Laboratory-scale investigation on the role of microalgae towards a sustainable treatment of real municipal wastewater

S. Petrini, P. Foladori and G. Andreottola

ABSTRACT

Engineered microalgal-bacteria consortia are an attractive solution towards a low-cost and sustainable wastewater treatment that does not rely on artificial mechanical aeration. In the research conducted for this study, a bench-scale photo-sequencing-batch reactor (PSBR) was operated without external aeration. A spontaneous consortium of microalgae and bacteria was developed in the PSBR at a concentration of 0.8–1.7 g TSS/L. The PSBR ensured removal efficiency of 85 ± 8% for chemical oxygen demand (COD) and 98 ± 2% for total Kjeldahl nitrogen (TKN). Nitrogen balance revealed that the main mechanisms for TKN removal was autotrophic nitrification, while N assimilation and denitrification accounted for 4% and 56%, respectively. The development of dense microalgae–bacteria bioflocs resulted in good settleability with average effluent concentration of 16 mgTSS/L. The ammonium removal rate was 2.9 mgN L^{-1} h^{-1}, which corresponded to 2.4 mgN $gTSS^{-1}$ h^{-1}. Although this specific ammonium removal rate is similar to activated sludge, the volumetric rate is lower due to the limited total suspended solids (TSS) concentration (three times less than activated sludge). Therefore, the PSBR footprint appears less competitive than activated sludge. However, ammonium was completely removed without artificial aeration, resulting in a very cost-effective process. Only 50% of phosphorus was removed, suggesting that further research on P uptake is needed.

Key words | microalgae, municipal wastewater, nutrients removal, photobioreactor, sustainability

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INTRODUCTION

The conventional wastewater treatment plants (WWTPs), based on the activated sludge configuration, ensure high efficiency in pollutant removal. However, WWTPs are today facing some serious issues: (1) large energy consumption, mainly associated with mechanical aeration of bioreactors; (2) increasing amounts of excess sludge, which cause expenditures for treatment and disposal, and environmental impacts; (3) greenhouse gases (GHG) emissions like CO_2, CH_4 and N_2O, which contribute to the growing problem of global warming.

In regard to energy consumption, WWTPs have been estimated to use about 1% of the national electricity consumption (Cao 2011; Shen *et al.* 2015; Zhang *et al.* 2016). A large amount of the energy is used in WWTPs to meet stringent targets on effluent water quality. At the same time, since energy mainly originates from fossil sources, WWTPs contribute to environmental problems such as global warming and climate change. In this context, actions aimed at maintaining good quality effluents but reducing WWTP energy consumption are imperative.

As regards excess sludge production, restrictions in disposal routes may occur in many countries, leading to a progressive increase in costs. Sludge treatment and disposal may generate expenditures from 250 € to more than 1,000 € per tonne of dry mass (mean 470 ± 280 €/t in European countries; Foladori *et al.* 2010).

These aspects entail a paradigm shift in the configurations of conventional WWTPs towards solutions that are more environmentally and economically sustainable. A promising emerging alternative way to treat wastewater is to couple bacteria with microalgae (Judd *et al.* 2015). In fact, in the presence of light, through photosynthesis, microalgae produce oxygen to support bacterial activity (oxidation of organic matter and ammonium), contribute to CO_2 mitigation by photosynthetic fixation, and permit nutrients recycling by assimilation of N and P. In return, bacteria

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produce carbon dioxide, which is essential for photosynthesis. The exploitation of microalgal-bacteria symbiosis, together with sunlight, may lead to a low- or no-energy treatment process that does not rely on artificial mechanical aeration (Quijano et al. 2017), resulting in a process more sustainable than WWTPs based on activated sludge (Molinuevo-Salces et al. 2010).

Among the best-known systems based on microalgal-bacteria consortia, high rate algal ponds (HRAPs) are the most widespread. HRAPs are open shallow ponds with long hydraulic retention times (HRTs) (3–10 d) and low depth (0.1–0.3 m) (Posadas et al. 2015). Although they are characterized by low-energy consumption compared to conventional WWTPs (Godos et al. 2009), they require a large use of land (Gonzalez-Fernandez & Muñoz 2017). This high footprint is the major drawback in countries where land is scarce and expensive. Moreover, HRAPs can only be controlled to a limited extent, so that efficient N removal may be difficult to achieve in sensitive areas.

