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CHLOROPHYTE ALGAE REMOVAL CAPACITY IN TRICKLING FILTER WASTEWATER UNDER CRITICAL NUTRIENT CONDITIONS

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ABSTRACT

This study demonstrates the efficacy of chlorophyte algae (*Microsistis spp., Chlorella spp., Closteriopsis and Sphaerocystis*) in removing nitrites, nitrates, phosphates, and ammonium from residual water at the outlet of the trickling filter at the Vijayawada Municipal Corporation in Andhra Pradesh, India, under critical conditions (values maximum and minimum). After 12 days of natural growth of the algae in the samples when exposed to sun radiation, the effectiveness of their removal was measured. During the experimental phase, changes were made to the maximum and minimum of nutrients produced by the plant. For a maximum concentration of ammonium, it was reduced by 25.55 percent, from 17.50 mg/l to 13.03 mg/l. Minimum phosphate levels dropped from 19.00 mg/l to 8.53 mg/l, or 55.10 percent, while maximum phosphate levels dropped from 204.00 mg/l to 155.89 mg/l, or 23.58 percent, in the samples analysed. Nitrate's upper and lower concentration limits are both decreased, with maximum and minimum values of 116.00 and 53.64 mg/l, respectively; this corresponds to a 53.76 percent reduction and a 53.76% reduction, respectively. This reduction from 76.00% to 41.97% only eliminates 44.78%. Nitrites show a drop of 56.78%, from 1.55 mg/l to 0.67 mg/l, while at their highest concentration. This article presents the results of a research undertaken for the purpose of demonstrating that a wastewater treatment system employing naturally occurring chlorophyte algae is a very realistic option for preventing the eutrophication of receiving water bodies..

Key words: Eutrophication, Phytoplankton, Algae, Treatment Plants, Photobioreactor

Introduction

There are studies dating back 50 years to investigate the removal of nutrients through the use of chlorophyte algae (Oswald, 1955). In the 1960s, through the work carried out by Oswald, the concept of using mass cultures of algae was introduced. on a large scale for wastewater treatment.

The removal of nutrients such as ammoniacal nitrogen and phosphorus, as well as DBO and COD, has been studied in Wastewater Treatment Plant of Vijayawada Municipal Corporation, Vijayawada, Andhra Pradesh, through Phytoremediation in biological wastewater treatment systems. However, there is no evidence

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that shows what the nutrient removal capacity of chlorophyte algae may be, when critical nutrient levels (high and low values) occur prior to the growth of algae in domestic wastewater, this being the objective of the study. (Yamashita T, 2014)

For the development of this article, the effluent from the trickling filter system of the treatment plant of the Wastewater Treatment Plant of Vijayawada Municipal Corporation, Vijayawada, Andhra Pradesh, India was used. During the study, the type of chlorophyte algae presents in said wastewater samples and their maximum growth time were identified, which was used as the period of maximum observation of the level of nutrient removal when the samples were modified to their maximum levels. and minimums of phosphate, nitrate, nitrite and ammonium.

Four types of chlorophyte algae were identified, these being *Microsistis Sp, Chlorella Sp, Closteriopsis and Sphaerocystis*. It is important to indicate that of the four species identified, the alga *Chlorella Sp.,* is the one that presents the greatest interest in its nutrient removal processes in domestic wastewater, determining that it achieves its highest volume of biomass after 12 days.

From the analysis of the article carried out, it is concluded that there is a maximum removal of phosphate of 55.10% when it has minimum values at the beginning of 19.00 mg/l, while for nitrates, its maximum removal of 53.76% occurs when the nitrates are in its maximum value of 116.00 mg/l. Nitrites present their maximum removal of 56.78% when presenting maximum initial values of 1.55mg/l. In the same way, a 25.55% ammonia removal occurs when it has maximum values of 17.50 mg/l. (Greenan C.M. 2006)

Background

The use of chlorophyte algae for nutrient removal has a long history of development, so much so that it has been investigated for more than 50 years (Oswald, 1955, Taziki, et al., 2015).

