EVALUATION OF COMBINED ACTIVATED SLUDGE – MICROALGAE SYSTEM FOR WASTEWATER TREATMENT

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The biotechnological principle of using combined microalgae – activated sludge system for wastewater treatment consists in bidirectional metabolic fluxes that can be established between the photoautotrophic microalgae and heterotrophic bacteria. Thereby, the oxygen released during the photosynthesis process by the microalgae species is used by bacteria to degrade organic matter, the resulted degradation products (mainly CO_2) being used in turn as nutrients by the microalgae for cell development.
Since the microalgae biotechnology has been recognized as a viable solution for wastewater treatment, it was used mainly in tertiary treatment processes. However, during the last decade, several researchers focused their studies on using the microalgae for secondary wastewater treatment, high treatment performances for domestic and industrial wastewater being attained.
The main drawback of this biotechnology is represented by the poor microalgae cells recovery. The currently applied methods (centrifugation, filtration, flocculation/coagulation etc.) are involving high costs, contamination with heavy metals, filter clogging etc. In order to solve this problem, several researches were conducted in this field and, until now, bio-flocculation method seems to be the most feasible solution.

Keywords: microalgae-activated sludge system, microalgae recovery, protozoan community, wastewater treatment

INTRODUCTION

In the last decade has been registered an increase of scientific reports addressed to the use of microalgae biotechnology for biological wastewater treatment. The performed studies have emphasized both the advantages and drawbacks of the proposed alternative solution. Regarding the benefits it was identified that the use of the microalgae species can cover not only economic sector of wastewater treatment processes, through the elimination of aeration costs [1], but also social and environmental one, through production of renewable resources (bioactive compounds, bioenergy etc.) [2] and promotion of environmental services by greenhouse gas (GHG) mitigation [3].

As in conventional wastewater treatment, some drawbacks were also identified for this alternative, one of these being poor settling capacity of microalgae due to the small cells size (usually lower than 30 µm) [4]. In the last years several methods addressed to the microalgae cells recovery has been proposed, the most used being: centrifugation, filtration, sedimentation and coagulation/ flocculation [5]. However, these procedures rise high costs or/and are not friendly for the environment [6]. Taking into account these problems, Salim and collaborators [7] proposed an efficient low-cost solution represented by bio-flocculation. This method consists in the use of auto-flocculating microalgae in consortium with non-flocculating species of the same taxonomic category. According to authors, the microalgae cells recovery was significantly increased without adding any chemicals in the culture medium.
Due to the physiological characteristics of the autotrophic microalgae, such as the ability to uptake high amounts of nutrients (mainly nitrogen and phosphorus) [8], these taxa were mainly applied in tertiary wastewater treatment [9]. However, researches performed in this field underlined that the use of microalgae species seems to be advantageous when taxa are involved in the secondary wastewater treatment step [8] due to the risk of the increase of organic matter concentration in the effluent during the photosynthetic activity [10].
In order to ensure the functionality of the microalgae-bacteria consortium during biological wastewater treatment, bidirectional metabolic fluxes must be established between taxa. This relationship is mainly materialized in the oxygen (O_2) and carbon dioxide (CO_2) exchange between photoautotrophic microalgae and heterotrophic bacteria, chemicals resulted from photosynthetic activity and organic matter degradation, respectively [11]. Thus, providing proper development of the consortium can be obtained high wastewater treatment performances in case of both nutrients and organic matter removal.
The aim of the experiment was to analyze the influence of microalgae-bacteria biomass on nutrient and organic matter removal efficiencies from dairy industry wastewater. Another goal of the research was to assess the settleability of microalgae cells during treatment. The diversity of the protozoan species was also monitored, given the bio-indicators role of these microorganisms in conventional wastewater treatment.

