Removal of Nutrients by Algae from Municipal Wastewater Contaminated with Heavy Metals.

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Bachelor thesis
May 1st, 2015
Environmental Engineering

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ABSTRACT

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Bachelor's thesis 47 pages, appendices 11 pages.
May 1st ,2015

Selected species of algae (green algae and blue green algae) were cultivated in municipal wastewater using PBR(photo-bioreactor)bottles. Uptake of nutrients by these algae species was measured on different dates. From the results of the experiments, it was observed that a combination of certain blue green algae species (cyanobacteria) was able to remove most of the nutrients from the wastewater. The presence of heavy metal ions in the wastewater also affected the nutrient-absorbing capacity of different algae species. Further research on several blue green algae species would be helpful in utilizing these algae species in treatment of municipal wastewater.

Key words: algae; wastewater; pH; temperature; nutrients; nitrogen; phosphorous

CONTENTS

ABBREVIATIONS OF TERMS

PBR - Photo-bioreactor bottles

SP- Algae species

PAS (Photo Iluminescent Algae System)

Ni – Nickel

Cu – Copper

PO_3^- - Orthophosphate

NO_3^- -N - Nitrate

TN- Total Nitrogen

TP-Total Phosphorus

ml- Millilitre

Syke- Suomen ympäristökeskus

Min- Minimum

Max- Maximum

Ave- Average

TAMK- Tampere University of Applied Sciences.

ENVE- Environmental Engineering students group

1 Introduction

The word ‘algae’ refers to a simple but very diverse form of organism which are found in almost every parts of the planet. From single cell autotroph floating in the freshwater to large seaweed such as giant kelp in oceans which can be hundred feet in length, algae is the simplest phototroph which show the greatest diversity of any major division of the plant kingdom (Hemsley, 2000) These simples form of autotrophs can be both prokaryotes (lack nucleus or membrane bound organelles and eukaryotes (contain membrane bound nucleus and organelles).

Within fresh water or oceans, algae can be seen as microscopic single cell floating on the surface known as planktonic algae or they may be seen at the bottom attached with sediments, rocks known as benthic algae. Algae contain chlorophyll and other pigments that can trap the light from the sun and use light energy to make their own food by process called photosynthesis. These diverse form of organism serve as a primary producers in both marine and fresh water by providing oxygen as a byproduct of photosynthesis, by being a food source for zooplanktons, small insects, snails and in case of large filamentous macro -algae serving as a habitat for small animals and fish and many other aquatic life.

Algae are also good bio indicators which means that their presence can provide useful information on physical and chemical characteristics of the waterbody at a particular site (Sigee, 2010).Many algae species are available all the year and they quickly response to the change in the environment due to pollution. They are a good indicator of water quality and many lakes are characterized based on their dominant phytoplankton groups (Chowdhury, 2013) .Algae are also known to clean waterbody by their presence. Recent studies have revealed that algae are a good source for biological purification of wastewater and they are able to clean wastewater by accumulating nutrients, heavy metals, organic and inorganic toxins, pesticides and radioactive matters in their cells. Biological treatment system by micro algae is now thought to be as effective as conventional wastewater treatment system and low cost alternative in treatment of municipal wastewater (Chowdhury, 2013)

1.1 Uses of Algae in wastewater treatment

Uses of algae is diverse. New technologies and discoveries have found several uses of algae in so many fields that it is easy to count where algae cannot be used rather than where it can be used. Some of the major fields were algae use is a booming market are feed manufactures, pharmaceutical companies, cosmetic companies, chemical industries, bio fuel producing companies, pollution control in many industries where CO_2 is produced as pollutant gas, in wastewater treatment either along with conventional water treatment plant or separately to treat wastewater that is rich in nutrients that come from poultry farming, pig and cow farming before the effluent reaches agricultural crops. (Oilgae, 2015)