There are studies on the removal of nutrients from wastewater by immobilization systems but limited to Chlorella with alginate, Scenedesmus oblique immobilized in k-carrageenan and Scenedesmus intermedius immobilized in calcium alginate. These studies have evaluated only the quality of the final effluent, and few have determined nitrogen (Moreno Marin et al., 2008).

The efficiency of reduction of ammoniacal nitrogen, total phosphorus and chemical oxygen demand (COD) in synthetic and semi-synthetic wastewater has been evaluated using an axenic strain of nostoc sp., and a microbial consortium, immobilized on alginate beads of calcium and in suspension (Moreno, 2008).

In India, there are similarly studies about Phytoremediation through the evaluation of growth rates of *Scenedesmus quadricauda* cultivated in household effluents, such as the study carried out in different locations. One of the latest studies carried out comes from the doctoral thesis carried out in 2012. It explores a culture strategy to improve the decantation of systems based on chlorophyte algae, concluding that the activated sludge can be used as a bacterial inoculum to improve decantation of biomass for this algae-bacteria system. This study explores a better understanding of an algae-bacteria system by analyzing the compositions of the bacterial community and offers new options to solve the problems of chlorophyte algae culture (Su, 2011).

In another study carried out in India that dealt with the removal of nutrients by 3 cultures of free and immobilized microalgae in 2013 (Aravantinou, et al 2013), the removal capacity of ammonium (NH_4^+) and orthophosphate (PO_4^{-3}) with free and immobilized cultures was studied of microaglas, using for this purpose water from the Wastewater Treatment Plant and two clonal cultures of *Chlorella vulgaris* and *spirulina subsalsa*. A study reported that the maximum removal capacity was for *Chlorella vulgaris* with 50% removal for NH_4^+ mg/l and 74% for PO_4^{-3} mg/l (Ruiz J, et al 2011).

Description of the study area

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This research was carried out based on the characteristics of the wastewater effluent from the trickling filters of the Wastewater Treatment Plant of Vijayawada Municipal Corporation, Vijayawada, Andhra Pradesh, India (figure 1).