Closed photobioreactors (PBRs) make it possible to overcome these limitations because: (1) they can be built vertically to minimize land requirements and (2) the process conditions can be easily controlled to guarantee high pollutant removals and efficient nitrification (Muñoz & Guieysse 2006; Karya et al. 2013). In recent years, engineered PBRs have gained increasing attention in the treatment of municipal wastewater (Su et al. 2011) since they could become a cost-effective and sustainable solution in the future.

At the moment, there are only a few studies on wastewater-borne microalgal bacteria consortia developed in PBRs (Kang et al. 2018). Therefore, little is known about the removal process and the operational aspects of these systems. Indeed, more detailed knowledge is required about: (i) kinetics of biomass, (ii) efficiency of separation between biomass and treated wastewater, (iii) energy savings, (iv) sludge production.

The aim of this study is to provide a comprehensive picture of the performances of a stable wastewater-borne microalgal bacteria consortium acclimatized for 2 years in a bench-scale sequencing PBR (PSBR) treating real municipal wastewater. Since no external aeration was provided, oxygen was supplied only by microalgal photosynthesis. Instead of exploiting sunlight, artificial light was supplied in order to guarantee a constant light intensity and a stable photoperiod. In this way, the influence of daylight variations was avoided and the interpretation of data was easier.

The focus of the research reported was on the following key factors that affect the entire performance of the PBRs and the sustainability of the process: (1) removal efficiencies of chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN) and total phosphorus with the aim of meeting the EU discharge limits; (2) biomass macrostructure and settling properties; (3) kinetics of ammonium removal; (4) online parameters, such as dissolved oxygen (DO) and pH, for real-time control of the process; (5) opportunities for energy saving.

MATERIALS AND METHODS

Influent wastewater

Influent pre-settled wastewater was collected from a full-scale municipal WWTP (Trento Nord plant, Italy). No filtration of the wastewater was performed; in this way, the microorganisms naturally present in the influent wastewater acclimatized, resulting in a microalgal–bacterial consortium.

Photo-sequencing batch reactor

A bench-scale cylindrical photobioreactor with a working volume of 2 L was used (Figure 1). The system was operated as a sequencing batch reactor (PSBR) equipped with peristaltic pumps, a magnetic mixer and a lamp controlled by timers.

Sunlight entered the laboratory but the reactor was never directly exposed. In fact, light was supplied by a cool-white lamp (8 led × 0.5 W; Orion, Italy). The light intensity provided, measured as photosynthetically active radiation (SQ-520 quantum sensor, Apogee Instruments, USA), was 25 ± 5 μmol quanta · $m^{-2} \cdot s^{-2}$. The photoperiod was set at 16 h of light and 8 h of dark.

The PSBR cycle (48 h) comprised four phases: (1) Feed, 0.08 h; (2) React, 47.5 h; (3) Settlement, 0.5 h; (4) Draw, 0.08 h. The React phase consisted of two photoperiods of

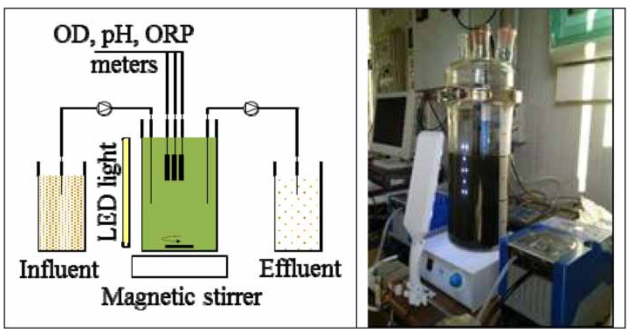

Figure 1 | Scheme of the PBR.

16 h light/8 h dark, resulting in a sequence of periods with high and low DO values. Wastewater was fed at a rate of 0.7 L/cycle. No external aeration was provided, so that oxygen was supplied only by photosynthesis. Temperature of mixed liquor was 22.2 °C on average during the 7-month experimentation.