In most of the wastewater treatment plant whether it is 'domestic wastewater, municipal wastewater or industrial wastewater' chemical methods (use of chemicals) and biological method(use of anaerobic and aerobic bacteria in digestion of organic matter) are used but now interests have grown that algae could be used in different stages along with wastewater treatment plant or separately by itself depending upon the effluent requirement .The problem with conventional wastewater treatment includes high cost of chemicals, maintenance and high energy input . Use of algae in wastewater treatment plant is cost effective, requires low energy input and the process is sustainable. Many researches on algae have shown that algae are able to absorb not only nutrients but also heavy metals particles in wastewater. This property of algae is very beneficial in treatment of wastewater especially in domestic and municipal wastewater which is rich in nutrients like nitrogen and phosphorous and many traces of heavy metal ions (Oilgae, 2015).

Treatment of wastewater by using algae cultivation can be used in several stages with conventional wastewater treatment plant. If the wastewater is domestic like grey water from houses and the effluent is to be discharged directly into lakes or ponds, algae treatment facility could work without any further use of chemicals. If the wastewater is municipal wastewater coming from septic tanks or industrial wastewater that contains many toxic chemicals and metal ions, then additional treatment process like chemical process and future biological process may be required along with algae treatment. Algae based treatment processes are mainly used for the removal of nitrogen and phosphorous in municipal and industrial wastewater (Oilgae, 2015).

Many wastewater effluent coming from dairy industries, animal feed industries, pig industries, agriculture run off water contain high amount of nutrients and fertilizers which can pollute the fresh water body like lakes, ponds and rivers if this water is not treated and directly discharged. Algae treatment plant can play a very vital role in capturing all those excess nutrients and fertilizers and make water safe to be discharged at fresh water body. The algae cultivated can be harvested in any waste water treatment plant and sent into the bioreactor to produce biogas like methane that can be used to produce electricity to fuel the whole treatment plant again. Apart from biogas, harvested algae is also rich in nutrient that can be used as fertilizer in agriculture fields, also used to feed aquatic plants and fish. The example is set up in Australia where farmers in diary industries are now actually able to use diary effluent (wastewater) as a valuable resource. Algae cultivation is being done on PAS (Photo-Iluminescent Algae System) which is a system of thin film plastic which contains fluorescent dyes that alter and change the incoming sunlight that helps algae grow. The diary effluent is sent through the algae under PAS and with the help of light and nutrients in effluent algae grow produce methane gas as renewable energy source. The digester also concentrated nutrient stream that can be used as fertilizers for growing crops and animal feed (Algae Enterprises, 2015)

1.2 Factors that affect Algae growth in water.

1.2.1 pH and temperature.

pH is the measurement of hydrogen ion concentration in water. pH plays a very important role in aquatic life living in freshwater or marine water. It determines the solubility and biological availability of many nutrients (nitrogen, phosphorous , carbon etc.)and metal ions(zinc, copper, lead etc.) to aquatic plants and animals (Department of Ecology ,Washington, n.d.). Many metals tend to dissolve in low pH and be readily available in water. They have toxic effects on aquatic plants and animals. Likewise, availability of different nutrients like phosphorous and nitrogen in different forms is also determined by the pH value .Slight change in pH can increase the nutrient availability level in lakes or

ponds and cause rapid growth of many plants and algae giving rise to eutrophication problem (Kemer, 2013). pH is also an indication of what kind of algae is dominating the water body. Lower pH due to increased CO_2 dissolved in water gives' green algae an competitive advantage over blue green algae' (Weiner, 2008).

pH in fresh or marine water body may change due to several reasons that can be manmade or natural. Manmade causes include pollution through mining, combustion in vehicles that release CO_2, sulfur oxides, nitrogen oxides and when water reacts with these compounds there is an acid rain which has low pH less than 5,0. Natural causes include photosynthesis in surface by many phytoplankton and other aquatic organisms during day time used more CO_2 which can cause pH to rise. Likewise, at night photosynthesis stops and CO_2 from atmosphere dissolves in the water and lowers the pH (Kemer, 2013)

