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MICROALGAE–BACTERIA SYSTEM FOR BIOLOGICAL WASTEWATER TREATMENT

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Abstract. Use of microalgal–bacterial consortium in biological wastewater treatment can represent a feasible alternative for sustainable wastewater treatment requirements and support for algal productivity. The advantages emerge from the ability of microalgal taxa to perform photosynthetic process and achievement of nutrients exchange between microalgae and bacteria cells. A mixed consortium wild-type microalgae (such as *Chlorella* sp.) and bacteria was tested for biological treatment of dairy industry wastewater in stirred tank batch bioreactor. The aim of the experiment was to assess the feasibility of the biological system in terms of treatment performances, growth rate and microalgae removal. At the end of the treatment cycle (96 h) the removal efficiencies of organic matter (COD–Cr), total nitrogen (TN) and total phosphorus (TP) were 91, 68 and 38%, respectively. The maximum microalgal and microalgal–bacterial system growth rate were 0.13 and 0.10 day^{-1}, respectively. The highest removal of microalgal cells was about 63% recorded after 72 h of batch treatment. The use of microalgal–bacterial consortium for wastewater treatment can be promoted as a cost-efficient biotechnology in terms of high organic matter and nutrients removal by aeration costs elimination, the major drawback so far being represented by the poor microalgae cells removal from effluent.

Keywords: microalgae–bacteria system, dairy wastewater treatment, microalgae recovery.

AIMS AND BACKGROUND

Conventional treatment of wastewater results in continuous production of waste activated sludge mainly disposed inside the treatment plants premises or landfilled. However, these solutions are not feasible on long term due to the necessity to identify new landfilling sites and potential contamination risk of ecosystems with pathogens, heavy metals, etc. Other alternatives to reuse waste activated sludge are unconsidered or limited especially due to the costs problems.

Treatment of dairy industry wastewater in aerobic conditions involves high aeration costs and produces high amounts of waste activated sludge. Thereby, the

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oxygen generated by photosynthetic microalgae taxa can represent a viable alternative for aeration costs elimination in the aerobic treatment and partial replacement of activated sludge, microalgal biomass being valued as a renewable resource for a wide range of applications. Thus, the main purpose of this study was to evaluate the treatment performances for dairy industry wastewater using microalgae–bacteria system, major drawbacks being also presented.

Eutrophication problems generated by the nutrients concentrations above maximum discharge limits in the aquatic ecosystems led to the idea to develop tertiary treatment step for wastewater treatment using algal species in order to remove the remaining nitrogen and phosphorus from the incompletely treated effluents[1]. Later, concerns arising as a result of high accumulation of waste activated sludge and implementation of more stringent regulatory tools for the wastewater treatment field promoted the use of microalgae cells in second biological treatment step due to the following reasons:

- elimination of aeration costs due to the oxygen released by microalgal taxa[2] saving the energy requirements for intensive aeration (valued at about 0.5 kWh m^{-3} of treated effluent[3]);
- microalgae cells are able to accumulate large amounts of nitrogen, phosphorus[4] and heavy metals[5];
- replacement of activated sludge with high valued algal biomass which can be used to produce: valuable chemicals for different industrial sectors (pharmaceutical, food, cosmetics, etc.)[6], fertilisers in agriculture[7], biofuels: bioethanol[8], biohydrogen[9], biodiesel[10], microalgal biomass being identified as a viable third source of renewable energy[11] after terrestrial crops and lignocellulosic agriculture and forest residues[7].