METHODS

Microalgae culturing

The wild-type microalgae inoculum was sampled from sequential batch reactor fed with dairy industry wastewater, and cultivated for 40 days in a "*Chlorella* Broth" nutritive medium [12] consists of the following chemicals: KNO_3 (2.5 g/L), KH_2PO_4 (2.45 g/L), $MgSO_4 \cdot 7H_2O$ (2.4 g/L), K_2SO_4 (0.217 g/L), $FeSO_4 \cdot 7H_2O$ (1.5 mg/L), $MnSO_4 \cdot H_2O$ (1.4 mg/L), H_3BO_3 (0,28 mg/L), $ZnSO_4 \cdot 7H_2O$ (0.22 mg/L), $Na_2MoO_4 \cdot 2H_2O$ (0.05 mg/L) and $CuSO_4 \cdot 5H_2O$ (0.0078 mg/L). Culture's pH was adjusted to 7.50 by using NaOH 1%. Inoculum culturing was performed in 250 mL Erlenmeyer flasks using an incubator-shaker (INNOVA®44 R, New Brunswick Scientific, USA), being set the following operational parameters:

temperature - 25^{0}C, stirring - 100 rotation per minute (rpm) and photoperiodicity – 12 hours light : 4 hours dark. Each flask contained 120 mL of culture medium and 30 mL of non-autoclaved dairy wastewater in order to keep the adaptability of the microalgae species to wastewater characteristics. The ranges of the physical-chemical indicators of the used dairy wastewater were the following: pH: 7.4 – 7.7; dissolved oxygen (O_2): < 0.5 mg/L; chemical oxygen demand (COD): 1.33 - 2.02 g O_2/L; ammonium (NH_4^+): 23 - 67 mg/L; nitrite (NO_2^-): < 0.9 mg/L; nitrate (NO_3^-) : < 4 mg/L; phosphate (PO_4^{3-}) : 36 - 95 mg/L.

Experimental operation

The experiment was conducted in 250 mL Erlenmeyer flasks containing 150 mL of dairy wastewater influent. The main physical-chemical characteristics of the influent were as following: pH - 7.8; O_2 - 0.5 mg/L; COD - 1.54 g O_2/L; NH_4^+ - 46 mg/L; NO_2^- - 0.4 mg/L; NO_3^- - 1.3 mg/L; PO_4^{3-} - 93 mg/L. The test consisted of 5 experimental variants conducted simultaneously for 96 hours. 3 experimental variants were differentiated by various biomass concentrations of cultivated microalgae-bacteria inoculum: 0.25; 0.50 and 1.0 $g_{dry\ weight}$/L. In other two variants was added 0.05 and 0.10 $g_{dry\ weight}$ conventional activated sludge/L mixed with 0.25 and 0.50 $g_{dry\ weight}$/L microalgae-bacteria inoculum, respectively. In case of mixed activated sludge-microalgae system, the biomass concentrations were selected in order to keep initial microalgae-activated sludge biomass ratio of about 5:1 taking into account the results reported by Su and collaborators [13]. The activated sludge was obtained from municipal wastewater treatment plant. The experiment was performed in incubator-shaker (INNOVA®44 R) at the same operational conditions set during the microalgae culturing, excepting stirring parameter that was increased to 115 rpm.

Analytical methods

COD, NH_4^+, NO_2^-, NO_3^-, and PO_4^{3-} indicators were analyzed according to the following ISO standards: SR 6060-1996, SR EN 14911-2003 and SR EN 10304/1-2009 (in case of the last three parameters), respectively. pH and O_2 variations in the liquor were investigated according to the SR EN ISO 10523-2012 and SR EN 25814-1999 standards, respectively. The microalgae growth rate (day^{-1}) was assessed according to the method reported by Wang and collaborators [8]. Microalgae settleability (%) was measured using the method reported by Salim and collaborators [7]. Sampling frequency for analysis of all mentioned above parameters was 24 hours. Microalgae-bacteria biomass (g dry weight/L) was determined by filtration of 10 mL of culture mixture through dried and weighed glass microfibre filter (934-AH^{TM}) with sample maintenance at 90^{0}C for 24 hours [14]. Activated sludge concentration (g dry weight/L) was analyzed according to the SR ISO 12880-2002 standard. Microscopic investigations of the microalgae-activated sludge flocs and protozoan community were performed using trinocular light microscope (B1, Optech, Germany).