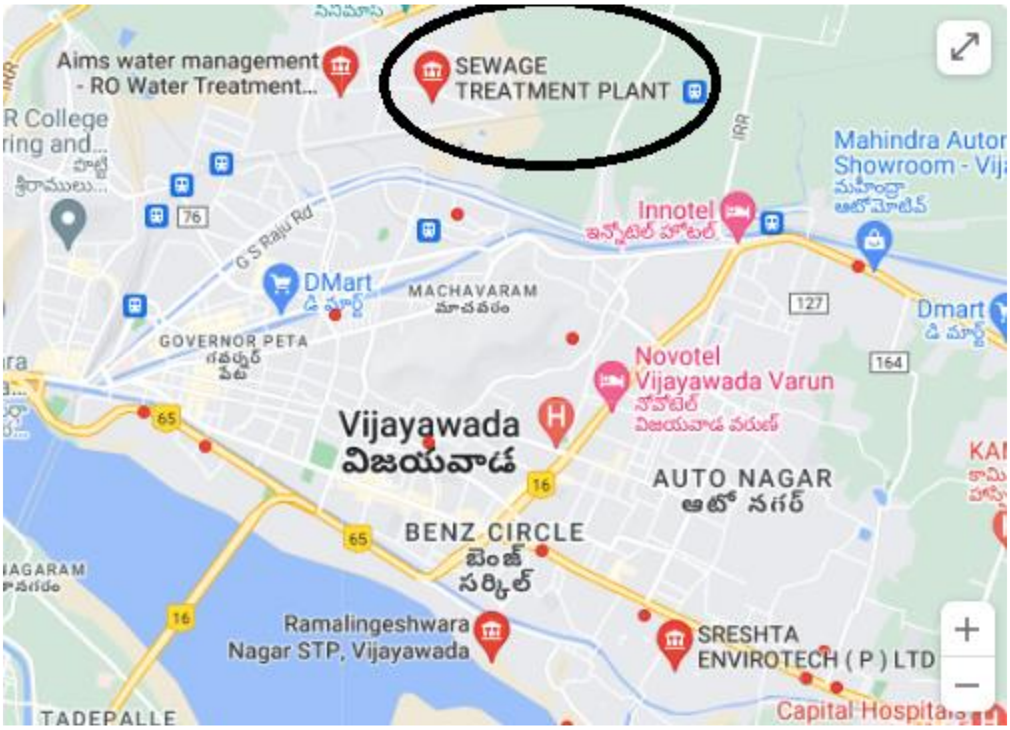

Figure 1. Location of the Wastewater Treatment Plant

Methodology

The methodology used consisted of collecting water samples at the outlet of the third trickling filter of the domestic wastewater treatment plant of the Wastewater Treatment Plant of Vijayawada Municipal Corporation, Vijayawada, Andhra Pradesh, India to know the maximum and minimum values of nutrients produced by it and then make combinations. between both limits for each of the nutrients analyzed and verify nutrient removal levels after 12 days.

Number of samples to be carried out

The number of samples was defined considering four parameters to be evaluated (nitrites, nitrates, ammonium and phosphates) in maximum and minimum values, which would give us a total of 8 samples. In order to provide statistical support, 3 sampling repetitions were carried out, with which the total number of samples was 24.

Collection of samples.

The collection of the samples to carry out both the identification of algae, their counting and the collection of samples for the alteration was made specifically in effluent from the third trickling filter (figure 2), before entering the secondary settler.

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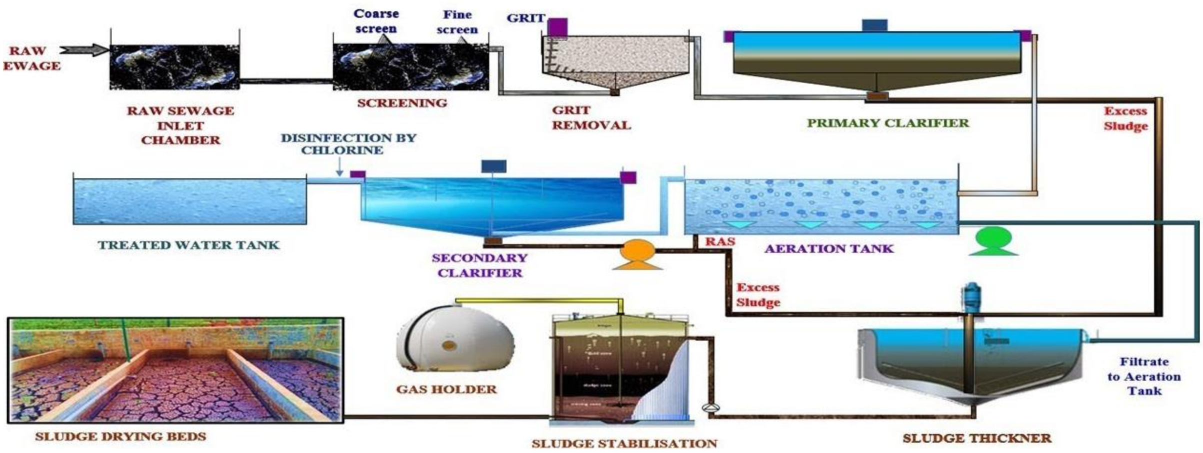

Figure 2. Scheme of Domestic Wastewater Treatment Plant and sampling point

Algae identification

Before carrying out the algae count phase in order to see their growth, a prior identification of the algae species present in the residual water from the effluent of the trickling filters was carried out and thus know the type of species, and then compare them. with those already registered and classified according to the texts and monitoring prior to the plant.

Algae count

The study considered the algae count after several days of isolating a water sample in a container exposed to natural environmental factors. The result obtained in previous studies in terms of counting was that the seventh day was the day of maximum growth or "boom" and then the algae culture began the decay stage as long as there are no external factors that affect the sample, such is the case of variation of organic load, rain or solar radiation among others. The algae count was carried out with a Sedgewick Rafter counting chamber.