Thereby, according to last reason, wastewater treatment plants can also act as a cost-efficient microalgae biomass production system decreasing the environmental footprint by reducing competition for fresh water resources and replacing the commercial fertilisers with nutrients taken directly from wastewater[12,13], costs for both water resources and nitrogen and phosphorus requirements being evaluated at 10–20 % of the total costs for algae growing[14]. Other advantages resulted from microalgae use in wastewater treatment process are represented by: good removal of coliform bacteria[15], contribution to greenhouse gas (GHG) mitigation through direct uptake of carbon dioxide (CO_2) from the atmosphere or flue gases from heavy industries[7,16] and replacement of fossil fuels[4]. For instance, Borkenstein and collaborators[17] reported 3.25 g CO_2 l^{-1} uptake from flue gas (derived from a cement plant) by 2 g l^{-1} dry weight of *Chlorella emersonii*.

Many studies were reported involving the use of microalgae–bacteria consortium in wastewater treatment process. For instance, the use of *Chlorella pyrenoidosa* for soybean processing wastewater treatment conducted, after 120 h of hydraulic retention time (HRT), to the following removal efficiencies for soluble chemical

oxygen demand (COD), total nitrogen (TN), ammonium nitrogen ($N–NH_4^+$) and total phosphorus (TP): 77.8 ± 5.7, 88 ± 1.0, 89.1 ± 0.6 and 70.3 ± 11.4%, respectively[18]. Su et al.[19] reported removal efficiencies of 98.2 ± 1.3, 88.3 ± 1.6 and 64.8 ± 1.0 % for COD, total Kjeldahl nitrogen and phosphate (PO_4^{3-}), respectively, using a mixed algal-bacterial culture for municipal wastewater treatment within 8 days HRT.

The performances obtained by partial replacement of activated sludge with photosynthetic microalgae species are the result of the trophic cooperation established between bacteria and microalgae. The photoautotrophic microalgae release oxygen through photosynthesis[20] which is used by the bacteria, with heterotrophic metabolism, to degrade organic matter. The compounds released from degradation processes are used by the microalgae taxa[21] to support metabolic pathways, conducting to the development of complex biological symbiotic system characterised by bidirectional flows.

The major drawback resulted from the implementation of such biotechnology is represented by the poor settling ability of microalgal cells due to the small cell size (< 30 μm) and poor aggregation property conducting to low sedimentation velocity ($< 10^{-6}$ m s^{-1}) (Ref. 22). Frequently used methods for microalgae recovery are: centrifugation, sedimentation, filtration, ultra-filtration, flotation and coagulation/flocculation[6,7,23], most of them being unfeasible due to the contamination risks with metals, rapid filters clogging and high harvesting costs, many authors underlining the necessity to develop a cost-efficient technology for algal biomass recovery[10,23,24]. In order to solve this problem, several studies focused on identifying the efficient method for good microalgal cells recovery. One of them is represented by bio-flocculation. Salim and collaborators[25] attained an improvement of microalgal biomass recovery using non-flocculating microalgal species (such as *Neochloris oleoabundans*) in cohabitation with autoflocculating microalgae (*Tetraselmis suecica*). Another efficient method that promotes the reduction of algal flocculants costs is based on using naturally-available precipitating ions from water, such as Mg^{2+}, which at proper pH value can increase the settling rate of microalgal cells by 100 times more than unflocculated ones[26].

EXPERIMENTAL

The experiment was conducted in a stirred tank bioreactor BIOSTAT®, in batch mode, at a HRT of 96 h with 50 rpm rotation speed, at room temperature (20–31°C). Around the bioreactor, a cool-white circular lamp was used with 25 690 lm m^{-2} light intensity and 15:9 h light:dark photoperiod. Bioreactor was fed with 3 l of dairy industry wastewater with the following physicochemical characteristics: pH – 7.11, O_2 (< 0.5 mg l^{-1}), COD–Cr (1426 mg O_2 l^{-1}), NH_4^+ (51.2 mg l^{-1}), NO_2^-

(< 0.1 mg l^{-1}), NO_3^- (70 mg l^{-1}), PO_4^{3-} (33 mg l^{-1}), TN (72 mg l^{-1}) and TP (13.5 mg l^{-1}). Initial microalgal-bacterial biomass was 1.14 g dry weight l^{-1}.

