ROMANIAN ACADEMY

BUCHAREST INSTITUTE OF BIOLOGY

Domestic wastewater treatment using selected photosynthetic microorganisms and *Chlorella sorokiniana* UTEX 1230

PhD THESIS SUMMARY

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Bucharest, 2020

Content:

Introduction

The study of this paper was made by analyzing the physico-chemical parameters of wastewater and implicitly the outlet of the Constanta Nord treatment plant, one of the two treatment plants of Constanța, which serves most of the northern part of the city and the resort Mamaia. It is designed to serve 255,000 equivalent inhabitants for the summer and 185,000 equivalent inhabitants for the winter. A particular feature of this wastewater is the seasonal loading both in the flow parameters and in the pollutant load. The treatment plant is positioned on a tongue of land between the Black Sea and Lake Tabacariei with an area of about 100 ha and which together with Lake Siutghiol form a lake complex (Ilelenicz 1999, Antipa 1941).

Positioned in such a sensitive area and given that the effluent discharges into the natural receiver Black Sea at 4000 meters offshore at adiabatic -18 meters, and having a short pipeline for accidental discharges at 500 meters offshore, the authorities imposed limits the most severe of the legislation in force. Thus arose the need for drastic treatment of domestic wastewater, especially in terms of parameters total phosphorus, total nitrogen and CCO considered to be the most dangerous water pollutants, producing the phenomenon of eutrophication and thus damage to marine habitat including the marine microbiota.

In order to reduce the influential loads of the treatment plants, their management must be reconsidered. Currently, the phosphorus content in the effluent of treatment plants is reduced mostly by chemical precipitation in the tertiary treatment phase. However, the use of chemicals is associated with high costs and leads to excessive production of activated sludge, considered by waste legislation, increasing the risk of pollution. These shortcomings in conventional tertiary wastewater treatment have led to the introduction of more sustainable approaches. Numerous studies have shown that phytoremediation, ie the application of algae biomass for wastewater treatment, is an effective measure to reduce the concentration of nutrients by up to 95% (Arbib et al. 2014, Gao et al., 2016, Wang et al., 2017).

In addition to wastewater treatment, algal biomass is perceived as a raw material for the production of bioenergy, nutrients and perfume. Thus, through a successful processing after its use, algae biomass can not only provide a low-cost treatment of wastewater, but is also a potentially profitable approach to wastewater management. However, despite promising application possibilities, certain obstacles prevent the treatment of algae-based wastewater from large-scale exploitation and become a cost-effective alternative to conventional methods. Current technology for separating biomass from water algae significantly increases total treatment costs (Milledge et al., 2013). Finally, the ever-changing chemical content of wastewater poses new challenges, requiring wastewater treatment with problematic and unknown contamination, while their influence on algae growth is often obscure (Su et al., 2012).

Objectives and importance of wastewater treatment with photosynthetic microorganisms

- Selection of photosynthetic microorganisms from the endogenous microbiota in order to use them in wastewater treatment
- Removal of treated wastewater nutrients using green microalga *Chlorella sorokiniana* UTEX 1230 immobilized
- Removal of nutrients from wastewater using activated sludge and *Chlorella sorokiniana* UTEX 1230

- Removal of nutrients using photosynthesizing microorganisms naturally selected from wastewater
- Replacement of chemical dephosphorylation, step in the treatment technology, with another solution less dangerous for humans and the environment. Currently, this dephosphorylation is done because the phosphorus in domestic wastewater is in high concentration, and through the biological treatment process with activated sludge, it is reduced by about 40%. Sometimes this reduction is insufficient for the treated water to comply with the country's legislation on pollutant loading limits for industrial and urban wastewater when discharged into natural receptors. Under these conditions, the treatment technology provides a tertiary stage of phosphorus removal by chemical processes, flocculation, most often using ferric chloride, a very corrosive and very poisonous solution for the aquatic environment.
- It has been known about activated sludge for over 100 years that it has an increased efficiency for sewage treatment, it is commonly used in the treatment of very large quantities of domestic water, requiring a relatively short retention time and high efficiency. . However, the problem with this treatment method is the consumption that this technology generates, very high electricity costs and the inability of the sludge to remove phosphorus when the quantities are large. An objective of this study was to observe the process of photosynthesis that occurs and to what extent it helps or affects the activated sludge. A positive result could help in the future to reduce the costs of electricity consumption used in traditional treatment to maintain aeration in the reactors of treatment plants, using air-generating turbines that are high consumers of electricity.

Original contributions

Chapter 2 contains methods and ways of working in determining the indicators analyzed in water. All methods used are standardized and the test results have been verified with certified reference materials. The calibration curves were made for the results to be subject to statistics and the standard deviations were determined so that the accuracy of the results falls within the 5-10% confidence interval.