Environmental Protection agency (EPA) sets pH range (6,5-9,0) suitable for the growth and survival of aquatic plants and animals (Weiner, 2008) .The optimum pH for growth of algae is believed to be 8,2-8,7 but most species of algae grow well in pH range between 7 and 9 (FAO,Fisheries and aquaculture department)

Temperature is one another parameter that is very important in growth of algae and many other aquatic life. Change in temperature also influences other parameters like pH ,conductivity, salinity, total dissolve oxygen, photosynthesis, compound toxicity etc. that can change the physical and chemical properties of water. (Christine K. , 2014).The optimum temperature for growth of cultured algae is believed to be from 20-25 ºC. Many cultured species of micro algae would do fine and grow well in temperature range between 16-27 ºC. Temperature below 16 ºC and above 35ºC may not be suitable for algae growth. (FAO,Fisheries and aquaculture department).

1.2.2 Sunlight and Carbondioxide

Sunlight and carbon-dioxide together are another important factor for growth of algae. These two are the core elements needed for photosynthesis in algae. Algae including cyanobacteria (blue green algae) have chlorophyll present in their cells .These chlorophyll capture light energy from the sun which convert the inorganic carbon to organic carbohydrate (glucose), lipids and proteins. Photosynthesis process in plants and algae releases

oxygen so it is also called oxygenic photosynthesis (Hemsley, 2000) .Water temperature and turbidity in lakes both affect the photosynthesis in algae. With increase in water temperature photosynthesis in most algae speeds up although there are slightly different optimum temperature requirement for different algae species. Likewise, high turbidity in lakes slow down the photosynthetic rate in algae as amount of sunlight entering into several depth of lakes decreases((Fitch, 2014).

Carbon dioxide in fresh or marine water is available to many phytoplankton like algae in the dissolved gas form. Most of the carbon dioxide in water enters through the atmosphere .Sunlight on the other hand is available only during the day so photosynthesis process peaks during day time and declines during night (Fitch, 2014).

1.2.3 Nutrients

Nutrients play a very important role in growth of cells and enzymatic process in algal cells. Some inorganic nutrients like nitrogen, phosphorous, carbon are primary nutrient requirement in algae cells. There are also many other micronutrients and trace metals which are needed in lesser amount which are silica(Si), magnesium(Mg), sodium(Na), potassium(K), calcium(ca), sulfur(s), copper (Cu), manganese(Mn), cobalt(Co). In this section we will focus mainly on primary nutrients like nitrogen and phosphorous and how are they important to algal growth.

Nitrogen

Nitrogen is one of the essential nutrient required for all living cells including algae for growth and reproduction. It is very crucial element which is found in amino acids that forms the proteins, in nucleic acids that makes up DNA. Plants and other living organisms cannot directly absorb atmospheric N_2 as nutrient because of the strong bond in N_2 molecule which is hard to break. So, atmospheric nitrogen must be broken down to several other chemical forms .This process is called nitrogen fixation and many bacteria which are present in water, soil pores, roots fix the atmospheric nitrogen to other chemical forms

so that it can be absorb by plants either in soil or in water. Some fixation of nitrogen also occurs during lighting process (Weiner, 2008).

In nitrogen cycle, nitrogen is converted to various chemical forms like ammonia, ammonium ion, nitrite, nitrate and free nitrogen. The fixed nitrogen in soil pores in the form of ammonia and nitrogen oxides is absorbed by the roots of plants and legume plants to make proteins, DNA and other organic nitrogen compounds. When these plants are eaten by the animals and when they excrete or die, organic nitrogen enters into the soil in the form of ammonia. This process is called ammonification. Ammonia is first converted to nitrate by bacteria and nitrates are gain converted into nitrite further by oxidation and the process is called nitrification. Nitrates in the soil are again converted to molecular nitrogen through nitric oxide by denitrifying bacteria and the process is called denitrification through which nitrogen again returns to the atmosphere (7activestudio, 2014)

Most algae and aquatic plants receive nitrogen in the form of nitrate and ammonia. Blue green algae (cyanobacteria) are actually able to fix atmospheric nitrogen into ammonia.

