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NITROGEN AND PHOSPHORUS REMOVAL FROM MUNICIPAL WASTEWATER USING CONSORTIA OF PHOTOSYNTHETIC MICROORGANISMS

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Abstract

In this paper there are presented experiments aiming to investigate the nutrient removal, either from the influent or from efluent of a wastewater treatment plant. The time of contact was 7 days. In order to determine the ratio volume of water – algal biomass we increased the volume of water and the retention time to 4 days. In the study photosynthetic micro-organisms were used, either free or immobilized, aiming to put them to work for consuming the nutrients. In relation mass / volume of 10 grams wet weight of free photosynthetic micro-organisms in 1,000 milliliters input water, removal efficiency of total nitrogen was 15% on the first day, 29% on the second day and 33% on day 4, while total phosphorus removal efficiency was 31% in the first day, 57% on second day and 80% on day 4. In relation mass / volume of 10 grams wet weight of free photosynthetic micro-organisms in 1,000 milliliters effluent water, removal efficiency of total nitrogen was 40% on the first day, 66% on day 2 and 79% on day 4, while the removal efficiency of total phosphorus was 22% on day 1,50% on day 2 and 67% on day 4. Experiments with photosynthetic micro-organisms immobilized in an artisanal cage showed greater efficiency of removing nutrients (nitrate decreased by 70% and phosphorus by 50%) compared to experiments with free photosynthetic micro-organisms (nitrate was removed by 64% and total phosphorus by 39%) subjected to the same experimental conditions.

Key words*: photosynthetic microorganisms, nitrogen, phosphorus, removal, wastewater.*

INTRODUCTION

Wastewaters are unique in their chemical profile and physical properties as compared with fresh and marine waters (Jin et al., 2014). The research of cultivation of algae on waste streams for wastewater treatment was conducted as early as the 1950s, and the symbiotic algal-bacterial relationship in waste stabilization pond was first proposed in which algae were used as tiny aeration devices to provide large amount of O_2 through photosynthesis for aerobic bacteria to oxidize and degrade the organic compounds in wastewaters while heterotrophic bacteria concomitantly release CO_2 and the nutrients needed by microalgae during photosynthesis (Oswald et al., 1957).

Organic matter from wastewater contains, in addition to significant amounts of carbon, also various compounds of N, S, P, H and O characteristics for each stage of the treatment cycle. As the amount of organic C and N decreases, these compounds are different depending on the treatment process used (aerobic or anaerobic). This phenomenon is best reflected in the nature of the resulting final compounds. In aerobic processes, predominate products are oxidized (nitrates, sulfates, etc.) and in anaerobic processes in particular ammonia, methane and sulfides. Resulting nitrogen compounds can be harmful to the quality of the receiving water effluent treatment plants. They may act in natural waters as fertilizer that stimulates the growth of algae and other aquatic plants, causing an accelerated eutrophication (Zarnea, 1994). This nutrient enrichment or eutrophication can profoundly alter the structure and function of aquatic ecosystems, potentially endangering human health, biodiversity and ecosystem sustainability. Therefore, both nitrogen and phosphorus in wastewater should be properly treated or reused thereby reducing their contaminant effects in aquatic ecosystems (An et al., 2003).

The N/P ratio and initial nutrient concentration are considered to be the

significant factors that affect algal growth and nutrient removal efficiency (Hee et al., 1991).
In the past, special attention has been focused on nitrogen and phosphorus removal from municipal wastewater using biological, physical and chemical methods (Blackall et al., 2002; Mallick, 2002). However, some harmful substances cannot be effectively eliminated because the conventional treatment technology used in wastewater treatment plants is insufficient for removing these specific compounds (Ternes, 1998; Saçan and Balcioglu, 2006). More often, the effluents from the wastewater treatment plant fail to meet with the national or local environmental standards. Recent studies have demonstrated that microalgae have a great potential for the removal of nitrogen and phosphorus from wastewater (An et al., 2003; Blackall et al., 2002; Mallick, 2002; Órpeza et al., 2009). Microalgae can be used for treatment of wastewater due to their capacity to assimilate nutrients including both nitrogen and phosphorus (Noüe et al., 1992; Shi et al., 2007).
The aim of this paper is to monitor in laboratory microcosms the nutrient removal ability of selected photosynthetic microorgansims, either free or immobilized, using either input waste water (after mechanichal purge) or effluent water (after traditional activated sludge treatment).