Chapter 3 contains the experimental results regarding the selection of photosynthetic microorganisms from the endogenous microbiota for use in wastewater treatment. The isolation of consortia of photosynthetic microorganisms capable of using as nutrients the various chemical forms of nitrogen and phosphorus from the treated urban waters, was done using the culture medium BG 11 (10%), in which inoculations were made.

a. from mechanical purification water (water entering the treatment plant passed through coarse filters, desanding and grease removal)
b. of mechanically, biologically and chemically treated water (waste water, biologically treated with activated sludge and chemically treated with iron sulphate for the chemical reduction of phosphorus)

Selection in the appropriate matrix (wastewater or treated water) of consortia of photosynthetic microorganisms capable of using as nutrients the various chemical forms of nitrogen and phosphorus in oulet wastewaters. In order to make more efficient the removal of nitrogen and phosphorus nutrients, different volumes of outlet wastewater sample were experimented in contact with both algal biomass left free in the form of flakes and algal biomass immobilized by artisanal methods. As the results were gratifying and encouraging, it was decided to try to treat domestic wastewater, previously untreated in any form, to see what removal of nutrients can be done starting from their very high concentrations, taking into consideration the fact that this new matrix has other characteristics in terms of organic loading. In the case of samples left in contact for three days, the dephosphorylation yield was 99%, respectively the percentage of nitrogen removed was 73%, and in the case of samples left in contact for five days the elimination of nutrients on average was 94% in in the case of phosphorus and 99% in the case of nitrate. In experiments with longer contact time, less favorable results of phosphorus elimination of only 87% and 100% nitrate were found, the main cause being the release of phosphorus by the decomposition of dead photosynthetic microorganisms in the water subsequently analyzed. After four days of initial contact, an increase in phosphorus concentration can be observed. This increase can be explained a) the death of microorganisms, b) the formation of new photosynthesizing cells that were not attached to the other consortia and were taken together with water, dispersed in it during analysis at the mineralization bath.

Table 1. Elimination of nitrate and total phosphorus from wastewater treated with previously selected algal biomass

Contact period	Initial concentration (mg/l)		Final concentration (mg/l)		Percent removed (%)	
	P total	NO_3^-	P total	NO_3^-	P total	NO_3^-
3 days	0,919	24,3	0,018	6,630	99	73
4 days			0,016	0,743	99	97
5 days			0,060	0,214	94	99
6 days			0,094	0,237	90	99
7 days			0,127	0,174	87	100

Figure 1 shows the nutrient removal efficiency in the experiment using wastewater as a matrix. The content of chemicals in the water sample was quite high, as the water was only mechanically treated, especially the content of total phosphorus and ammonium (directly influencing the concentration of total nitrogen), so the removal efficiency of ammonium from the sample was not very high under the conditions of the experiment, it would probably have needed a higher amount of biomass for a higher yield. The total nitrogen removal yield was 15% on the first day, 29% on day 2 and 33% on day 4, while the phosphorus removal efficiency was 31% on day 1, 57% on day 2. and 80% on day 3. The remaining nutrients were successfully removed with a yield of over 50%, a considerable percentage taking into account the high concentrations of previously untreated water. A significant decrease in chemical oxygen consumption (COD) was also observed reaching 43% on day 4. This observation indicated that lighting may have a beneficial effect on organic absorption by algae. This decrease suggests that the bacteria may have mineralized the organic compounds to obtain carbon and energy to support their metabolism, and that some carbon sources may have existed in the form of very small particles. It has been reported that algal biomass cannot use carbon particles with a large particle size because algae cells and the membrane used to transport nutrients were smaller than the size of large particles (White et al., 2000).