Phosphorous

Like nitrogen, phosphorous is also one of the essential nutrient for growth of plants and animals. Plants and algae need phosphorous to make ATP, it is also needed to make DNA and phospholipids in the cell membrane. Orthophosphate(PO_4^{3-}) is the only form used by most plants (including algae) and other organisms to obtain phosphorous in water (Weiner, 2008).

The conversion of phosphorous into different chemical forms is called phosphorous cycle but it does not involve atmosphere like in nitrogen cycle. Most of the phosphorous present in the environment is in the form of inorganic orthophosphate present in rocks and minerals in soil or in water body .The weathering of rocks and minerals helps plants and algae to absorb inorganic phosphorous and convert them to organic form. When these plants are eaten by animals, phosphorous again enters the soil or water when animals urinate, excrete or when they die. The organic phosphorous in soil or water is again converted to

inorganic phosphorous by decomposition with the help of bacteria (Beverly Biology, 2014)

Nitrogen and phosphorous are considered to be limiting nutrient in growth of algae. When one of these nutrients is in excess amount, it triggers phytoplankton growth number. In summer and spring times, when there is excess of nitrogen and phosphorous available in the water body algae bloom is a common problem called eutrophication. Excess of nutrients naturally or artificially(run off of fertilizers from agricultural land that contain phosphate) in water body helps algae to grow rapidly and decrease the light penetration and dissolve oxygen amount .This has direct effects on many aquatic animals like fish, crabs, snails and many more. There might also be a shift of algae species from green algae to more harmful blue green algae (Andrew R Dzialowski, 2005).These blue green algae produce toxic that can lead the death of almost all aquatic living organisms, animals that drink the toxic water and even humans by drinking toxic water or using it. Some toxins like Hepatotoxins affect the liver is produced by some strains of cyanobacteria like Anabaena, Nodularia, Microcystis, Oscilatoria etc. There are also some toxins that affect the nervous system, gastrointestinal system, kidney (WHO, 2015).

2 Aim of this work

The aim of this project was to cultivate different mixture of algae species in photo-bioreactor bottles and compare their ability to remove nutrients and heavy metals from municipal wastewater.

This project is a continuation of previous research done in TAMK by different students on various species of algae to purify wastewater. We tested with the selected mixture of algae species which were already known to work better in treatment of wastewater.

This thesis mainly focuses on nutrients absorption by algae from municipal wastewater.

3 Methods and materials

In the beginning wastewater was brought from the wastewater treatment plant Vinikanlahti, Tampere and then it was then poured in PBR(photo bioreactor bottles) to approximately 3000 ml .Three holes were made on the cap of each PBR bottle. One hole for injection of nutrients for the growth of algae, second hole for the filtration so that the air could come out of the bottle but nothing would enter inside and the third hole for the aeration of wastewater.

3.1 Transferring mixture of algae in PBR bottles and labelling them.

The second stage was to transfer the algae and heavy metal (in our case Ni or Cu) inside the new designed bottles. We named PBR bottles as belonging to set A, B, C and D. In each set there are six bottles. Each set has 2 columns 'first and second' and each column contains 3 bottles. The three bottles on first column of each set has (wastewater + algae mixture) in it while the other three bottles on second column of each set has (wastewater+ algae mixture +heavy metal) in it. To make sure that we won't be confused while taking samples from each bottles to test for nutrients and heavy metals uptake, we labelled them as below:

TABLE 1: Labelling of bottles.

Set A		Set B		Set C		Set D	
Colm 1	Colm 2	Colm 1	Colm 2	Colm 1	Colm 2	Colm 1	Colm 2
A_{1a}	A_{2a}	B_{1a}	B_{2a}	C_{1a}	C_{2a}	D_{1a}	D_{2a}
A_{1b}	A_{2b}	B_{1b}	B_{2b}	C_{1b}	C_{2b}	D_{1b}	D_{2b}
A_{1c}	A_{2c}	B_{1c}	B_{2c}	C_{1c}	C_{2c}	D_{1c}	D_{2c}

In the table above A_{1a}, A_{1b}…….. D_{2c} represent the labelling of the bottles.