Monitored nutrient limits

Carrying out the study required knowledge of the minimum and maximum values of the existing nutrients in the residual water from the output of the trickling filters of the studied plant. To define these limits, a monitoring of 3 repetitions was carried out during 3 days, starting from 8 in the morning until 3 in the afternoon. The days chosen, as well as the hours, were the days that showed the same number of students who were present at the Wastewater Treatment Plant of Vijayawada Municipal Corporation order to obtain the same flow and the same behavior with similar patterns of the updated and real maximum and minimum values of the plant.

Sample modification

The maximum and minimum nutrient values identified according to the procedure of the previous item, were used as a reference to modify the residual water samples from the output of trickling filters, so that it presented said critical values, and thus determine the absorption level. of nutrients under these conditions.

To modify the samples, solutions were used as follows:

- NH_4Cl in a solution of 0.0381 gr diluted in 1 liter of distilled water in order to obtain the concentration of 1 ml = 1.2200 mg /$NH3$. And in a solution of 15.6500 gr diluted in 1 liter of distilled water to obtain the concentration 1 ml=5 mg /$NH3$
- KNO_3 in a solution of 0.7218 gr diluted in 1 liter of distilled water to obtain the concentration of 1 ml = 0.1000 mg/NO_3. And in a solution of 36.0900 gr diluted in 1 liter of distilled water to obtain the concentration of 1 ml = 5 mg/NO_3

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- $NaNO_2$ In a solution of 1.2320 gr diluted in 1 liter of distilled water to obtain the concentration of 1 ml = 0.2500 mg/NO_2. And in a solution of 2.464 gr diluted in 1 liter of distilled water to obtain the concentration of 1 ml = 0.500 mg/NO_2
- KH_2PO_4 In a solution of 21.9500 gr diluted in 1 liter of distilled water to obtain the concentration of 1 ml = 0.500 $mg/\ PO_4$. And in a solution of 43.9gr diluted in 1 liter of distilled water to obtain the concentration of 1 ml = 10 $mg/\ PO_4$

Having already dosed the solutions, we proceeded to collect the water sample at the outlet of the trickling filter. It was collected at 8 in the morning, which is the time with the least amount of nutrients present in the sample. Once having the sample with values even lower than the registered minimums brought to this state through dilution, the sample was modified to the minimum values.

The total samples were 15 liters divided into 250 ml transparent glass bottles for each sample.

Each of the containers was labeled with its respective description of alterations based on the minimum and raised to its maximum according to the label.

RESULTS

The results, tables, graphs and statistical data that were obtained through techniques and experiments in this study are shown in a particular way according to their study phases and are broken down below algae present

A limited number of algae coincident with studies previously carried out in the same treatment plant were found. The species found were the following:

1. *Microsistis Sp*
2. *Chlorella Sp.*
3. *Closteriopsis.*
4. *Sphaerocystis.*

Amount of algae found

The algae count of this investigation was carried out simultaneously with 3 wastewater samples for 12 consecutive days, with the objective of observing their growth and obtaining an average growth, as well as the maximum day of growth according to the type of observed algae This count was performed using a Sedgewick Rafter counting chamber.

It was observed that the Chlorella alga presents a differentiated growth from the rest of the species identified in the samples, since as shown in figure 1, it has a sudden growth from day 1 to day 7 but in the same way it continues to grow until day 12. but at a slower pace.