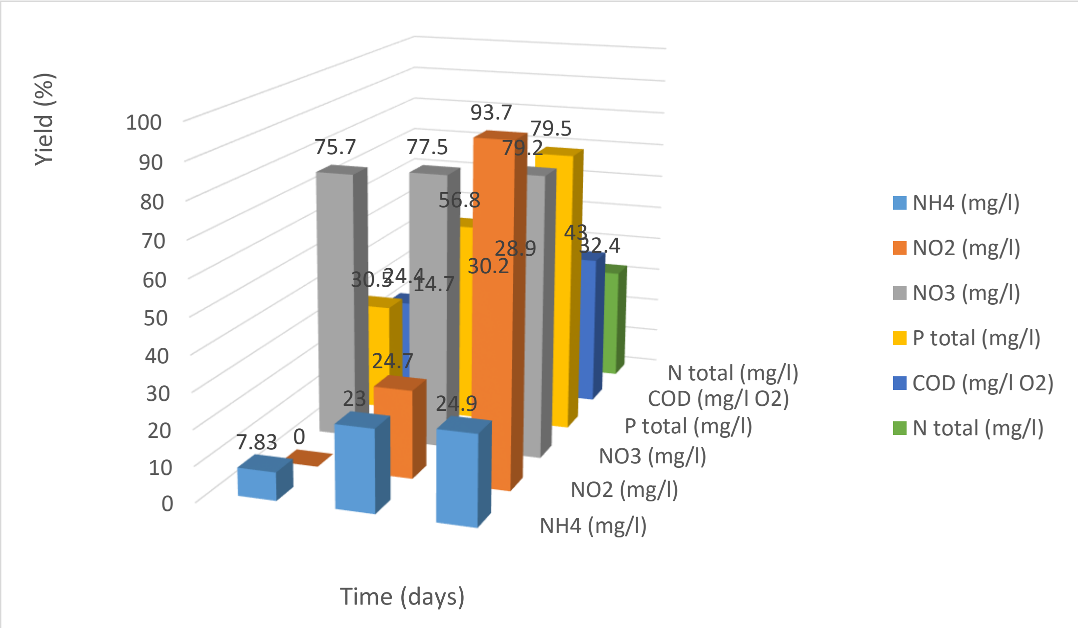

Figure 1. Nutrient removal efficiency from wastewater

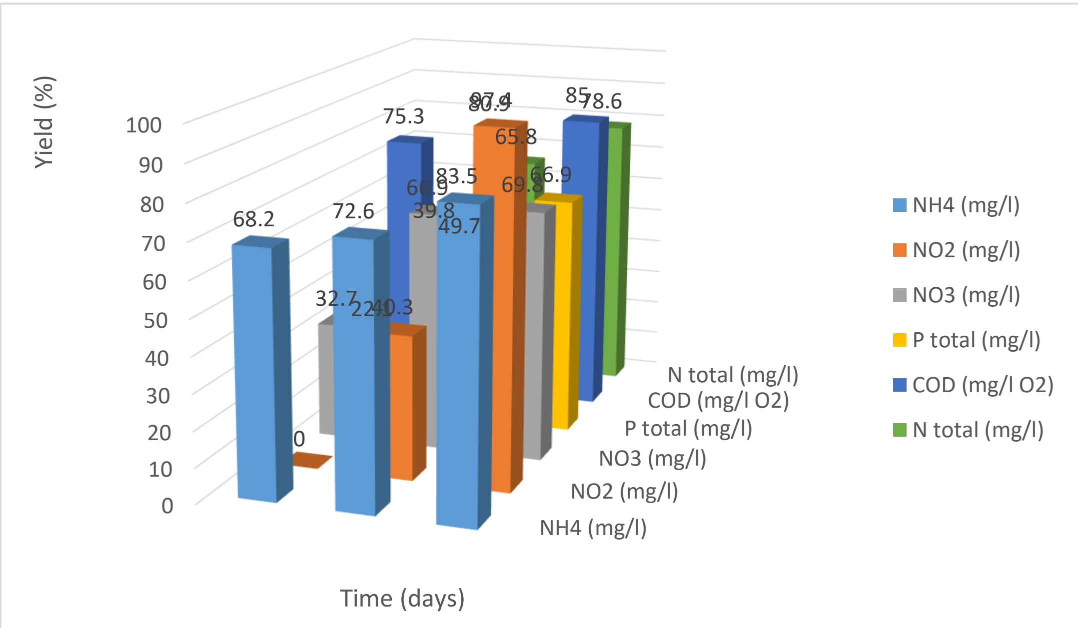

Figure 2. Nutrient removal efficiency from treated wastewater

Figure 2 shows the elimination yield on nutrients, given the relatively low concentrations of previously treated wastewater, a very high elimination efficiency is observed for most of the indicators analyzed, from the first day of the experiment. The biggest surprise came from the indicator of the organic substance which decreased from the first hours of contact by over 75%. Phosphorus decreased gradually, linearly until 48 hours after contact, subsequently its concentration was not significantly altered. Comparing personal research with data from the literature, we found that in previous research, Chlorella vulgaris was inoculated with treated water from a treatment plant and the study shows a removal of total phosphorus and total nitrogen concentrations of 60% in 2 days (Rawiwan et al., 2012).

Chapter 4 included the results of investigations aimed at removing nutrients from treated wastewater using green microalgae *Chlorella sorokiniana* UTEX 1230 immobilized. The experiments were performed using *Chlorella sorokiniana* UTEX 1230 immobilized in BG11 agar medium - remained in contact with treated wastewater under the same environmental conditions (light, temperature, etc.) and contact time of up to 5 days. The environmental conditions and preselected parameters of the experiment were: the volume of 2 liters, 1 liter and 0.7 liters of purified wastewater sample, remaining in contact with the biomass of the corresponding microalgae of 141.81 mg total chlorophyll of *Chlorella sorokiniana* UTEX 1230.

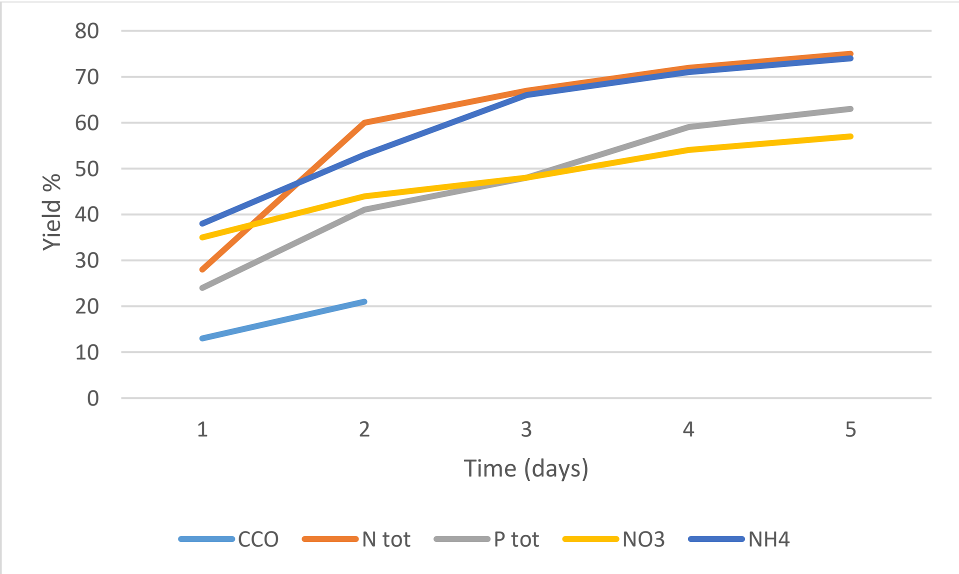

Figure 3. Nutrient removal yield using a volume of 1 liter of treated wastewater and immobilized Chlorella sorokiniana UTEX 1230 biomass, a mass corresponding to 141.81 mg total chlorophyll.