The project was carried out at TAMK's green house building .There were already algae species cultivated in separate 500 ml of bottles. These algae species were provided by Finnish Environment Institute (SYKE) to TAMK. These 12 species of algae were:

TABLE 2: Algae species cultivated in 500 ml bottles provided by SYKE

Bottle (500 ml)	Algae Species with their phylum name
SP1	Selenastrum capricomutum (Chlorophyta)
SP2	Pediastrum simplex (Chlorophyta)
SP4	Anabaena cylindrical (Cyanophyta)
SP6	Scenedesmus sp.(Chlorophyta)
SP7	Chlorphyta sp (Cyanophyta)
SP8	Purpuraemus sp.(No information)
SP10	Haematococcus (Chlorophyta)
SP11	Planktothrix rubescence (Cyanophyta)
SP12	Chlorella pyrenoidosa –(Chlorophyta)
SP13	Desmodesmus subspicatus (Chlorophyta)
SP14	Golekinia brevispicula (Chlorophyta)
SP15	Crucigenia tetrapedia – (Chlorophyta).

We can see from the above table that most of the algae species provided are green algae (chlorophyta) and some blue green algae(cyanobacteria) with phylum name cyanophyta.

From earlier experiments carried out on wastewater treatment at TAMK, it had already been known that mixture of algae species work better than the single species in wastewater treatment so based on the same principle we transferred mixture of different algae species in each set of PBR bottles. The figure below gives a clear picture about which algae species was transferred to which set of PBR bottles.

SP1.Selenastrumcaprico-mutum	Set A
SP2. Pediastrum simplex	
SP4. Anabaena cylindrical	
SP6. Scenedesmus sp.	
SP7.Chlorphyta sp (Pekari Strain)	
SP8 .Purpuraemus sp.	
SP10. Haematococcus	
SP11. Planktothrix rubescence	
SP12. Chlorella pyrenoidosa – Green algae	
SP13.Desmodesmus subspi-catus	
SP14. Golekinia brevispicula	
SP15. Crucigenia tetrapedia	

SP1.Selenastrum caprico-mutum	Set B
SP2.Pediastrum simplex	
SP6.Scenedesmus sp.	
SP10.Haematococcus	

SP4.Anabaena cylindrical	Set C
SP6.Scenedesmus sp.	

SP4.Anabaena cylindrical	Set D
SP11.Planktothrix rubescence	

FIGURE 1: Representation of mixture of algae used in different set of bottles.

IMAGE 1: The setup of the experiment at greenhouse TAMK.

The image above shows the experiment set up at greenhouse TAMK. The first six bottles of set A starting from left contain all mixture of alga species(most green +some blue green algae) ,second six bottles of set B contain mixture of algae species SP1,SP2,SP6 and SP10(all green algae). The third six bottles of set C contain mixture of algae species SP4 and SP 6(green + blue green algae) and the last six bottles of set D at far right corner contain algae species SP4 and SP11(blue green algae).

It was quite challenging to transfer mixture of different algae species in different set of bottles. For first six bottles in set A to obtain mixture of algae species, 50 ml of each algae species from 12 cultivated bottles was collected in a separate 600 ml bottle and out of that 100ml (600/6) was transferred to each six bottles make sure mixture of algae was transferred equally in all six bottles. Similarly for second six bottles of set B, 50ml of 4 algae species was first taken and diluted with 400 ml of water to make the total volume 600ml and again 100 ml was transferred to each six bottles. Likewise to obtain the algae mixture for third set of six bottles in set C, 50 ml of 2 algae species was taken and diluted with 500 ml of water to make the total volume 600 ml and 100 ml was then transferred to six bottles. For the last six bottles in set D, the procedure was exactly same as for the six bottles in set C; only the difference was the mixture of algae used.

