in the enrichment medium or was due to the native preference of the microalgal inoculum. During experiments,  $\text{NO}_2^-$  ions were below the detection limit ( $<0.1 \text{ mg/L}$ ).

### 820 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity

Taking into account the results obtained in the first phase of the experiment, as well as higher initial biomass concentration used for the second phase (about 1.9 g dry weight  $\text{L}^{-1}$ ), the HRT was decreased to 72 hours. After increasing the light intensity, high COD removal efficiency was recorded in the first 24 hours of treatment, a performance that is close to that achieved for the same indicator after 72 hours of HRT at lower light intensity (Figure 3(a)). Moreover, good performances were achieved within 48 hours of HRT – similar to the time required by the conventional wastewater treatment plant operating at the milk processing factory from where the influent was sampled.

No notable increase of  $\text{NH}_4^+$  removal efficiency was recorded compared to other indicators, maximum removal

efficiencies in every batch treatment ranging between about 39 and 86% (Figure 3(b)). Su *et al.* (2012) also reported similar low  $\text{NH}_4^+$  removal efficiency compared to good COD removal efficiency. Moreover, as in the previous phase, an increase of  $\text{NH}_4^+$  concentration in the liquor was noticed after 48 hours of HRT, possibly caused by the bacteria decay in the last hours of treatment, a hypothesis that can also explain the increase of  $\text{PO}_4^{3-}$  concentration at the end of the first, second and fourth batches. Also, in the last two batches, a decrease of  $\text{NH}_4^+$  removal efficiencies was noticed in the first hours of treatment, results that can be explained by higher influent  $\text{NH}_4^+$  concentration of 65.3 and 68.9 mg/L recorded for batch number five and six, respectively, compared to 21.9 mg/L registered in the fourth batch.

Compared to the first phase, no increase of  $\text{PO}_4^{3-}$  concentration in the liquor was recorded after the first 24 hours of HRT of every batch treatment, possibly due to the equilibrium established for  $\text{PO}_4^{3-}$  supply between biomass and liquor. Moreover, an increase of  $\text{PO}_4^{3-}$  removal efficiency was obtained, maximum values ranging between 68 and 99% (Figure 3(c)). These results could be positively influenced by the increase of microalgae growth rate and microalgae–bacteria biomass concentration (which was higher than 1.90 g dry weight  $\text{L}^{-1}$ ). Also, improvement of the phosphorus uptake from the liquor seems to be directly correlated with the light intensity increase as has been stated by Powell *et al.* (2009). As in the case of  $\text{NH}_4^+$ , a decrease of  $\text{PO}_4^{3-}$  removal efficiencies was recorded for the last two batches. This can be also explained by the wide fluctuation

of influent  $\text{PO}_4^{3-}$  concentration, which increased from 2 mg/L (for the fourth batch) to 25.2 mg/L (registered in the fifth batch) and decreased again to 6.6 mg/L in the sixth batch.  $\text{NO}_3^-$  attained during the experiment a maximum value of 3 mg  $\text{NO}_3^-/\text{L}$  and, as in the first phase,  $\text{NO}_2^-$  ions were below detection limit.

### Dynamics of the protozoan community

Investigation of the protozoan structure and composition during the experiment revealed the existence of two biological development stages. The first stage, identified during the wastewater treatment at  $360 \mu\text{mol m}^{-2} \text{s}^{-1}$  light intensity as an unstable development biological stage, was mainly characterized by high species richness and continuous modification of the protozoan community. A total number of 12 species of flagellates, amoebae and ciliates was recorded as shown in Table 1. The reduction of the protozoan population was noticed for the last 24 hours of every batch. Such decline of protozoan species can be a result of prolonged HRT that forced taxa to grow in two different conditions: one characterized by high loads of nutrients and organic matter corresponding to the first hours of the batch, and another one identified in the last hours of each batch and characterized by low organic matter concentration and decrease of bacterial biomass, a condition that was unfavorable for the protozoan species developed in the first part of the batch treatment. No difference was found between the protist population diversity identified in the first biological stage and that reported for a conventional activated sludge system.