MATERIALS AND METHODS

Isolation of the photosynthetic microorganism consortia was made in BG_{11} medium using for the inoculation either purified water (effluent of the wastewater treatment plant) either waste water (input of the wastewater treatment plant); thus obtained consortia were further enriched by growing them together and mixed in the effluent water of the traditional activated sludge wastewater treatment plant and further in the waste water from sewage. The experiments were performed with either free or immobilized cells. Immobilization of the cells was made on solid substrate in a pyramid mesh made of plastic. Chemical analysis were performed according to ISO standards: spectrophotometric methods - ammonia SR ISO 7150-1/2001, nitrate SR ISO 7890-1:1998, nitrite SR ISO 6777:2002, total phosphorus SR EN ISO 6878:2005.
Laboratory equipment used consists of: Equipment for the determination of the dissolved oxygen - oxygen meter WTW type OXI730; Hach Lange DR 6000 Spectrophotometer - DR6000 spectrophotometer UV-VIS provides peak performance for both routine laboratory tasks and demanding photometric applications. This system is designed to work effectively in professional laboratories. Intelligent software assists the manager laboratory calibration routine quality assurance and custom application development; Hach Lange Thermostat LT 200 - for standard and special digestions; MultiLab mobile luxmeter Model: 545 light measurement with detachable sensor.
Microcosms with free photosynthetic microorganisms: The first experiment was done using a volume of 300 milliliters with effluent from wastewater treatment plant, held in contact with algal biomass; analyzed water was changed from three to seven days, determining the concentrations of nutrients in the initial phase and final phase.
The second experiment was done using a volume of 1500 mL and was kept in contact with microalgae biomass for 4 days to determine a suitable hydraulic retention time.
The third experiment was done in order to compare the efficiency of the microcosms with free photosynthetic microorganisms and microcosms with immobilized photosynthetic microorganisms using a volume of 1500 milliliters kept in contact with microalgae biomass for 2 days.
The fourth experiment lasted 4 days, using influent waste water (after mechanical treatment stage) and effluent (after traditional activated sludge treatment), volume of 1 liter left in contact with microalgae mass of 10 grams (wet weight), at an average temperature of 22 degrees Celsius, artificially illuminated by a fluorescent tube of 60 cm with a luminous intensity of 1150 lumens about 91.5 candelas for 12 hours, alternating with 12 hours of dark.
Microscopic observations: Photosynthetic microorganisms have been inspected at

optical microscope by using alkaline methylene blue as a metachromatic regent to test the presence (or absence) of intracellular deposits of phosphorus, in the form of polyphosphate, and Lugol to test the presence (or absence) of polysaccharides.

RESULTS AND DISCUSSIONS

The first experiment was done using a volume of 300 milliliters with effluent from wastewater treatment plant North Constanta, in an Erlenmeyer flask, held in contact with algal biomass; We found that samples of effluent loaded with nitrogen and phosphorus mixed with microorganisms microalgae and cyanobacteria for several days had a significant efficiency lowering concentrations of the nitrogen and phosphorus; multiple experiments were made in order to determine the best contact time, so contact time was of 3 days in some experiments, 5 days for others and longest contact time was of 7 days, determining the concentrations of nutrients in the initial day and final day.

In the case of samples left in contact for three days, efficiency of phosphorus removal was 99 % and the percentage of nitrogen removed was 73 %, and in the case of the samples left in contact for five days removal of nutrients in the medium was 96 % in the phosphorus and 99 % in the case of nitrate. In experiments with longer contact were found less favorable outcomes phosphorus removal of only 87 % and 100 % nitrogen.

In the second experiment we used the effluent of the wastewater treatment plant North Constanta, in a flask with 1,500 milliliters volume and was kept in contact with microalgae biomass for 4 days to determine a suitable hydraulic retention time. Initially the effluent was charged with 8.9 mg/L nitrate, 0.484 mg/L ammonium and 0.844 mg/L total phosphorus and after 2 days nitrate decreased by 94%, ammonium decreased by 93% and total phosphorus by 54%. After another 2 days (4 days after contact) decreased by 99.2% nitrate, ammonium decreased by 97% and total phosphorus decreased by only 61% difference between the concentration of day 2 and day 4 being very close.