The whole project was divided into two test run or experiments. Both the experiments done were exactly the same ; we tested nutrients and heavy metal uptake by algae from

wastewater .The only difference was in first test run Ni was used as testing metal and in the second test run Cu was used as testing metal.

The Ni or Cu concentration to be used in wastewater was 30 mg/L based on the previous experiment done at TAMK (Benchraka, 2010) .Since each bottle were filled with 3 litres of wastewater we had to add 90 mg of Ni or Cu to make the concentration 30mg/L. Clean 100 ml plastic pipe lets were used to add the metal concentration.

3.2 Total Nitrogen analysis

Total Nitrogen analysis was done by using total nitrogen kit by HAACh LANGE. The kit is named as total nitrogen 138 kit. The range for the total nitrogen analysis is (0-16) mg/L. This Haach method of analysing total nitrogen is quite simple and the procedure is very clearly given in the pamphlet. There are two main stages digestion which takes one hour and then reading the sample in Haach reader.

3.3 Total Nitrate Nitrogen (NO_3^- -N) analysis

Total nitrate nitrogen test was done by Cadmium Reduction Method 8039. Later Haach reader was used to get the readings .The range for measurement according to the standard was 0.3-30.0 mg/L NO_3^- -N. Every time we tested for Total Nitrate Nitrogen we did two test for each sample to make sure that the readings would match close to each other. For somehow if the readings were not close then we had to test third time and make sure we get the correct readings.

3.4 Total Orthophosphate (PO_4^{3-}) analysis

In the similar way, we also tested the reactive Orthophosphate (PO_4^{3-}).The test was done according to the PhosVer 3(Ascorbic Acid) Method[1] and the reading was done in Haach reader. The measuring range for orthophosphate is 0, 2 to 2, 50 mg/L PO_4^{3-} .

3.5 pH, temperature and conductivity measurement

PH, temperature and conductivity was measured using compact meter named as ‘Five Easy PH meter’ and ‘Five Easy Conductivity meter’ made by company ‘Mettler Toledo’. Both pH and conductivity meters are an electrical devices which consist of a probe also known as glass electrode which is connected to the compact electric meter that measures and displays pH and conductivity readings. The probe is a cylindrical rod like structure made of glass and at the bottom of the probe there is a bulb. The bulb is very sensitive and consists of the sensor that detects the pH or conductivity of the solution. The probe has to be dipped into the solution to measure pH or conductivity. The temperature of the solution is also displayed when measuring pH or conductivity.

Both pH and conductivity meters were first calibrated before use and the method to calibrate them could be found in the pamphlet that come with the meters. PH and conductivity was measured every time we did the nutrients analysis.

IMAGE 2: Five Easy PH and Conductivity meter (Aa6opa, 2015)

3.6 Light measurement

The light or illuminance was measured using lux meter which measured the incoming light in all the algae bottles and it could measure the light up to 1 square meter of area. The experiment was carried out in winter so in some days there was no sunlight. Sodium lights were used in the green house to provide light for the growing algae and these lights were automatic and they would shut down automatically in the evening until next morning.

3.7 Harvesting of algae

The harvesting of the algae was done at the end of each experiments. From the beginning of the experiment until the start of harvesting, growth of the algae and their ability to remove excess nutrients from wastewater was observed by testing the nutrients content in wastewater twice in every week.

Harvesting the algae at the end was necessary to determine how much nutrients and metal algae have absorbed at the end of the experiment. The first step was to take all the wastewater from PBR algae bottle in 45 ml of centrifuge tubes and centrifuge to remove all the water from it for drying. We emptied one algae bottle at a time. The remaining solid mass in centrifuge tube was then taken out with the help of a spoon into a porcelain cup. There was always a small amount of algae left in the centrifuge bottle so ethanol was used to remove that.as ethanol evaporates much faster than water when drying.