RESULTS AND DISCUSSION

Variation of dissolved oxygen (O_2) concentration

During testing was emphasized in case of all experimental variants an increasing tendency of dissolved oxygen concentration in the liquor. Also, no significant differences were noticed between variants regarding the indicator's patterns (Fig. 1, a). Maximum value of 10.3 mg O_2/L was recorded for the experimental variant inoculated with 0.10 g activated sludge/L.

After 48 hours of treatment was noticed a decrease of oxygen supply in case of all experimental variants. The most pronounced decline of parameter's value was recorded in case of the experimental variant characterized by highest initial activated sludge concentration. This result can be explained by high oxygen uptake for organic matter degradation by heterotrophic bacteria. An increase of oxygen level after 72 hours of treatment could be caused by the decrease of bacteria's activities due to the low available organic matter concentration remained in the liquor (as will be shown in further paragraphs). Too prolonged hydraulic retention time (HRT) (that limited the availability of the biological system for organic matter and nutrients) can be responsible for the decrease of the oxygen concentration value after 96 hours of treatment.

Variation of pH values

Similar with dissolved oxygen indicator, an increasing trend of pH level was also registered for all experimental variants, the parameters' values ranged between 7.75 and 9.23 (Fig. 1, b). High level of the indicator's value compared with that reported for conventional wastewater treatment is caused by the CO_2 and HCO_3^- uptake during the photosynthetic activity of microalgae [15]. No significant differences were noticed between experimental variants inoculated with initial 0.25 and 0.50 g/L microalgae-bacteria biomass. However, after the first 24 hours of wastewater treatment, in case of experimental variants without activated sludge inoculation, a direct correlation between initial microalgae concentration and pH value was registered. After 96 hours of treatment the indicator's values for all experimental variants were about 9.17, except variant with highest initial activated sludge concentration, which increased up to 8.90. Thus, it can be assumed that metabolic activity of the bacteria had a buffer effect on the pH (mainly through nitrification process). From the Figure 1 (b) can be also noticed that concentration of activated sludge is directly correlated with buffering ability of pH value from liquor.

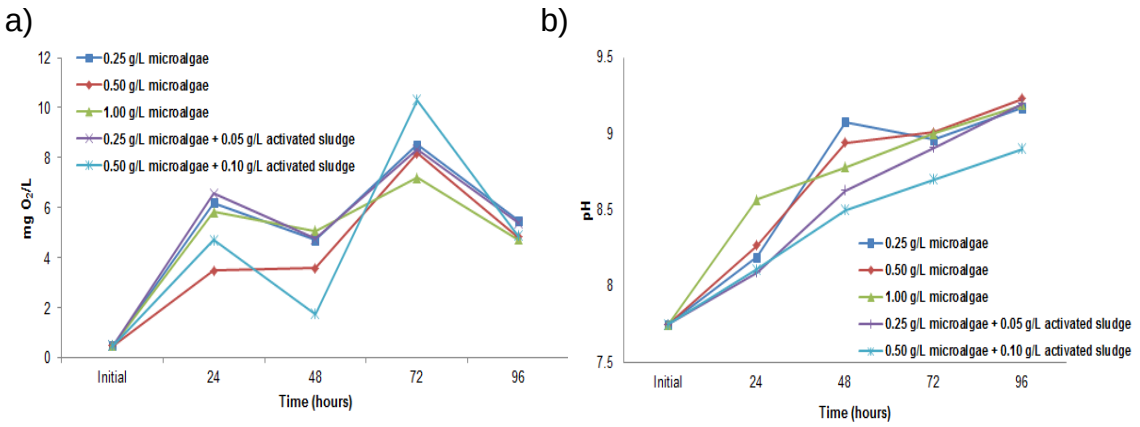

Figure 1. Variation of dissolved oxygen (O_2) concentration (mg/L) (**a**) and pH values (**b**) during dairy wastewater treatment.