The third experiment was made on 2 samples of water from the effluent of the treatment plant of 1500 milliliters left in contact with free photosynthetic microorganisms (Table 1), and with an immobilized cells in a pyramid mesh made of plastic (Table 2).

Table 1. – Nitrogen and phosphorus removal by free photosynthetic microorganisms (experiment 3)

	Day 0	Day 1	Day 2	Removal ratio %
NO_2 (mg/L)	0.027	0.155	0.01	100
NO_3 (mg/L)	16.2	2.62	2.61	83
NH_4 (mg/L)	0.108	0.007	0.001	99
P total (mg/L)	0.237	0.134	0.096	60

Table 2. - Nitrogen and phosphorus removal by photosynthetic microorganisms immobilized in pyramid mesh made of plastic (experiment 3)

	Day 0	Day 1	Day 2	Removal ratio %
NO_2 (mg/L)	0.027	0.753	0.022	100
NO_3 (mg/L)	16.2	5.26	2.55	84
NH_4 (mg/L)	0.108	0.001	0.001	99
Ptotal (mg/L)	0.237	0.047	0.037	85

The fourth experiment lasted 4 days, using influent wastewater (after mechanical treatment) and effluent (after traditional activated sludge treatment), volume of 1 liter left in contact with microalgae mass of 10 grams (wet weight), at an average temperature of 22 degrees Celsius, artificially illuminated by a fluorescent tube of 60 cm with a luminous intensity of 1150 lumens about 91.5 candelas for 12 hours, alternating with 12 hours of dark. The content of chemical elements in the water sample was big enough, especially the content of phosphorus and ammonia (directly influences the concentration of total nitrogen), so removal efficiency of ammonia in the sample was not large in terms of the experiment, probably would have needed a bigger amount of biomass a higher yield. The results are shown in Table 3 and 4, respectively.

Table 3. – Experiment 4 - Nitrogen and phosphorus removal by free photosynthetic microorganisms using influent water (after mechanical purge)

	Day 0	Day 1	Day 2	Day 4	Removal ratio %		
					Day 1	Day 2	Day 4
NH_4 (mg/L)	21,7	20,0	16,7	16,3	7,83	23,0	24,9
NO_2 (mg/L)	0,174	1,06	0,131	0,011	-	24,7	93,7
NO_3 (mg/L)	5,38	1,31	1,21	1,12	75,7	77,5	79,2
P total (mg/L)	5,12	3,56	2,21	1,05	30,5	56,8	79,5
COD (mg/LO_2)	90,6	68,5	63,2	51,6	24,4	30,2	43,0
N total (mg/L)	20,4	17,4	14,5	13,8	14,7	28,9	32,4

As seen in table 3, removal efficiency of total nitrogen was of 15% on the first day, 29% in the second day and 33 % on day 4, while the removal efficiency of phosphorus was 31% on day 1, 57% in day 2 and 80% on day 4.
The remaining of the nutrients have been removed successfully yield over 50%, taking into account our considerable percentage of high concentrations of nutrient from the input water (previously treated only mechanical).
It appeared that there were some inhibitory factors in the initial stage. This phenomenon was also found during the first days of growth of *Chlamydomonas reinhardtii* in wastewater (Kong et al., 2010). Wastewater often has high concentration of nutrients, much of the N in the form of NH4 -N which can inhibit algal growth at high concentration (Wrigley and Toerien, 1990). In addition, the presence of toxic heavy metals and organic compounds in wastewater, especially in industrial wastewater, is another critical inhibition factor for microalgal growth (Chinnasamy et al., 2010).Was observed a significant decrease in the chemical oxygen demand (COD), reaching 43 % in day 4.

In a study using *Chlorella* sp. (Changfu Wang et al.2013) showed higher removal ratios of total nitrogen in influent wastewater than effluent wastewater. The removal rate of NH4 -N was higher than 83% in influent wastewater. The removal rate of total phosphorus was of 90% in influent and 60% in effluent wastewaters.
Experiment have continued changing just the matrix to the effluent of the wastewater treatment plant North Constanta, to see the efficiency for a quantity of 10 grams algal biomass in a liter of effluent water (less loaded with nutrients). We used only free photosynthetic microorganisms in form of flakes, at an average temperature of 22 degrees Celsius, artificially illuminated with fluorescent light with a luminous intensity of 1150 lumens approximately 91.5 candelas, for 12 ore alternating with 12 hours dark. The concentrations of nutrients in this water ware much lower. The nitrate concentration is the only one that's higher because of nitrification stage, the water suffered in the conventional treatment process with activated sludge.