Before transferring the algae from centrifuge tubes to the porcelain cups, the porcelain cups were first weighted. This was done by placing the cups in an oven for 4 hours at 100 degree Celsius. After that, they were cooled into a desiccators for half an hour and dry weight of those cups were taken in a weighting machine. Immediately after the weight of dry porcelain cups were taken, algae was transferred to the cup from centrifuge tube and weight of wet algae and cup was recorded.
The cups with wet algae biomass were then again placed into oven at 55 degree Celsius for 24 hours for drying. After 24 hours of drying the porcelain cups with dry algae biomass were again weighted.

IMAGE 3: Porcelain cups with wet algae biomass in oven for drying.

As mentioned earlier the main aim of harvesting algae was to determine nutrients and heavy metals left in algae biomass at the end of experiment but unfortunately the exact method to test nutrients absorption in biomass was not found. Regarding metal absorption in biomass and the process of its extraction is mentioned in Adedayo Bello thesis work, 2015.

4 Results and discussions

4.1 Orthophosphate(PO_4^{3-}) results.

TABLE 3: PO_4^{3-} concentration(mg/l) in different PBR algae bottles with its removal percent in the first test run.

SET A	Initial readig	After 4th day	After 6th day	After 13th day	After 17th day	% Removal
Blank(mg/l)	19,35	29,05	28,47	25,03	28,43	
Metal(mg/l)	19,35	38,07	33,73	31,13	34,47	
SET B						
Blank(mg/l)	10,50	5,77	3,87	3,90	3,90	62,85
Metal(mg/l)	10,50	8,43	8,27	7,33	7,43	29,23
SET C						
Blank(mg/l)	3,65	1,28	1,73	2,27	1,97	46,02
Meta(mg/l)	3,65	1,58	0,97	1,70	1,50	58,90
SET D						
Blank(mg/l)	8,75	0,90	0,83	1,77	1,28	85,37
Metal(mg/l)	8,75	1,53	1,23	1,47	1,15	86,85

The above table 3 shows the orthophosphate concentration in wastewater measured in different days and its removal percent by algae for both the blank and metal samples in different sets. Here in this table and the following tables in other sections, ‘blank’ word denotes the PBR algae bottle with (wastewater+algae+nutrients) in it and ‘metal’ word denotes the PBR algae bottle with (wastewater+algae+nutrients+heavymetal) in it.

In the above table and the following tables below that represent the first test run , initial reading of PO_4^{3-} , NO_3^-N and TN in wastewater is taken from initial reading of second test run. This is done because in second test run’ initial reading’ was taken after several mixture of algae species were added in the wastewater while in first test run we took the initial reading of wastewater before any mixture of algae species were added which can be seen in appendix 1. Since same mixture of algae species were added in wastewater in both test run ,we suppose it is worth taking initial reading of wastewater after different mixture of algae species were added.

It can be noticed that there was no PO_4^{3-} removal in set A while in set B, C and D removal of PO_4^{3-} was on average(including blank +metal) above 45% , 50% and 85% respectively. Cyanobacteria species in set D were able to absorb more phosphate than other algae species. Likewise, removal percent of PO_4^{3-} is seen more in 'blank' bottle than in the' metal' bottle.

TABLE 4: PO_4^{3-} concentration(mg/l) in different PBR algae bottles with its removal percent in second test run.

SET A	Initial reading	After 2nd day	After 6th day	After 9th day	%Removal
Blank(mg/l)	19,35	4,38	5,05	3,77	80,53
Metal(mg/l)	19,35	6,95	6,48	4,17	78,47
Set B					
Blank(mg/l)	10,50	1,48	1,80	1,28	87,78
Metal(mg/l)	10,50	3,00	1,48	1,80	82,86
SET C					
Blank(mg/l)	3,65	1,75	1,27	0,68	81,28
Metal(mg/l)	3,65	1,83	1,05	1,30	64,38
SET D					
Blank(mg/l)	8,75	1,63	0,87	0,58	93,33
Metal(mg/l)	8,75	1,87	0,85	0,98	88,76

The table 4 above is also an illustration of orthophosphate concentration in wastewater contained in different algae bottle sets. It can be seen that there was an average 81 %, 85%,73% and 91% phosphate removal from wastewater in both blank and metal samples of set A,B C and D respectively.