Microalgae growth rate

Plotting the variation of the microalgae chlorophyll absorbance (at 680 nm) was pointed out the decrease of chlorophyll content in the first 48 hours of treatment in case of experimental variant inoculated with highest microalgae-bacteria biomass (Fig. 2). Taking into account that no lag phase was recorded in case of other variants, can be supposed that initial concentration of 1.00 g microalgae-bacteria/L promoted photoinhibition process at the used experimental light intensity. However, after the lag phase, the microalgae growth rate reached a maximum value of about 0.29 day^{-1}. The following value of about 0.22 day^{-1} was registered for experimental variant with initial 0.25 g microalgae-bacteria/L, being with 0.06 units higher than that inoculated with 0.50 g microalgae-bacteria/L.
Experimental variant inoculated with 0.05 g activated sludge/L reached a microalgae growth rate of 0.23 day^{-1}. Lowest growth rate was 0.16 day^{-1} recorded for variant with highest activated sludge inoculum. This result could be attributed to the higher concentration of activated sludge biomass influencing negatively the chlorophyll concentration through shadow effect.

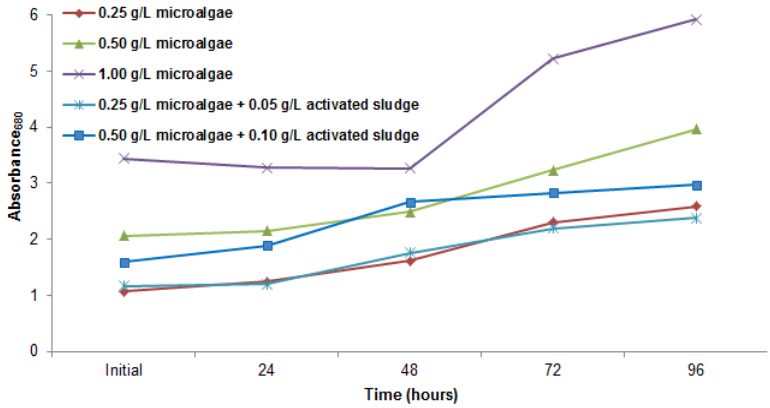

Figure 2. Variation of microalgae growth rate (day^{-1}) during dairy wastewater treatment

COD and nutrients removal

COD removal efficiencies are presented in Figure 3 (a). High removal efficiencies were recorded only after 24 hours of dairy wastewater treatment, experimental variants inoculated with activated sludge showing the best values of 79% and 82%. No difference was noticed between experimental variants inoculated with 0.25 and 0.50 g microalgae-bacteria/L, COD removal efficiencies reaching 69% for both cases. However, use of 1.00 g microalgae-bacteria/L improved COD removal efficiency, being recorded after 24 hours of treatment a value of 76%, in spite of lag phase registered in the first 48 hours of treatment. At the end of the wastewater treatment no significant differences between experimental variants were recorded, COD removal efficiencies ranging between about 90% and 92%.

Analysis of NH_4^+ removal efficiencies revealed high values (ranging between about 72% and 90%) after the first 24 hours of the experiment (Fig. 3, b). Highest performance was achieved by the experimental variant inoculated with 0.10 g activated sludge/L. After 72 hours of HRT the highest value of NH_4^+ ions removal efficiency was 94% for experimental variant with highest microalgae inoculum concentration. However, at the end of the treatment cycle was noticed an increase of NH_4^+ in the liquor possibly due to the too prolonged HRT.

Increase of NO_3^- concentration in the liquor after the first 24 hours of wastewater treatment pointed out the occurrence of nitrification process (Fig. 3, c). After comparing the indicator's concentrations between all used experimental variants a direct correlation between used activated sludge inoculum and NO_3^- concentration was noticed. For instance, in the last hours of treatment, in case of experimental variant inoculated with maximum activated sludge concentration, was recorded about 63.2 mg NO_3^-/L.