Table 4. – Experiment 4 - Nitrogen and phosphorus removal by free photosynthetic microorganisms using effluent water (after traditional activated sludge treatment)

	Day 0	Day 1	Day 2	Day 4	Removal ratio %		
					Day 1	Day 2	Day 4
NH_4 (mg/L)	1,70	0,540	0,466	0,280	68,2	72,6	83,5
NO_2 (mg/L)	0,077	0,340	0,046	0,002	-	40,3	97,4
NO_3 (mg/L)	13,1	8,82	4,34	3,96	32,7	66,9	69,8
P total (mg/L)	0,970	0,756	0,488	0,321	22,1	49,7	66,9
COD (mg/L O2)	20,20	4,98	3,85	3,04	75,3	80,9	85,0
N total (mg/L)	4,45	2,68	1,52	0,953	39,8	65,8	78,6

Nutrient removal efficiency was 79% for total nitrogen and 67% for total phosphorus; also chemical oxygen demand (COD) was reduced by 85%, as seen in Table 4. In previous research, *Chlorella vulgaris* was inoculated with final effluent of wastewater treatment

plant and 60% of nitrogen and phosphorus concentrations were removed from the system in 2 days (Rawiwan B. et al. 2012).

The production of biomass and growth rate of *Chlorella vulgaris* - reached a maximum cell density of 11.5x106 cells mL^{-1} and growth rate of 0.54 d^{-1} using KNO_3 as nitrogen source (Jeanfils J. et al 1993). Other studies, reported a lower density of 16 x106 cells mL^{-1} with similar inoculums size (1x 106 cells mL^{-1}) (Lau P.C et al 1995). Similarly, Lau et al. 1994 reported a growth rate for *C.vulgaris* of 0.364 d^{-1} , and Lau P.C. et al. 1997 reported a cell density of 26.5x106 cells mL^{-1} and growth rate of 0.362 d^{-1}, both studies used Bristol medium with 40 mg L^{-1} NO_3-N.

We used an oxygen meter to measure changes in the concentration of dissolved oxygen from water samples , both in light conditions (due to photosynthesis) and in the dark, by measuring the consumption of oxygen caused by the breath of the aerobic microbiota present in the samples.

We analyzed samples of effluent water, water that came from activated sludge bioreactors where oxygen is blown through a ventilation system so the water sample already have a fairly high content of dissolved oxygen. The water temperature during the tests was 19 degrees Celsius. As a result of the calculations carried out under light conditions we achieved an increase in oxygen concentration of approximately 0.02 mg/L O_2 per minute, reaching to be 2.20 mg/L O_2 in 10 minutes, starting at a concentration of 2 mg/L dissolved O_2; We alternated periods of darkness followed by observing the drop in the dissolved oxygen concentration of about 0.2 mg / L O_2 in 10 minutes. Oxygen concentration decreased more slowly in the dark than the speed with which occurred in light conditions. Once we changed the temperature above 22 degrees Celsius, analyzing the dissolved oxygen concentration we found slow growth in light conditions, increasing only 0.07 mg / L O_2 in 10 minutes.

Microscopic observations were, so far, focused on the presence (or absence) of polyphosphate inclusions and on the presence or absence of amidon/glycogen inclusions.

In Figure 1 (a and b) one can see the images of the photosynthetic consortium treated with alkaline methylene blue for (specific) labelling of polyphosphate granules (and other acidic structures).

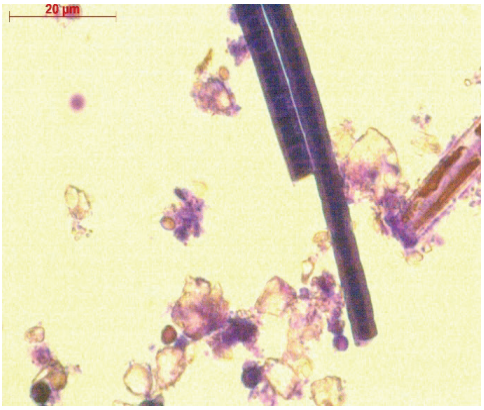

Figure 1a - Photosynthetic microorganisms grown in BG_{11}, labelled with alkaline methylene blue.