As shown in Figure 3, the nitrogen elimination efficiency was 35% on the first day, 44% on the second day, 48% on the third day, 54% on the fourth day and 57% on the fifth day; the total phosphate removal efficiency was 24% on day 1, 41% on day 2, 48% on day 3, 59% on day 4 and 63% on day 5. The total nitrogen removal rate was 28% on day 1, 60% on day 2, 67% on day 3, 72% on day 4 and 75% on day 5; while ammonium was removed by 38% on day 1, 53% on day 2, 66% for day 3, 71% on day 4 and 74% on day 5. The removal of organic carbon by analyzing the chemical oxygen demand of COD was of 13% after 24 hours, by 21%

less after 48 hours and in the following days the concentration started to increase, which may mean that the organic compounds in the water increased. Analyzing Chlorella sorokiniana in the effluent wastewater of a treatment plant from a silk factory with a retention time of 7 days, a reduction of nutrients of 87% was reported for CCO, 40% for phosphorus and total nitrogen 21% (Li et al., 2019). The best and fastest efficacy was observed in the first 48 hours, then the concentrations began to fluctuate, increasing slightly and then decreasing.

Chapter 5 contains the results of the removal of nutrients from wastewater using activated sludge and *Chlorella sorokiniana* UTEX 1230. The purpose of this chapter was to analyze the effect of the simultaneous use of algae and activated sludge, having different ratios / in different quantitative ratios, compared to the efficiency of only activated sludge (classical process) to treat domestic wastewater. In the experiment, a combination of activated sludge and *Chlorella sorokiniana* was used to treat domestic wastewater. At this time, the usual treatment practice is to blow air using electric turbo blowers to generate the nitrification process. This process uses about 70% of the total electricity consumption of the treatment plant, and the use of green microalgae is designed following the international literature, where the subject is growing in popularity in recent years (Whitton et al., 2015, Posadas et al. , 2013, Tang et al., 2018, Chen et al., 2019, Fan et al., 2020, Katam et al., 2020, Mujtaba et al., 2019, Chen et al., 2017, Arun et al., 2019, Sforza et al., 2018, Roudsari et al., 2014, He et al., 2013). The use of microalgae could become a way to supplement the oxygen in the eastewater treatment process using activated sludge. The results could help the wastewater treatment process, lowering its costs and contributing to a more economically and technologically efficient technology, using the oxygen produced naturally by Chlorella sorokiniana through photosynthesis. The positive impact of this treatment cycle could reduce greenhouse gas emissions and help fight global warming.

The experiment was carried out in the laboratory, the water used being freshly harvested domestic wastewater from the entrance of a traditional treatment plant, the same amount of water was used in all experiments modifying only the biomass used, namely activated sludge, *Chlorella sorokiniana* or mixed between the two , using different ratios to see which is the most favorable ratio between activated sludge biomass and microalgae biomass for the treatment process, at laboratory level. The graph of total phosphorus concentration (figure 4) has a decreasing tendency in all experiments, the most efficient being the experiment using *Chlorella sorokiniana* / activated sludge in a ratio of 3: 1 with a reduction yield of 95%, following the ratio of 1: 1 with yield of 90%, the lowest total phosphorus reduction yield was observed in the

Chlorella sorokiniana alone sample of 21%. Fan et al., 2020, in different sludge / *Chlorella sorokiniana* ratios of 1: 0.5, 1: 1, 1: 2, 1: 4 after 7 days of contact, had a phosphorus removal efficiency of 86% , 87%, 92%, 96% and 82%, respectively.

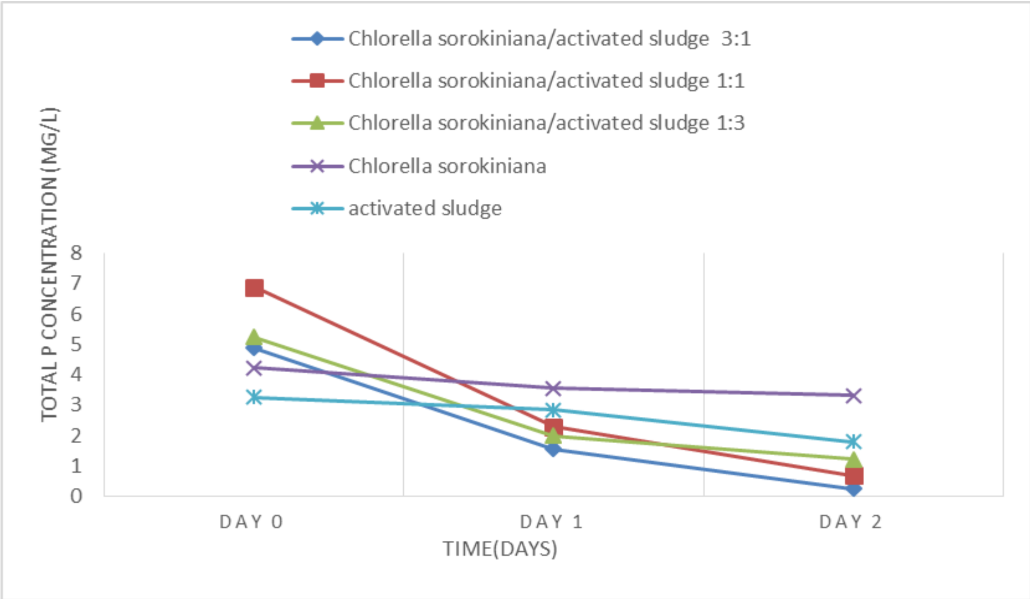

Figure 4. Time evolution of total phosphorus concentration in laboratory open -bioreactors

Amini et al., 2020 reported that in the reactor with activated sludge in ratio of 5: 1 obtained the highest removal efficiency for ammonium and phosphorus (respectively 88.0 ± 1.0% and 84, respectively, 0 ± 1.0%)

In the figure 5 one can see the decreasing concentration of total nitrogen of 83 % (3/1 and 1/3 *Chlorella sorokiniana*-activated sludge mass ratio), of 77 % for activated sludge alone, 66 % for 1/1 *Chlorella sorokiniana*-activated sludge mass ratio and of 54 % for *Chlorella sorokiniana* alone.