Microalgae-bacteria consortium

Microscopic observations were performed using a Nikon Optiphot EFD-3 Microscope equipped with epifluorescence apparatus (Nikon, Japan). Observations under blue and green light excitation were used to distinguish: (1) photosynthetic microorganisms, emitting red autofluorescence due to their pigments; (2) heterotrophic bacteria emitting green fluorescence after staining with SYBR-Green I (Invitrogen, USA). The biomass naturally developed in the PSBR consisted of a consortium of eukaryotic microalgae, prokaryotic cyanobacteria and bacteria, acclimatized for a 2-year period (2016–2017). Total suspended solids (TSS) were maintained in the PSBR at a concentration ranging from 0.8 g TSS/L to 1.7 g TSS/L (average TSS of 1.2 g TSS/L during the 7-month experimentation).

Analytical methods

Analyses of total COD, TSS, TKN, NH_4^+-N, NO_2^--N, NO_3^--N, total P and PO_4^{3-}-P were performed on influent and effluent wastewater according to *Standard Methods* (APHA 2012). Soluble COD (sCOD) was measured after filtration on 0.45-μm-membrane. The analysis of TSS was also used to determine the biomass concentration in the PSBR. DO, pH and temperature were measured continuously with online probes (Multi3410 and pH3310 meters, WTW, Germany).

Track studies were performed in the PSBR to measure the profiles of N forms during a typical cycle. Samples were collected every hour, then filtered and analyzed for N-forms.

RESULTS AND DISCUSSION

High removal efficiency of COD and TKN

The microalgal-bacterial consortium in the PSBR resulted in a very effective symbiotic collaboration. Oxygen supplied by microalgal photosynthesis met the biological demand for the oxidation of the influent compounds. Therefore, a very high removal efficiency of COD and TKN was achieved, 85 ± 8% and 98 ± 2%, respectively. The mutualistic relationship between bacteria and microalgae sustained the organic matter removal and nitrification without mechanical aeration being necessary. At the same time, the CO_2 resulting from organic matter decomposition by bacterial activity was available for microalgal photosynthesis. In this way, wastewater biodegradation was achieved without an external supply of both O_2 and CO_2, while decreasing CO_2 emission from wastewater to atmosphere.

The profiles of influent COD and effluent sCOD concentrations, during the 7-month experimentation, are shown in Figure 2(a). The influent COD of 262 ± 97 mg/L on average was reduced to 33 ± 8 mg COD/L in the effluent, largely meeting the EU discharge limit (125 mg COD/L, according to EU Directive 91/271/EEC).

TKN concentration diminished from 54 ± 18 mg TKN/L on average in the pre-settled wastewater to 1.0 ± 0.8 mg TKN/L and 0.6 ± 1.0 mg NH_4^+-N/L in the effluent (Figure 2(b)). Despite the strong fluctuation of the influent TKN concentration, which surpassed 100 mg TKN/L, a complete and stable nitrification occurred in the PSBR

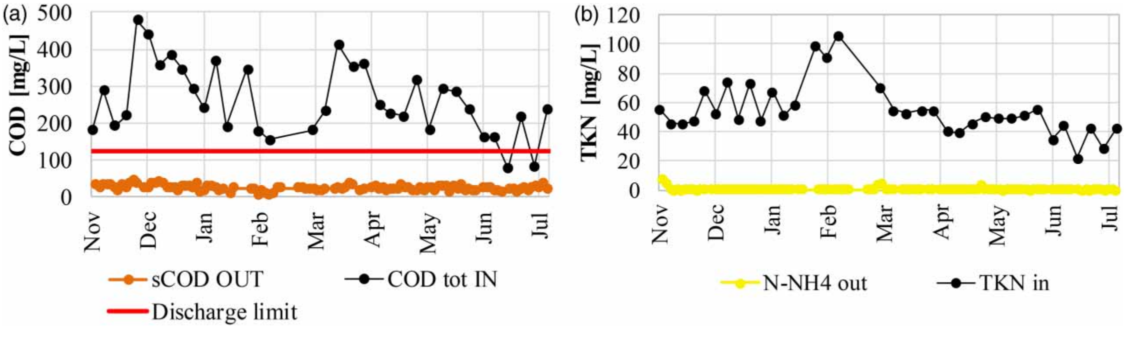

Figure 2 | Influent and effluent concentrations of: (a) COD and sCOD compared to EU discharge limits; (b) TKN and NH_4^+-N.

during all the 7 months of experimentation. Nitrites were negligible (0.1 ± 0.4 mg NO_2^--N/L) in the effluent, while nitrates were 13.7 ± 8.7 mg NO_3^--N/L on average.