**Table 1** | Diversity of the protozoan community identified during wastewater treatment at  $360 \mu\text{mol m}^{-2} \text{s}^{-1}$  (Phase I) and  $820 \mu\text{mol m}^{-2} \text{s}^{-1}$  (Phase II) light intensity

Bioindicators		Phase I				Phase II						
		Batch treatment				Batch treatment						
		1	2	3	4	1	2	3	4	5	6	
Flagellates	<i>Bodo</i> sp.	x	x	x	x	x	x	x	x	x	x	x
	<i>Polytoma</i> sp.	x	x	x	x	x	x	x	x	x	x	x
Testate amoebae	<i>Arcella</i> sp.				x							
Naked amoebae	<i>Entamoeba</i> sp. (cyst)		x	x	x							
Small free-swimming ciliates (<50 $\mu\text{m}$ )	<i>Cinetochilum</i> sp.	x	x			x	x	x	x	x	x	
	<i>Uronema</i> sp.				x							
	<i>Colpoda</i> sp.				x							
Large free-swimming ciliates (>50 $\mu\text{m}$ )	<i>Tetrahymena</i> sp.			x	x	x	x	x	x	x	x	
	<i>Stentor coeruleus</i>				x							
Crawling ciliates	<i>Chilodonella</i> sp.		x									
Stalked ciliates	<i>Vorticella microstoma</i>		x	x								
	<i>Podophrya</i> sp.		x	x	x							

At the beginning of the second phase of the experiment, a reduction of the protozoan richness to small flagellates and free-swimming ciliates was noticed, which led us to consider that the protozoan community attained a second, stable biological development stage. In a conventional wastewater treatment using an activated sludge system, the prevalence of such species is associated with low oxygen level and mediocre removal of biodegradable organic matter (Madoni 1994). However, in our experiment, the decrease of the trophic network complexity occurs in spite of high oxygen saturation and improved treatment performances, characteristics which are contradictory to the results reported for conventional activated sludge by Madoni (1994). Taking into account that the protozoan community reached steady state when the light intensity was increased, we assumed that oxygen level influenced the protozoan structure, as was also concluded by Tocchi et al. (2012) for a conventional activated sludge system. However, beside light intensity, we consider that other factors also contributed to this protozoan diversity, among them being solids retention time and the evolution of the microalgae–bacteria system, the last factor involving the dynamics of the microalgae–bacteria biomass ratio and microalgae metabolic activity. Thus, the stable protozoan trophic network developed during dairy wastewater treatment using the activated algae system can be different from other networks that were identified in conventional activated sludge systems.

## CONCLUSIONS

This work showed that when treating high strength dairy wastewater, the efficiency of using light by the photoautotrophic microalgae and the treatment performances of the microalgae–bacteria system can be strongly affected by the high bacteria development in a short period of time. The experiment revealed that this situation can be avoided by increasing light intensity. Thus, microalgae proved to be able to ensure the liquor's oversaturation in oxygen at  $820 \mu\text{mol m}^{-2} \text{s}^{-1}$  light intensity without any aeration costs. Also, with the development of the biological system at higher light intensity, better treatment performances were noticed. However, the  $\text{NH}_4^+$  removal efficiencies remained lower than those of COD; thus further research is needed in order to ensure equilibrium between nutrients and organic matter removal. Also, even if at higher light intensity an improvement of microalgal cells settleability was obtained, supplementary conditioning of the effluent is required for complete removal of the microalgae.

This work also showed that in the presented operational conditions, the protozoan community identified in the stable biological stage promoted by the microalgae–bacteria consortium was found to be different from that typically identified in conventional wastewater treatment with activated sludge. This underlines the necessity to gain more information about the behavior of the protozoan community when a microalgae–bacteria consortium is used for strong-wastewater treatment.

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