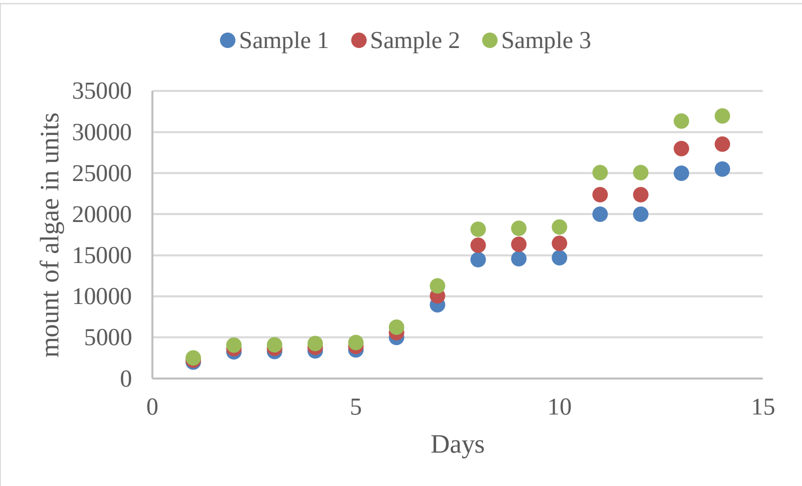

Figure 1. *Chlorella* algae growth

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The species of alga *Sphaerocystis* presents a maximum growth on the seventh day, completely declining its growth more and more. The study presents analysis of samples up to day 12 and to this day it presents a very significant reduction with respect to the peak that is visibly perceived in figure 2.

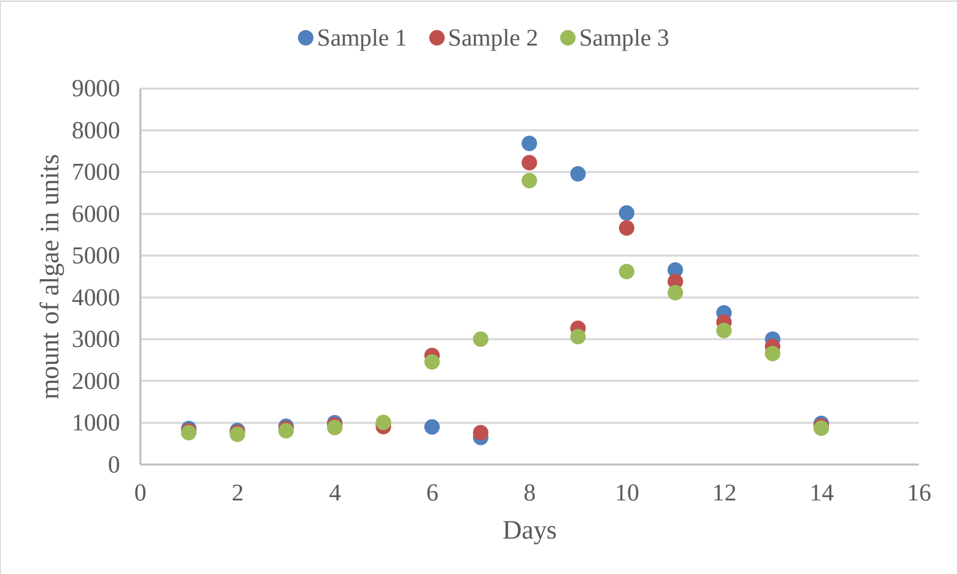

Figure 2. Growth of the *Sphaerocystis* algae

In the case of the *Closteriopsis alga* (Figure 3), the behavior is quite similar to that of the *Sphaerocystis* species in which maximum growth occurs on the seventh day and after that 7-day period the period of colony decay begins, since The last monitored day, which was day 12, is where the day with the least amount of algae occurs, after maximum growth.