As reported by Tiron O et al, 2015, where the treatment cycle was conducted for 24 hours, high removal efficiency of COD was of 98% in the first hours of the reaction, ammonium was removed with a rate of 1.8mg/g h, the transformation rate of ammonium into nitrate was 0.14 to 1.5 mg ammonium/g h and phosphorus removal was achieved from 11 to 85%.

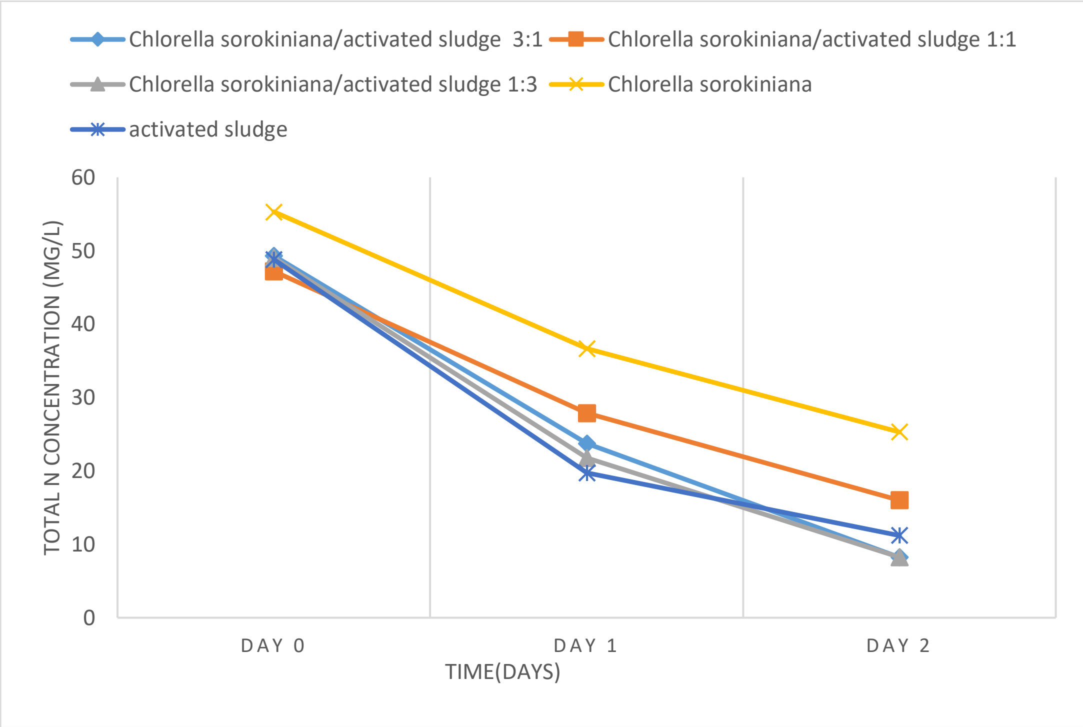

Figure 5. Time evolution of total nitrogen concentration in laboratory open -bioreactors

Another set of experiments was done by measuring and observing the concentration of dissolved oxygen. The three experiments can be seen in Figure 6, in order to draw a conclusion on the production and consumption of oxygen in water samples. In the first graph, the one that recorded the highest concentration of dissolved oxygen during the 48 hours, is the experiment using only Chlorella sorokiniana, the concentration records reaching over 20 mg / l oxygen. The laboratory diary recorded that the water sample was loaded with gas bubbles from the first minutes after mounting the experiment, adhering to all surfaces that were in contact with water. The vessel in which the experiment took place was not closed, the gas exchange between it and the ambient atmosphere being permanently allowed.

Graph 6 of the experiment using the mixture of 1: 1 activated sludge / *Chlorella sorokiniana* has an ascending representation, the oxygen concentration recorded being increasing and yet always being below the graph represented only by *Chlorella sorokiniana*, which may mean the consumption of active oxygen sludge. In the mixture, the sludge consumes the oxygen produced by *Chlorella sorokiniana*, the amount being about 3 mg / l oxygen if we make a comparison between the first two graphs.