Based on nitrogen balance in the PSBR, the mechanisms of TKN removal were: (1) biomass assimilation, which accounted for approximately 4% of removed TKN, (2) autotrophic nitrification, which converted all the remaining TKN into NO_3. Then heterotrophic denitrification during phases at zero-DO accounted for 56%. A fourth potential mechanism of N removal could be ammonia volatilization, but it was excluded because pH in the PSBR was 7.6 ± 0.3 on average, and thus not high enough to support the stripping. The high concentration of ammonium in municipal wastewater is an ideal substrate for microalgae and cyanobacteria (Krustok *et al.*, 2016), but the major mechanism for TKN removal in the PSBR was autotrophic nitrification. The percentage of TKN removed through biomass assimilation was very low (4%) because the microalgal processes designed for wastewater treatment (like this PSBR) are associated with low excess sludge production in comparison with processes aimed at maximizing the algal biomass productivity and harvesting.

Dense flocs of microalgae and bacteria ensured good settleability

A microalgal consortium treating unsterilized real wastewater would inevitably contain a mixed community of microalgae, bacteria and cyanobacteria. Microscopic observations of the microbial community spontaneously developed in the PSBR showed the presence of *Chlorella* sp., *Diatoms* sp. and filamentous cyanobacteria embedded in dense flocs together with a large amount of heterotrophic bacteria (Figure 3). Green and red fluorescence (Figure 3(b)) indicate the composition of the flocs: (i) green cells represent heterotrophic bacteria, stained with SYBR-Green I, (ii) red cells correspond to photosynthetic microorganisms because of red autofluorescence linked to photosynthetic pigments.

The structure of the microalgal-bacteria consortium results in an ideal self-sustaining system where oxygen was produced by photosynthetic microorganisms and immediately used by the adjacent heterotrophic bacteria. Moreover, the presence of dense microalgae–bacteria bioflocs ensured good settleability properties with an average effluent TSS concentration of 14 ± 17 mg TSS/L.

Various studies have reported wastewater treatment with microalgal-bacterial consortia better than with single bacterial or algal systems, with the further advantage of lower biomass harvesting cost compared to algal systems alone (Muñoz *et al.* 2005; Su *et al.* 2011; Liu *et al.* 2017).

Profiles of N forms during the typical PSBR cycle and online parameters (DO, pH)

Track studies were performed to monitor the profiles of NH_4^+-N, NO_2^--N, NO_3^--N during a typical cycle (Figure 4(a)) after the addition of influent wastewater. In the light phase, ammonia was removed rapidly, decreasing from the initial concentration of 13.8 mg NH_4^+-N/L to zero in approximately 6 h. At the same time, the concentration of NO_3^--N increased by 9.0 mg NO_3^--N/L. Therefore, a partial simultaneous nitrification–denitrification was observed in the PSBR because of local anoxic conditions that occur within the dense microalgal-bacterial aggregates or during phases with DO near zero (Figure 4(b)).

The ammonium removal rate was calculated by considering the slope of the straight line that interpolates the experimental NH_4^+-N concentrations over time. The

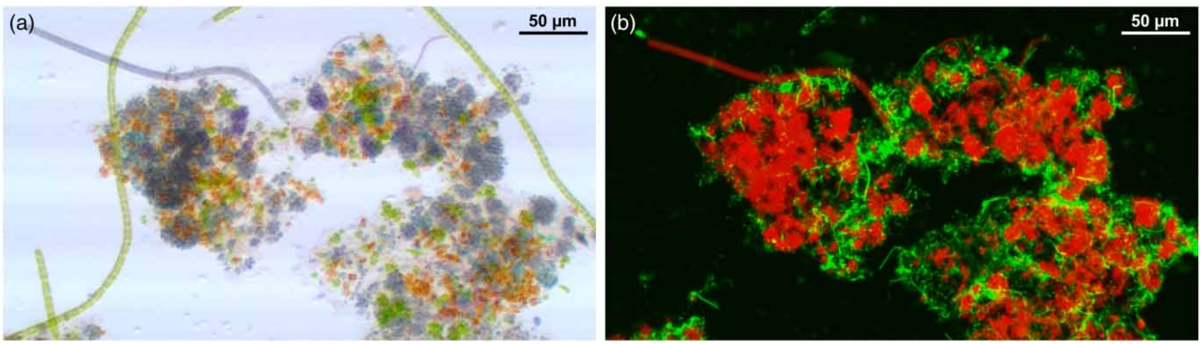

Figure 3 | Structure of the microbial community in the PSBR: (a) flocs under visible light; (b) flocs composed by photosynthetic microorganisms (red fluorescence) and heterotrophic bacteria (green fluorescence).