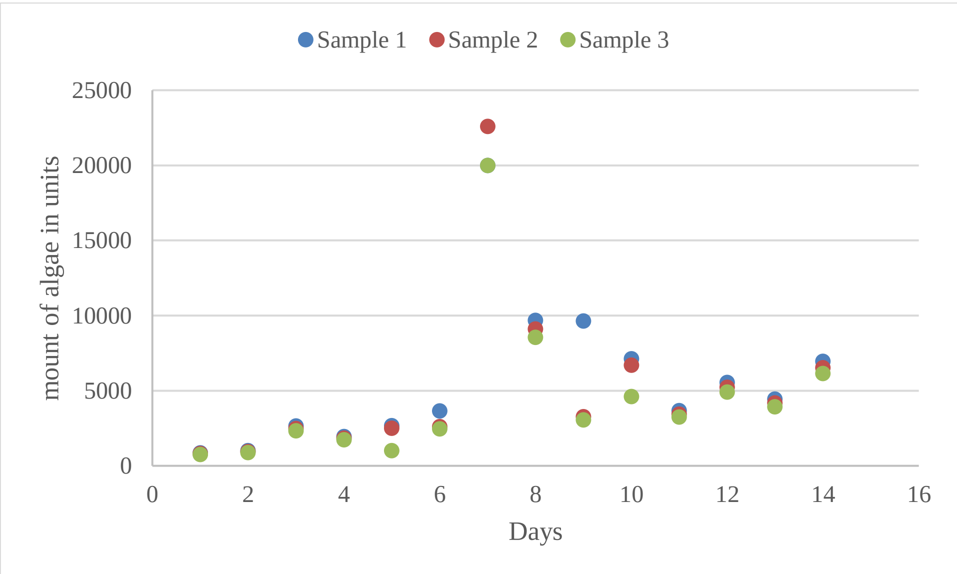

Figure 3. Growth of *Closteriopsis* algae.

Finally, the species of *Microsistis* algae (Figure 4) was counted, which presents the same behavior as the last 2 species counted with a maximum growth on the seventh day and then presents a decline until the last day of monitoring, being that last day the twelfth.

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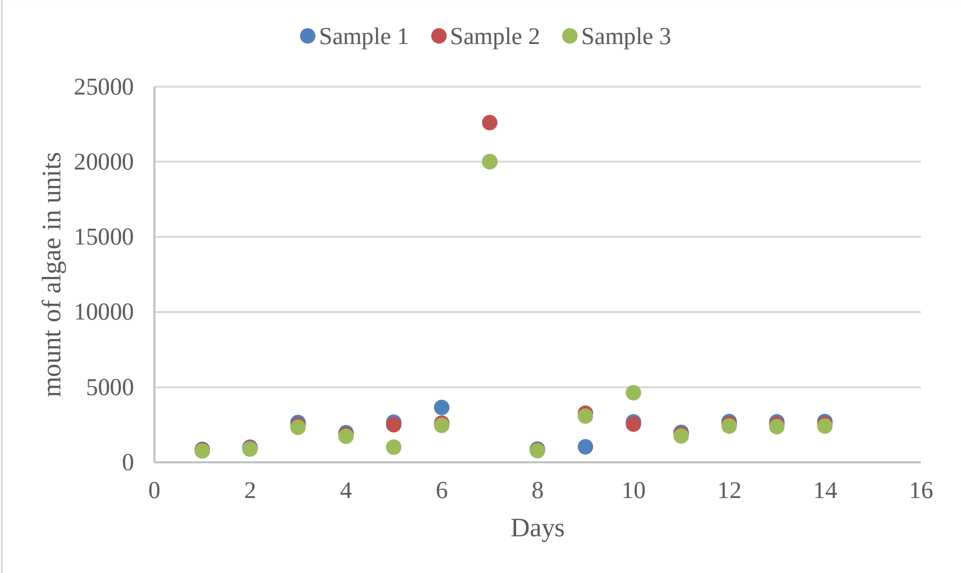

Figure 4. Growth of the *Microsistis* algae.

Maximum and minimum values of nutrients in the residual water of the treatment plant of the Wastewater Treatment Plant of Vijayawada Municipal Corporation, Vijayawada, Andhra Pradesh, India

After the sampling carried out, it was determined that the minimum average value of nutrients at the outlet of the trickling filter in the treatment plant of the Wastewater Treatment Plant of Vijayawada Municipal Corporation, Vijayawada, Andhra Pradesh, India are:

- Phosphates = 19.00 mg/l
- Ammonium = 1.00 mg/l
- Nitrite = 0.10 mg/l
- Nitrate = 76.00 mg/l

And their average maximum values are:

- Phosphates = 204.00 mg/l
- Ammonium = 17.50 mg/l
- Nitrite = 1.55 mg/l
- Nitrate = 116.00 mg/l