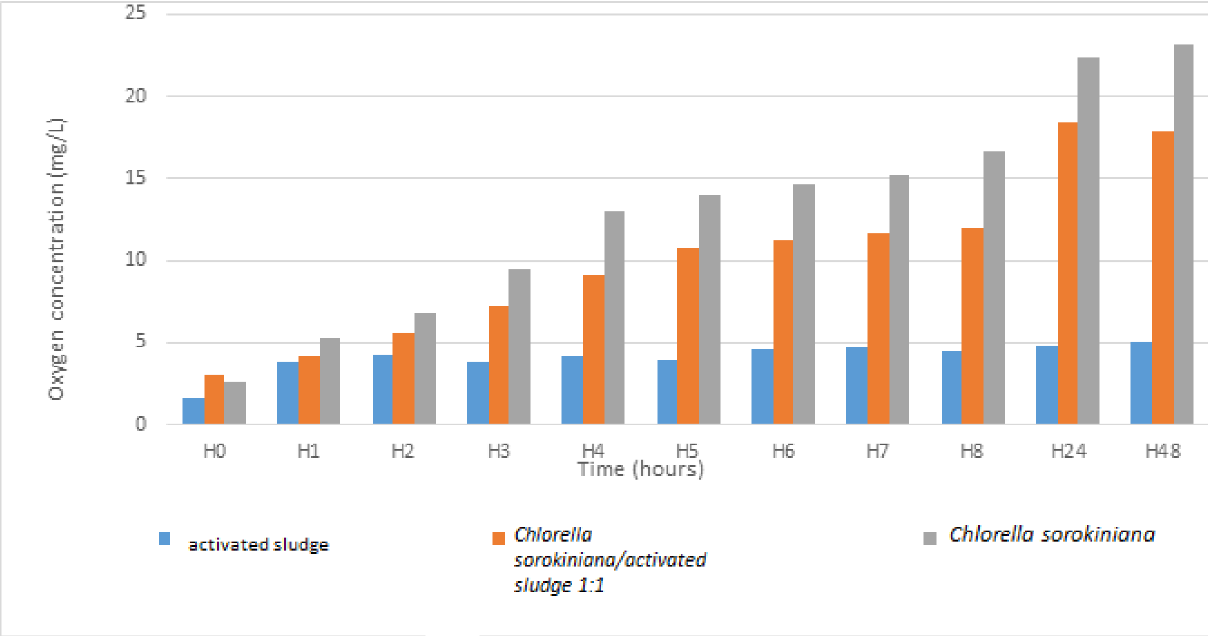

Figure 6. The influence of dissolved oxygen concentration over time

Chapter 6 presents the results of experiments for nutrient removal using photosynthesizing microorganisms naturally selected from wastewater. The photosynthetic microorganisms in these experiments were naturally selected from domestic wastewater, within 14 days of harvesting they were then isolated, rinsed with distilled water, weighed, to be inoculated into various samples of both domestic and treated wastewater. , harvested from the point of its discharge into the natural receptor. These were left free in the experiment, free in the water in which they were inoculated. It was also desired to observe the differences that occur in the case of continuous lighting, but also 12/24 lighting simulating the natural lighting conditions given the desire to be able to apply the most efficient method in large-scale treatment of domestic wastewater. The experiment was mounted in continuous lighting conditions and in 12/24 lighting conditions for both types of photosynthesizing microorganisms, respectively mixed of activated sludge and algal biomass in both wastewater and treated water. The contact time was set at 5 days to observe the changes that occur and to be able to establish an optimal hydraulic retention time. The graph below (Figure 7) shows the curves determined for each experiment separately for the CCO indicator. Observing the graphs, we can say that there is a phenomenon of decrease and then increase in all experiments, both in the case of continuous lighting and 12/24, but also in the case of experiments that used the mixture of microalgae with activated sludge, respectively photosynthetic microorganisms. After the first 24 hours, the experiment using the mixture of activated sludge and algal biomass in a ratio of 1:1, in conditions of discontinuous lighting, the chemical consumption of oxygen decreased, and after

48 hours to increase and the trend was maintained until the day the fifth. A change in the structure of the activated sludge was also observed, which showed a swelling phenomenon, which became slightly fluffy with a tendency to dissipate after the first 48 hours and its color changed from glossy oil gray to black. Most likely, the change in the structure of the sludge changed the concentrations of CCO in the water, filaments of activated sludge decomposing in the water caused the concentrations of CCO to increase. The activated sludge began to suffer after the first 24 hours, decomposing little by little until the fifth day. Following the graph of the experiment with photosynthetic microorganisms only, under the same conditions of illumination and temperature, we can say that they had a decrease in concentration more linear which continued after 48 hours, but from the third day there were also increases. of the CCO concentration, less obvious than in the case of the mixture which also contained activated sludge, however the trend remained upwards until the fifth day. Most likely, in the collected water samples were also found cells of photosynthetic microorganisms, or dead cells that have decomposed from the inoculated biomass, or cells in formation that have been caused by the movements of the water to detach from the biomass and thus have reached the water to be analyzed. The water thus collected was mineralized during the laboratory analysis and thus everything was extracted from the sample. In the first 48 hours it registered a rather significant decrease, of almost 50 mg / l.