Wild-type microalgae were withdrawn from a lab scale sequential batch reactor treating dairy industry wastewaters and were cultivated for 3 months in nutritive medium with initial 7.5 pH with the following chemical composition[27]: KNO_3 (2.5 g l^{-1}), KH_2PO_4 (2.45 g l^{-1}), $MgSO_4 \cdot 7H_2O$ (2.4 g l^{-1}), K_2SO_4 (0.217 g l^{-1}), $FeSO_4 \cdot 7H_2O$ (1.5 mg l^{-1}), $MnSO_4 \cdot H_2O$ (1.4 mg l^{-1}), H_3BO_3 (0.28 mg l^{-1}), $ZnSO_4 \cdot 7H_2O$ (0.22 mg l^{-1}), $Na_2MoO_4 \cdot 2H_2O$ (0.05 mg l^{-1}), $CuSO_4 \cdot 5H_2O$ (0.0078 mg l^{-1}). At every 24 h, 100 ml of culture medium were sampled, centrifuged (using an U 320 BOECO centrifuge) at 5000 rpm for 15 min, the resulted supernatant being analysed for: COD–Cr, NH_4^+, NO_2^-, NO_3^-, PO_4^{3-}, TN and TP according to the following standards: SR ISO 6060:1996, SR EN ISO 14911:2003, SR EN ISO 10304/1:2009 (for NO_2^-, NO_3^- and PO_4^{3-}), SR EN 12260:2004 and SR EN ISO 6878:2005, respectively.

The concentrate remained after centrifugation was washed with distilled water and centrifuged again at 5000 rpm for 15 min, the procedure being repeated twice. The concentrate was further re-suspended and analysed spectrophotometrically (with a LANGE DR 5000 spectrophotometer) at 680 nm in order to determine the microalgal cells recovery according to the following equation (adapted by Salim et al.[25]):

$$\text{recovery (\%)} = [(OD_{t0} - OD_{t30}) / OD_{t0}] \times 100\%, \quad (1)$$

where OD_{t0} is the optical density of microalgae cells before settling; OD_{t30} – optical density measured after 30 min of microalgae settling. Using the resuspended centrate was also analysed the chlorophyll *a* concentrations according to SR ISO 10260:1996 standard.

Microalgal-bacterial biomass was determined as described by Bellinger and Sigee[28]. The microalgal-bacterial system growth rate (GR, day^{-1}) was estimated according to the following equation:

$$GR = (\ln B_t - \ln B_0)/t, \quad (2)$$

where B_t (g l^{-1}) represents microalgal-bacterial biomass on day t and B_0 – initial microalgal-bacterial biomass. The microalgal growth rate was determined using second equation, the 'B' variable being changed with chlorophyll *a* concentration. Microscopic investigation of microalgal-bacterial flocs was performed using optical microscope (OPTECH B1).

RESULTS AND DISCUSSION

COD–Cr and nutrients removal efficiencies. Highest rates for COD–Cr (~ 88%) and nutrients (~ 65% for NH_4^+) removal were obtained in the first 48 h of batch treatment (Fig. 1). In terms of phosphate concentration dynamics, a slow increase

(with 3.6 mg PO_4^{3-} l^{-1}) was noticed during the first 24 h, while after 48 h a removal efficiency of about 42% was recorded. Nitrates (NO_3^-) were consumed almost entirely during the first 24 h (decreasing from 70 to < 0.1 mg NO_3^- l^{-1}), after 48 h of treatment a slow increase of parameter concentration being noticed. This can be indirectly correlated with the increase of dissolved oxygen concentration in the bioreactor (Fig. 2) that favoured nitrification. Also, considering the removal rates of NH_4^+ and NO_3^- recorded in the first 24 h can be concluded that NO_3^- was preferential inorganic nitrogen source for biological system in this experiment. The concentration of nitrites (NO_2^-) was below detection limit at all times.