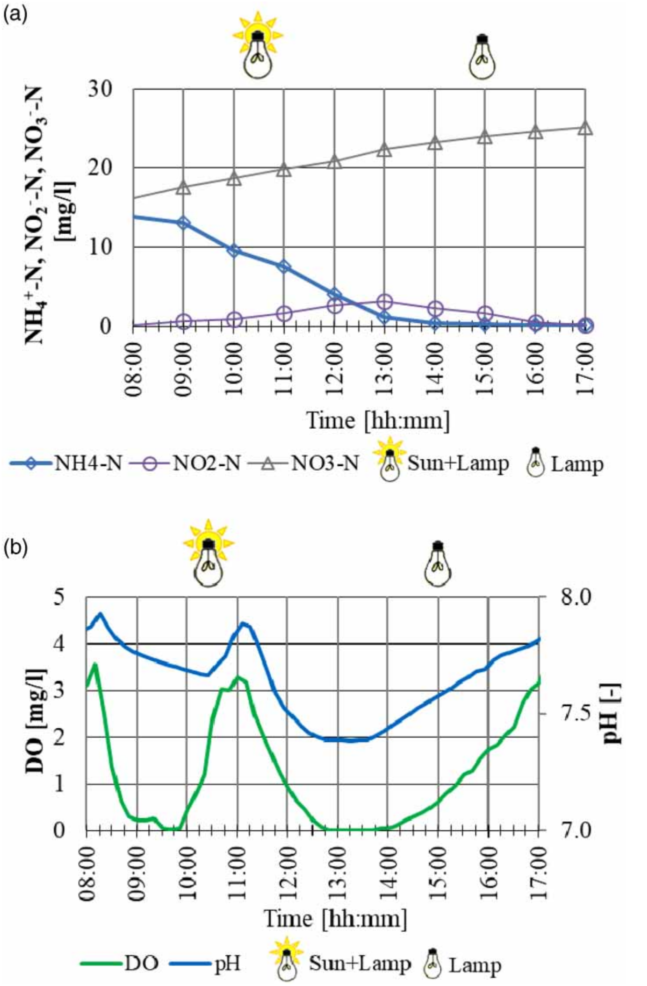

Figure 4 | Track study of a typical cycle in the PSBR: (a) profiles of NH_4^+-N, NO_2^--N, NO_3^--N; (b) profiles of online parameters DO and pH.

volumetric rate was 2.9 mg N L^{-1} h^{-1}, which corresponds to a specific rate of 2.4 mg N g TSS^{-1} h^{-1} at TSS concentration of 1.2 g TSS/L.

Ammonium removal occurred during the light phase and without external aeration. Oxygen was produced continuously in the system due to photosynthetic activity. However, in the presence of high oxygen demand for ammonium oxidation, DO concentration in the bulk liquid was close to zero for several hours (Figure 4(b)). The real-time measurements of DO and pH made it possible to identify the complete removal of ammonium. In particular, two simultaneous characteristic points were detected (Foladori *et al.* 2018a): (1) the 'Ammonia Valley', which is a relative minimum in the pH profile; (2) the 'DO breakpoint' which corresponds to a sudden increase in the DO profile not caused by a higher irradiation.

Comparing the ammonium removal rate of the PSBR with conventional activated sludge-based processes, the following aspects can be observed:

- The specific ammonium removal rate (in terms of mg N g TSS^{-1} h^{-1}) was similar to the typical nitrification rate assumed for activated sludge. Although algal process may present slow biokinetics (Judd *et al.* 2015), here the ammonium removal was supported by autotrophic nitrification.
- The TSS concentration in the microalgal-bacterial consortium was 1.2 g TSS/L on average, approximately three times less than activated sludge.
- The volumetric ammonium removal rate (in terms of mg N L^{-1} h^{-1}) was significantly lower than activated sludge, so that the PSBR appears less competitive in terms of footprint.
- The major advantage of the PSBR process is that ammonium was completely removed without external energy supply, resulting in a very cost-effective process.