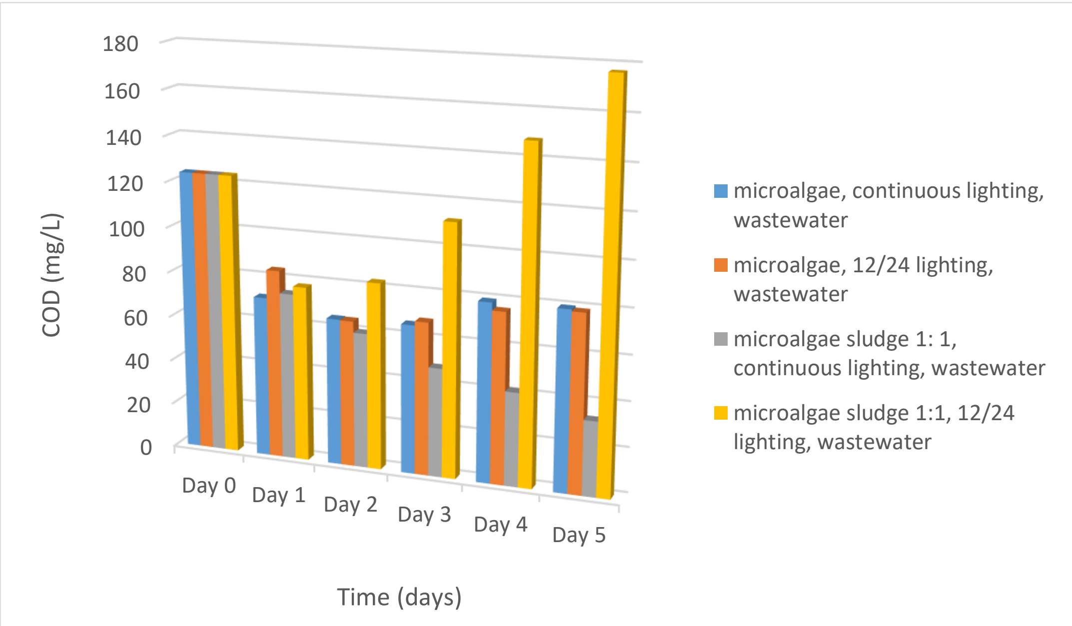

Figure 7. The evolution over time of the COD concentration in conditions of continuous and discontinuous lighting both using only algal biomass and mixed with activated sludge in wastewater

Figure 8 shows the variations in total nitrogen concentration recorded by the four mounted experiments. Most curves have similar trends, except for the experiment curve using mixed activated sludge and photosynthetic microorganisms under batch lighting. In this chart we notice a significant decrease in the first 24 hours and then a weak growth trend over the other four days. Given the observations made on the mud in the experiment after the first 24 hours in which the phenomenon of swelling was observed, the mud was suffering and received less oxygen than it would have needed, this behavior is not surprising.

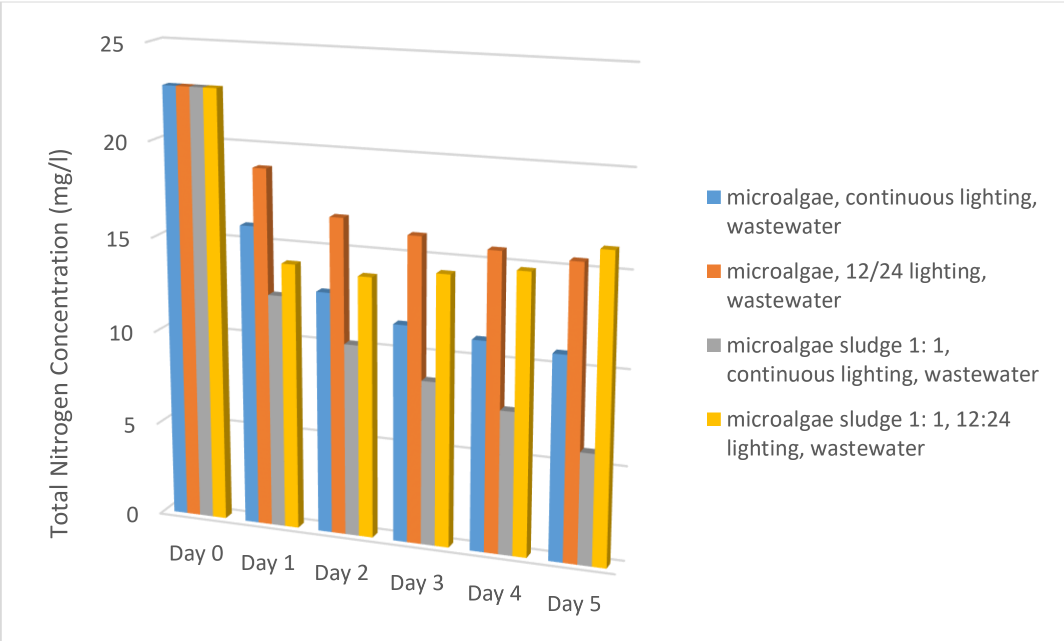

Figure 8. The evolution over time of the total nitrogen concentration in conditions of continuous and discontinuous lighting both using only algal biomass and mixed with activated sludge in wastewater

Figure 9 shows the total phosphorus concentration relative to time for the 4 experiments. Looking at the graphs you can clearly see an atypical curve and totally different from the others. That is from the experiment using the mixture of activated sludge and illuminated algal biomass 12 hours out of 24. It is clear a decrease in phosphorus concentration but only in the first 48 hours, the largest decrease occurring in the first 24 hours, and from a the third day the concentration of phosphorus in the water to be analyzed increases rapidly. We can assume that something happened and most likely, the activated sludge that suffered visibly, began to decompose in water dissolving. The lack of oxygen made him suffer and not develop normally. The experiment using only algal biomass under batch lighting had good results from the first 24 hours and continued to consume total phosphorus, recording significant reductions every day. Due to the high concentration of total phosphorus in domestic wastewater, it managed to reach a concentration below 1 mg / l in 5 days, the limit provided by the legislation in force regarding the discharge of treated water. In the case of experiments under continuous lighting, there were higher decreases but still comparable to the experiment only with illuminated algal biomass 12/24. The experiment using the activated sludge / microalgae mixture recorded a decrease in total phosphorus below the detection limit from day four, while the experiment only with algal biomass had a decrease comparable to the experiment with light-emitting photosynthetic microorganisms, total phosphorus consumption being just a little better. Most likely, algal biomass was more active in the presence of light, hence the difference in phosphorus consumption, while in the presence of darkness, the metabolism entered a slight slowdown