Ammonia nitrogen removal under maximum and minimum conditions

The combinations evaluated to establish the behavior of ammoniacal nitrogen under critical conditions were:

1) Ammoniacal nitrogen in maximum value (17.50 mg/l) and nitrites, nitrates and phosphates in minimum values (0.10 mg/l, 76.00 mg/l and 1.00mg/l respectively)

2) Ammoniacal nitrogen in minimum value (1.00 mg/l) and nitrites, nitrates and phosphates in maximum values (1.55 mg/l, 116 mg/l and 204 mg/l respectively)

In the nutrient removal results studied, quite interesting values were obtained, for example in the case of ammonium, this nutrient rose to the maximum value of 17.50 mg/l and the minimum was 1.00 mg/l (Table 1). After the 12 days of exposure to the environment in the flasks, a certain behavior of the values was noted that in the case of the samples that presented maximum values of the nutrient these had average removals of 25.55% based on the initial measurement, otherwise it is shown for the samples that contained the minimum of nutrients, these samples do not reflect a reduction of the nutrient, moreover, they have a higher value than that monitored at the beginning.

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Table 1. Average Ammonium Removal

Ammonium NH_4 condition	Initial Concentration (mg/l)	Final Concentration (mg/l)	Percentage Removed (%)
Maximum limit	17.5	13.3	25.55
minimum limit	1	1.25	-25

Phosphate removal under maximum and minimum conditions

To evaluate the behavior of phosphates, the following combination was analyzed:

1) Phosphate in maximum value (204.00 mg/l) and nitrites, nitrates and ammonium in minimum values (0.10 mg/l, 76.00 mg/l and 1.00 mg/l respectively)

2) Phosphate in minimum value (19.00 mg/l) and nitrites, nitrates and ammonium in maximum values (0.10 mg/l, 76.00 mg/l, 1.00 mg/l respectively)

The behavior of the samples that contained different limits of phosphates was shown to be different, since in both concentration limits of this nutrient removals were recorded (Table 2). The samples that had the minimum of phosphates presented average removals of 55.1% and the samples with the maximum registered showed average reductions of 23.58%.

Table 2. Average Phosphate Removal

PO_4- Phosphate Condition	Initial Concentration (mg/l)	Final Concentration (mg/l)	Percentage Removed (%)
Maximum limit	204	154.89	23.58
minimum limit	19	8.53	55.11

Nitrate removal under maximum and minimum conditions

In the case of nitrate removal in the 3 water samples, the results were positive for both minimum and maximum concentrations established by samples. In the case of those that presented the minimum nitrite concentrations, the average removal percentage is 44.78% and for the samples that contained the maximum content, the average removal is 53.78% (Table 3). Both very satisfactory results, especially the samples with the maximum content.

The combinations evaluated were:

1) Nitrates in maximum value (116.00 mg/l) and nitrites, phosphate and ammonium in minimum values (0.10 mg/l, 76.00 mg/l and 1.00 mg/l respectively)

2) Nitrites in minimum value (76.00 mg/l) and nitrites, phosphate and ammonium in maximum values (1.55 mg/l, 116.00 mg/l and 17.50 mg/l respectively)

Table 3. Average Nitrate Removal

NO_3- nitrates condition	Initial Concentration (mg/l)	Final Concentration (mg/l)	Percentage Removed (%)
Maximum limit	116	53.64	53.76
minimum limit	76	41.97	44.78

Nitrite removal under maximum and minimum conditions

In a similar way to the behavior of the removal of Ammoniacal Nitrogen is that of the removal of Nitrites. In these samples the removal behavior was negative in the case of those that contained the minimum

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of this nutrient, what is more, the tendency was to increase, The curious thing about this case is that it seemed to have a tendency to stabilize the nitrite values. In the case of the samples with the maximum amounts, there was an average removal of nitrites of 56.78% (Table 4)

The combinations evaluated were:

1) Nitrite in maximum value (1.55 mg/l) and nitrates, phosphate and ammonium in minimum values (76 mg/l, 19 mg/l and 1.00mg/l respectively)

2) Nitrite in minimum value (0.10 mg/l) and nitrates, phosphate and ammonium in maximum values.