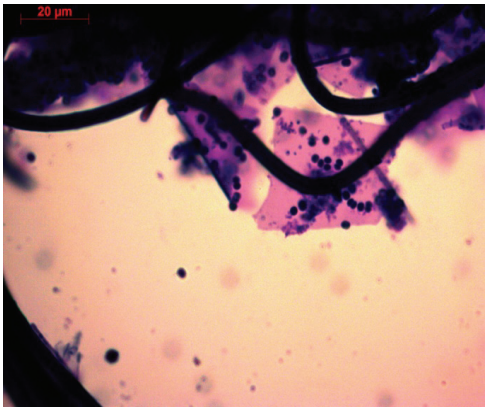

Figure 1b - Photosynthetic microorganisms grown in effluent water, labelled with alkaline methylene blue.

In both images one can see the absence of metachromatic (labeling of polyphosphate granules which suggest that the consortium do not accumulate polyphosphate in either of the growing condition (BG_{11} or effluent water). However, metachromatic label is visible in fig 5a, probably for acidic polysaccharides.

When it comes to polysaccharide inclusions, either glycogen or starch, one can see significant difference between the same consortium grown in BG_{11} or effluent water (Figure 2 a and 2 b).

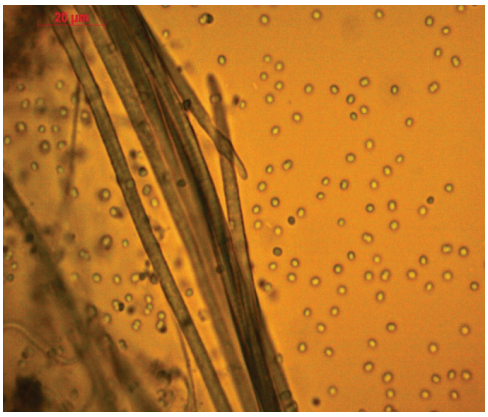

Figure 2a - Photosynthetic microorganisms grown in BG_{11}, labelled with Lugol solution, for polysaccharides inclusions.

One can see the absence of visible polysaccharides inclusions both in unicellular and filamentous photosynthetic microorganisms, when grown in BG_{11} medium

Figure 2b - Photosynthetic microorganisms grown in effluent water, labeled with Lugol solution for polysaccharides inclusions.

One can see the presence of visible polysaccharides inclusions filamentous photosynthetic microorganisms, when grown effluent water, suggesting that the intracellular accumulation of polysaccharides inclusions is sustained in these conditions of cultivation.

The signification of these results deserves further attention, including quantification of polyphosphate and of polysaccharides inclusions, in order to understand the mechanisms involved in N and P removal from effluent water by photosynthetic microorganisms.

CONCLUSIONS

Optimum contact time is of maximum 4 days, the most drastically decrease being recorded in the first two days; after two days, the changes in the concentrations of nutrients are rather low. A contact time of more than 4 days seems to influence the concentration of total phosphorus in the water, this starting to increase, the main cause of this could be the release of phosphorus by (injured/ dead) photosynthetic micro-organisms.

The efficient removal of the nutrients at the laboratory level using photosynthetic micro-organisms is both available on effluent (with low concentrations of nitrogen and phosphorus) but also on the influent if the concentration of biomass is higher enough. For 10 g WCW/1000mL influent water the removal efficiency of total nitrogen was 15% on the first day, 29% on the second day and 33% on day 4, while total phosphorus removal efficiency was 31% in the first day, 57% on second day and 80% on day 4.

For 10 g WCW/1000mL effluent, removal efficiency, calculated on initial concentration basis, of total nitrogen was 40% on the first day, 66% on day 2 and 79% on day 4, while the removal efficiency of total phosphorus was 22% on day 1, 50% on day 2 and 67% on day 4.

Experiments with photosynthetic micro-organisms immobilized in an artisanal cage showed greater efficiency of removing nutrients (nitrate decreased by 70% and phosphorus by 50%) compared to experiments with free photosynthetic micro-organisms (nitrate was removed by 64% and total phosphorus by 39%) subjected to the same experimental conditions.

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