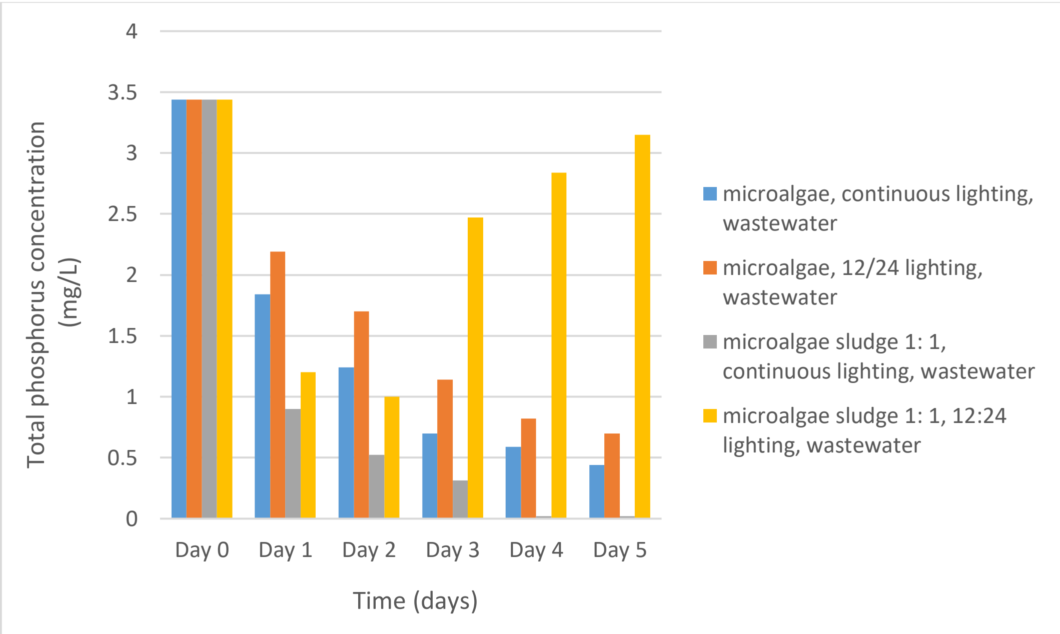

Figure 9. The evolution over time of the total phosphorus concentration under conditions of continuous and discontinuous lighting using only algal biomass and mixed with activated sludge in wastewater.

The phosphorus removal efficiency is maximum in the experiment of the activated sludge mixture, the photosynthesizing microorganisms continuously illuminated, reducing from the first 24 hours 74%, 85% in the next day, 91% in the third day and 99% in the four days and five. In the case of the one with discontinuous lighting, a yield of 65% and 71% was registered on the first day, respectively the second.

General conclusions

- The optimal contact time is up to 4 days using photosynthetic microorganisms, the most drastic decrease is recorded in the first two days, and after these two days, nutrient concentrations change quite a bit, while a contact time greater than 4 days seems to influence the concentration of phosphorus in the water, it begins to increase, the main cause may be the decomposition of photosynthetic microorganisms in the water subsequently analyzed.
- Efficient removal of nutrients is done with the help of photosynthetic microorganisms both in treated waters with lower concentrations of nitrogen and phosphorus but also in domestic wastewater, strongly concentrated in nutrients provided they significantly increase the amount of biomass.
- Analyzing the results of the daily concentrations removed from the immobilized *Chlorella sorokiniana* UTEX 1230 biomass, it can be concluded that after the second day of contact, in the case of all nutrients, there were no significant differences for the following days; establishing for this set of experiments an optimal hydraulic retention time of 2 days.
- The best nutrient removal efficiency recorded using photosynthetic microorganisms was 33% and 80% for total nitrogen and total phosphorus, respectively in wastewater and 79% and 67% for total nitrogen and total phosphorus, respectively in treated water
- The best nutrient removal efficiency using *Chlorella sorokiniana* UTEX 1230 was 90% and 98% for total nitrogen and total phosphorus, respectively, in treated water.
- The highest yield of COD removal was 91% at the ratio of 3: 1 activated sludge / *Chlorella sorokiniana*;
- The ammonium concentration had a very effective removal of 100% in the case of the *Chlorella sorokiniana* / activated sludge mixture in a ratio of 3: 1. In the case of the simple activated sludge experiment, the ammonium removal efficiency was 99%.
- The total phosphorus concentration had a greater decrease in the *Chlorella sorokiniana* / activated sludge mixture of 3: 1 with a reduction rate of 95%.

- The total nitrogen concentration decreased the most in the ratios of activated sludge / *Chlorella sorokiniana* of 1: 3 and 3: 1 registering an efficiency of 83%.

- The activated sludge had a dissolved oxygen consumption of approximately 3mg / l in the 1: 1 activated sludge / *Chlorella sorokiniana* mixture during the experiment using domestic wastewater

- In conclusion, the symbiosis between algal biomass naturally formed from domestic wastewater and activated sludge harvested from the treatment plant for experiments, is especially beneficial for removing nutrients from wastewater, but also financially, no longer needing a additional mechanical aeration under continuous lighting conditions, algal biomass producing very large amounts of oxygen, activated sludge using a fairly small part of the entire production.

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