High level of NO_2^- concentrations (about 6.4 mg/L) was registered after 24 hours in the experimental sample inoculated with 0.50 g microalgae-bacteria/L, decreasing at the end of the experiment below 0.1 mg/L. In case of experimental variants inoculated with activated sludge, maximum concentration reached in liquor by NO_2^- ions was about 0.7 mg/L.

During wastewater treatment, the variation of PO_4^{3-} concentration was characterized by alternative absorption/elimination processes. The best results of removal efficiencies were recorded after 24 hours, ranging between 68% and 81% in case of experimental variants inoculated with activated sludge, and 45% and 92% in case of remaining ones (Fig. 3, d). Similar with NH_4^+ variation, after 72 hours of HRT was recorded an increase of PO_4^{3-} concentration in the liquor, removal efficiencies decreasing to 42-53%.

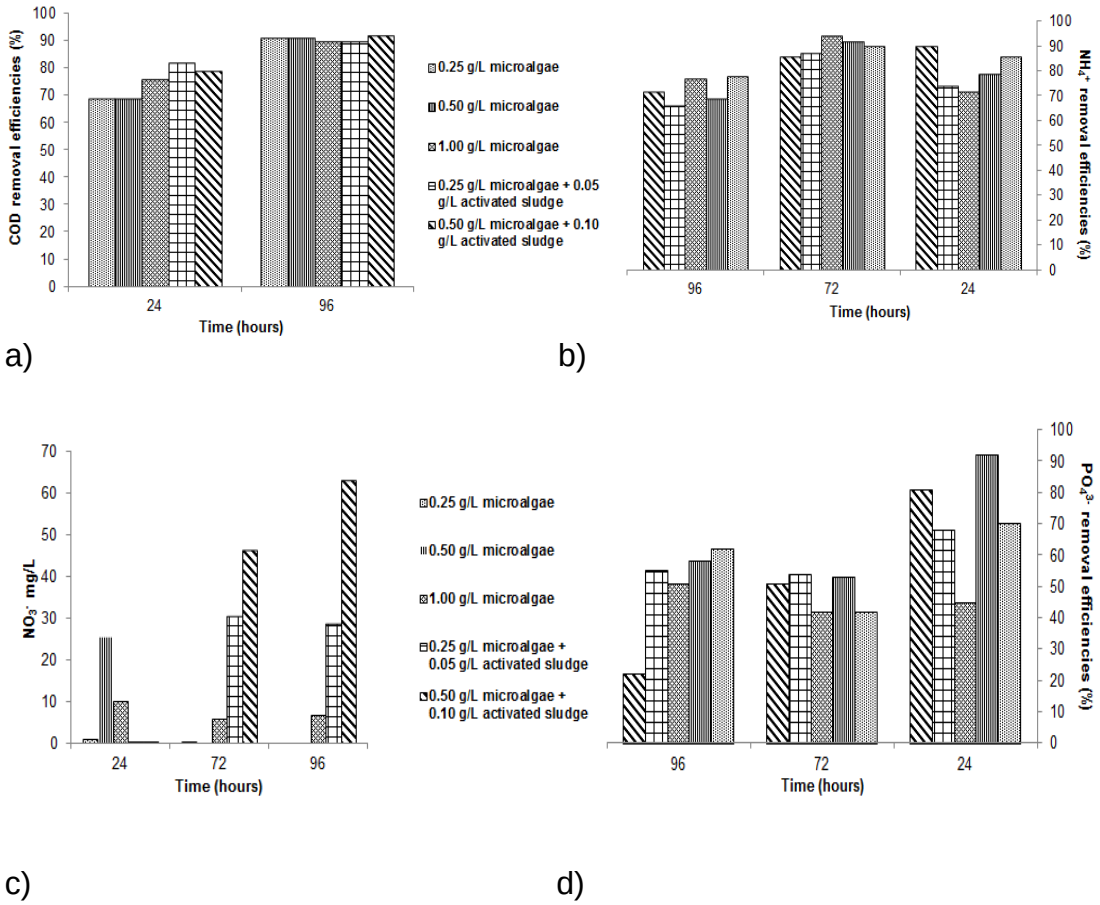

Figure 3. Variation of COD removal efficiencies (a), NH_4^+ removal efficiencies (b), NO_3^- concentrations (c), and PO_4^{3-} removal efficiencies (d)