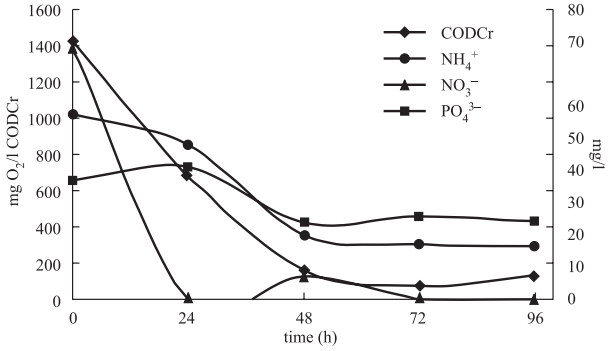

Fig. 1. Variation of COD–Cr, NH_4^+, NO_3^- and PO_4^{3-} concentrations during batch experiment

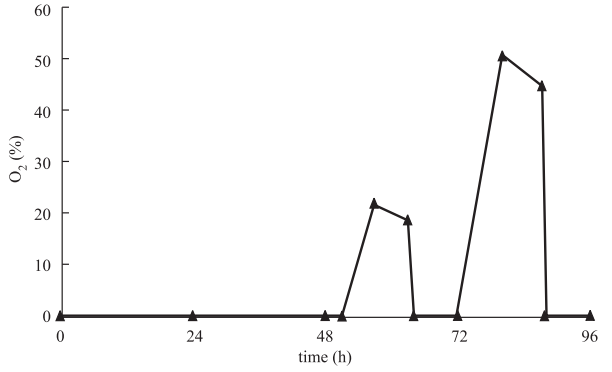

Fig. 2. Dissolved oxygen (O_2) saturation (%) in the bioreactor

Removal rates both for NH_4^+ and PO_4^{3-} decreased in the second part of batch treatment possibly due to the small amount of remaining COD–Cr that conducted to the decrease both of CO_2 released through organic matter degradation by bacteria and metabolic activity of microalgal cells. Moreover, at the end of the batch experiment, a negative growth rate of microalgal–bacterial biomass was recorded (Fig. 3).

In case of TN and TP about 68 and 38% removal efficiencies, respectively, were obtained after 96 h of batch treatment. Low removal efficiency for phosphorus, compared to nitrogen, can be explained by the lower requirements of this macronutrient for the microalgal cells[29].

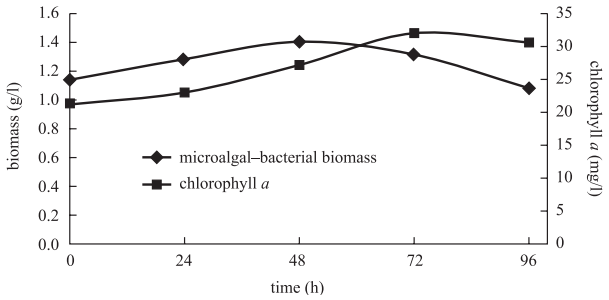

Fig. 3. Microalgal growth curve and variation of microalgal–bacterial biomass

Dynamics of dissolved oxygen (O_2). Increase of dissolved oxygen concentration was recorded only after 48 h, maximum saturation level being about 54% established between 72 and 96 h of treatment (Fig. 2). These results can be explained by the fact that oxygen generated by the microalgae in the first 48 h of batch treatment was entirely consumed by the bacteria. Decrease of COD–Cr with 88% reduced the bacterial activity and implicitly the oxygen uptake rate resulting in the accumulation of dissolved oxygen after 48 h. Moreover, after COD–Cr concentration was reduced, the oxygen concentration pattern followed the light: dark photoperiod.