Improvement of total N removal can be coupled with energy saving

Effluent nitrates from the PSBR remained relatively high, reaching an average concentration of 13.7 ± 8.7 mg/L in the experimentation. These values did not permit to reach the effluent total nitrogen (TN) of EU regulation for sensitive areas, which imposes a maximum annual average of 10 or 15 mg N/L depending on the Population Equivalent. Therefore, nitrate removal needed to be improved through optimization of denitrification, especially during dark periods when photosynthesis does not occur and anoxic conditions can take place (Foladori *et al.* 2018b). Applied strategies were the following: (1) feeding of influent wastewater during the dark periods, in order to couple the availability of COD and anoxic conditions; (2) reduction of mixing during the dark periods. In this way, TN concentration in the effluent was lowered to 6.3 ± 4.4 mgN/L, which enabled compliance with the European Directive. Another interesting approach could be the implementation of a shortcut N-removal. In this case, the temporary accumulation of nitrites, which occurs in the final part of ammonium removal (as shown in Figure 4(b)), could be exploited to shortcut the conventional nitrification–denitrification process, obtaining benefits in terms of energy and carbon supply.

Limited removal efficiency of phosphorus due to low sludge production

As regards phosphorus, only an average removal of 50 ± 19% was achieved in the PSBR during the 7 months of experimentation. The influent concentration passed from an average of 4.7 ± 1.8 mg P/L to an average of 2.3 ± 0.9 mg P/L in the effluent (Figure 5). To guarantee effluent TP under EU discharge limits for sensitive areas (maximum annual average of 1 or 2 mg N/L depending on the Population Equivalent), the removal should be improved. This is an open issue that requires further research. The algal systems are expected to incorporate a significant amount of P for their synthesis, but in this PSBR the synthesis of biomass was limited. Considering the excess sludge production in the PSBR of 0.03 g TSS/d, the direct consequence is a limited P uptake from wastewater. Other findings in the literature highlight that photobioreactors aimed at purifying wastewaters according to low discharge limits usually present lower applied loads and longer retention times, and thus excess sludge production is low (Judd *et al.* 2015).

Towards a cost-effective and sustainable wastewater treatment based on wastewater-born microalgal-bacterial consortium

This study has shown that the photosynthetic activity able to produce a sufficient amount of oxygen to support COD and TKN removal is the key variable of the process. Thus, light is a new factor to take into account, while DO concentration is not so meaningful when it is near zero for a long period of the cycle. Photosynthetic aeration is a very promising alternative in place of the mechanical aeration used in activated sludge. Furthermore, the challenge of applying a PSBR system is to exploit daylight, since it is a renewable energy and the cheapest and most sustainable option. However, there is still much to understand. Process parameters, consolidated for activated sludge, need to be redefined in the microalgal–bacterial consortia. In particular, further research is required to assess kinetic parameters and the optimal TSS content in PSBRs in order to avoid self-shading but increase the volumetric kinetics.

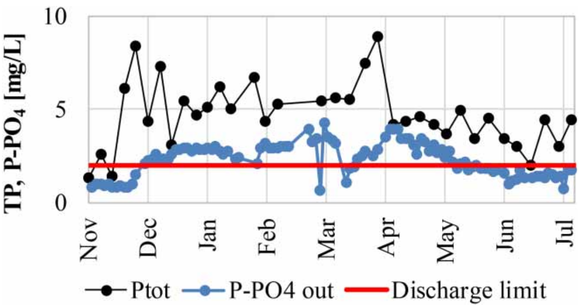

Figure 5 | Total P concentration in the influent wastewater and PO_4^{3-}-P in the effluent.

CONCLUSIONS

The microalgal-bacteria consortium proved to be effective for real municipal wastewater treatment.

In particular:

- photosynthetic oxygenation produced by a constant irradiation for 16 h/d (with intensity lower than natural solar irradiation) is sufficient to sustain complete nitrification and oxidation of organic compounds;
- the nitrification rate of the microalgal-bacterial consortium is comparable to that of mechanically aerated conventional processes (activated sludge);
- the system ensures high removal efficiency of COD and modest P removal;
- the biomass is characterized by good settleability.

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