Microalgae settleability

All experimental variants presented poor settling capacity of microalgae cells (being below 30% after 30 minutes of decantation) excepting variant inoculated with maximum activated sludge (0.10 g/L), in case of which was reached about 90% of microalgae cells recovery in only 2^{30} minutes. Thus, activated sludge inoculum had an important role in microalgae aggregation perhaps due to the provided higher content of extracellular polymers. However, even if both variants inoculated with activated sludge had initial biomass concentration ratio microalgae – activated sludge about 5:1, use of initial 0.05 g activated sludge/L seems to be insufficient to improve the microalgae settling.

Protozoan diversity

In the first 24 hours of wastewater treatment microscopic investigations revealed in all experimental variants the presence with high frequency of small free-swimming ciliates *Colpoda* sp., taxa strongly correlated with the influent quality and initial operational conditions [16]. Decrease of organic matter

concentration from the liquor, transition from anaerobic to aerobic conditions, due to the photosynthetic process, and as also increase of HRT conducted to the decrease in abundance of the free swimming ciliates, being replaced in time with crawling ciliates *Chilodonella* sp.. It was also noticed the appearance of testaceous amoebae *Arcella discoides,* that are used as bio-indicators for occurrence of nitrification process [17], and fixed solitaire ciliates *Vorticella microstoma*. The highest species richness of protozoan community was observed in case of experimental variant inoculated with highest activated sludge concentration. Thus, beside mentioned above species was also noticed the occurrence of free swimming ciliates *Coleps* sp., fixed solitaire ciliates *Podophrya* sp., and colonial fixed ciliates *Epistylis* sp. High diversity of the experimental variant with 0.10 g activated sludge/L could be attributed to the high solids retention time of the activated sludge which was in stable development phase before inoculation. Increasing HRT conducted to the increase of microalgae flocs' dimensions, frequency of developed flocs being directly correlated with used microalgae-bacteria initial concentration.

CONCLUSIONS

Use of microalgae for dairy industry wastewater, rich in organic matter and nutrients, seems to be a feasible alternative for biological treatment. During testing, it was proved that microalgae were able to ensure the oxygen supply needed for heterotrophic bacteria development, without aeration costs. Assessing the influence of different microalgae-bacteria biomass concentrations were not noticed significant differences in case of dissolved oxygen supply, the using microalgae-bacteria ratio being sufficient to provide the functionality of the consortium. Related to pH indicator, it was underlined that the increase of microalgae concentration determined high increase of pH value during the first 24 hours of treatment. This problem can be solved by increasing bacterial biomass in the system, which has a buffer effect on pH values mainly through nitrification process.

Conducted experiment also revealed that microalgae taxa, together with bacteria, are ensuring good treatment performances. Results showed that activated sludge biomass played an important role in the wastewater treatment process, the indicator value being directly correlated with COD removal efficiencies, NH_4^+ removal through nitrification, and microalgae cells recovery. Low bacteria biomass compared with microalgae level conducted also to the good treatment performances, but in a longer HRT. Nevertheless, it is important to ensure a balance between bacteria and microalgae biomass in order to ensure proper oxygen supply with simultaneous organic matter degradation process. Also, when high concentration of microalgae is used in the system, several operational conditions, such as proper light intensity and HRT, must be considered.

During the short term experiment (96 hours) the identified protozoan community reflected the quality of the influent and operational treatment conditions, the diversity of the identified species being similar with those reported for conventional treatment using activated sludge.

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