Microalgal growth rate. Microalgal growth curve (presented as chlorophyll *a* concentration evolution in time) and variation of microalgal–bacterial biomass are represented comparatively in Fig. 3, from where can be noticed that the decay phase for bacteria is attained faster than for microalgae. According to recorded results, the growth rate of microalgae and microalgae–bacteria system until decay phase was 0.13 and 0.10 day^{-1}, respectively, high fluctuations of indicator being noticed, in both cases, during the entire treatment cycle (Fig. 4) that can be associated with COD–Cr remaining concentration. Thus, during the first 24 h, due to the high initial load of organic matter and presence of oxygen released through photosynthesis, the growth rate of bacteria was higher than for microalgae. Along with the decrease of COD–Cr concentration (after 24 h), the growth rate of bacteria decreased while the development of microalgae cells was promoted for further 48 h due to the remaining nutrients and CO_2 availability resulted from organic matter degradation by bacteria. During the last 24 h of batch treatment, due to substrate limitations, microalgae decay was also noticed.

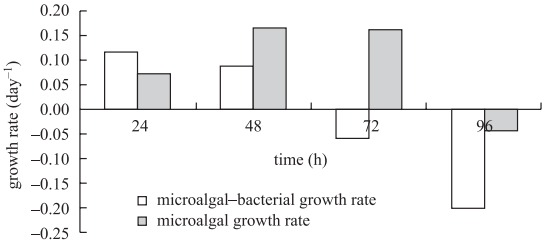

Fig. 4. Growth rate of microalgal–bacterial system and microalgae during the batch experiment

Microalgal cells recovery. Microscopic investigations performed on microalgal–bacterial flocks emphasised that from microalgae category *Chlorella* sp. was the prevalent taxa (Fig. 5*a*). Small size of this genus species (ranged between about 1.6 and 6.6 µm in diameter[28]) can determine the poor settling property. Monitoring the microalgal cells recovery during the batch treatment maximum value of about 63% was found after 72 h HRT (Fig. 5*b*,*c*). At the end of the experiment slow decrease of indicator value was recorded possibly due to the decrease of microalgal–bacterial biomass and the disturbance of microalgal–bacterial flocks stability, thus, resulting in an increase of free microalgal cells.

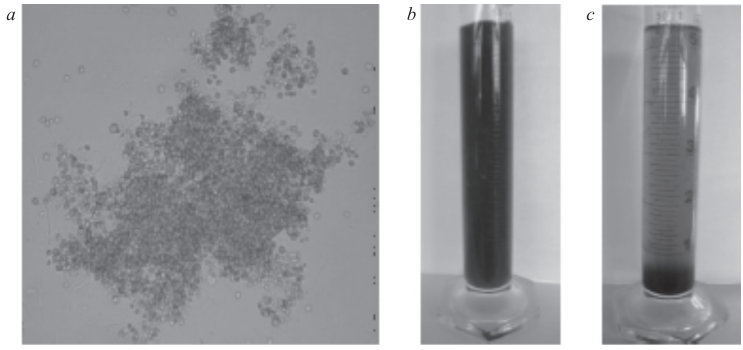

Fig. 5. Microalgal–bacterial flock with attached and free microalgal cells (*a*) (magnification 200×); microalgal–bacterial settling: initial (*b*); after 30 min of settling (*c*)

CONCLUSIONS

The experimental study underlined the feasibility of treating dairy industry wastewater in a microalgae–bacteria symbiotic system without aeration costs, the necessary oxygen being provided by the photosynthetic activity of microalgae. Good treatment performances for organic matter (~ 88 %) and nutrients (~ 65% for NH_4^+, ~ 42% for PO_4^{3-}, ~ 99% for NO_3^-) removal were recorded within first 48 h of batch treatment. However, the quality of treated wastewater resulted after

96 h of hydraulic retention time did not meet completely the quality standards imposed by national regulations, further research being necessary to improve treatment performances. The relatively poor settling ability and recovery (~ 63%) of microalgal biomass involves the necessity of using alternative solutions in order to solve this problem.

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