Table 4. Average Nitrite Removal

NO_2 nitrites condition	Initial Concentration (mg/l)	Final Concentration (mg/l)	Percentage Removed (%)
Maximum limit	1.55	0.67	56.78
minimum limit	0.10	1.21	-11.10

The removal of ammoniacal nitrogen (NH_3-N) achieved was 63.3% and phosphorus (phosphate PO_4^{3-}) 24.9%, suggesting that the interrelationship between the microorganisms present in the water with the chlorophyte algae (with evidence of presence at seven days of retention), consume ammoniacal nitrogen and phosphates. It is important to highlight that the result obtained was in water coming from the outlet of a trickling filter, which was left with a retention period of seven days, under natural conditions of light intensity and temperature.

These results coincide with the studies carried out by Caporgno, et al (2015), who using residual water from an anaerobic treatment system, achieved a removal of 59.7% nitrogen and 78% phosphorus. Similar results were obtained by Aloyce W. Mayo, (2013), in studies carried out on residual water from a system of stabilization ponds, in which he obtained a total nitrogen removal of 36% and nitrite removal of 57.8%.

It is important to note that samples 1, 2, 3 and 4 present a decrease in ammoniacal nitrogen, and an increase in nitrates, as well as an increase in nitrite. This behavior indicates that a nitrification process is being carried out in these samples where ammoniacal nitrogen in an aerobic process is transformed to nitrate.

The behavior of samples is as ammoniacal nitrogen increases, nitrates decrease, nitrites increase, and the growth of algae on average is less than the average growth of the first four samples, the same behavior occurs with chlorophyte algae. This behavior means that there is a process of assimilatory denitrification, and the microorganisms present in the water die to become organic matter. Subsequent to this, there is a transformation of organic nitrogen to ions of the ammonium cation, this process being known as ammonification.

In general terms, in each sample there was a reduction of phosphate. This means that the algae actually need this compound in order to grow.

Conclusions

The characteristics of the residual water coming from the effluent of the module of trickling filters are ideal to generate the growth of algae in a natural way,

It was possible to verify the natural growth of 4 species of algae which were *Microsistis* Sp, *Chlorella Sp,* Closteriopsis, *Sphaerocystis* with a maximum growth at 7 days, except for the species *Chorella Sp* whose maximum growth cycle occurred at the 12 days to then enter the phase of decay or death of the same.

To achieve significant removals of ammonium, it is required that this nutrient be at its maximum level, since under this situation the levels are reduced from 17.50 mg/l to 13.03 mg/l, that is, 25.55%, otherwise when it presents the minimum level this rather it increases from 1.00 mg/l to 1.25 mg/l, that is, it increases by 25%, therefore when the nutrient is at its minimum it does not present any reduction.

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In the case of phosphate, to achieve significant phosphate removals, they are achieved with the minimum levels, since they decreased from 19.00 mg/l to 8.53mg/l, that is, 55.10%, while the samples with maximum phosphate levels presented a reduction of 204 mg/l. l to 155.89 mg/l, that is to say a reduction of 23.58%

In the same way, a reduction is achieved in both limits for the case of Nitrate, but the reduction is significantly greater when this nutrient has a higher concentration, since the values are reduced from 116 mg/l to 53.64 mg/l, equivalent to 53.76% of removal while when it presents the lower concentration limit it only removes 44.78% by decreasing from 76.00% to 41.97%. The removal of nitrites presents a behavior similar to that of ammonium since the reduction occurs when the levels of nitrites are at their maximum, in this condition removals of 1.55 mg/l to 0.67 mg/l are achieved, that is to say, 56.78% contrary to the case of presenting minimum levels of nitrites, in this case the levels increase from 0.1 mg/l to 1.21 mg/l, that is, there is no removal and it increases by 1110%.

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