In second test run, the absorption rate of phosphate from wastewater by different algae species is much higher compared to the first test run and also blue green algae species in set D have shown the highest phosphate removal from wastewater. Metal concentration in wastewater also have affected nutrient absorbing capacity of different algae in different bottle sets. PO_4^{3-} is more removed in 'blank' bottles than in bottles with' metal' in them.

4.2 Nitrate nitrogen(NO_3^-N) results.

TABLE 5: NO_3^-N concentration(mg/l) in different PBR algae bottle with its removal percent in first test run.

SET A	Initial reading	After 4th day	After 6th day	After 13th day	After 17th day	% Removal
Blank(mg/l)	36,50	74,83	53,83	48,17	21,33	41,56
Metal(mg/l)	36,50	67,83	60,83	73,33	37,00	
SETB						
Blank(mg/l)	22,0	21,00	31,67	27,33	7,02	68,09
Metal(mg/l)	22,0	21,83	27,50	27,83	15,17	31,04
SET C						
Blank(mg/l)	18,50	20,67	21,83	24,83	4,17	77,45
Metal(mg/l)	18,50	13,67	21,50	23,83	5,03	72,81
SETD						
Blank(mg/l)	17,00	18	22,5	15,5	5,6	67,05
Metal(mg/l)	17,00	13,17	10,83	8,83	12,37	27,23

The above table 5 shows the NO_3^-N measured in wastewater in different days and its removal percent. We can clearly see that much of the NO_3^-N from wastewater from set B, C and D was removed. On an average (including both blank and metal)49%,75% and 47% nitrate nitrogen was removed from wastewater contained in set B,C and D respectively. 'Blank' sample in set A was able to perform better than 'metal' sample with above 40 % removal of NO_3^-N. In similar way, 'blank' sample bottles in other sets also have performed better than metal sample bottles.

TABLE 6: NO_3^-N concentration(mg/l) in different PBR algae bottle with its removal percent in second test run.

SET A	Initial reading	After 2nd day	After 6th day	After 9th day	% Removal
Blank(mg/l)	36,50	42,33	63,50	37,50	
Meta(mg/l)l	36,50	48,17	57,83	50,83	
SET B					% Removal
Blank(mg/l)	22,00	28,67	39,67	42,50	
Metal(mg/l)	22,00	39,17	42,33	35,83	
SET C					% Removal
Blank(mg/l)	18,50	29,83	37,33	35,33	
Metal(mg/l)	18,50	42,00	30,67	28,17	
SET D					% Removal
Blank(mg/l)	17,00	16,67	35,00	20,83	
Metal(mg/l)	17,00	47,50	32,17	39,83	

The table 7 above illustrates the measurement dates and nitrate concentration in wastewater algae bottles. Nitrate amount actually increased in the second test run at the end. There have been several fluctuations like increase and decrease in readings in different days of reading. It is very hard to conclude exactly why this reading was observed.

4.3 Total Nitrogen(TN) results.

TABLE 7: TN concentration(mg/l) in different PBR algae bottle with its removal percent in first test run.

Set A	Initial reading	After 4th day	After 6th day	After 13th day	After 17th day	% Removal
Blank(mg/l)	90,00	58,23	89,27	70,27	38,13	57,63
Metal(mg/l)	90,00	64,20	125,00	85,03	47,63	47,07
Set B						
Blank(mg/l)	54,80	46,13	40,77	35,60	30,83	43,74
Metal(mg/l)	54,80	65,47	59,53	44,50	36,63	33,15
Set C						
Blank(mg/l)	48,70	31,56	26,4	20,36	20,7	57,49
Metal(mg/l)	48,70	31,41	43,9	25,76	20,8	57,28
Set D						
Blank(mg/l)	45,50	25,30	24,30	14,80	11,07	75,67
Metal(mg/l)	45,50	33,37	47,03	39,60	29,27	35,67

The table 7 above shows the TN concentration in wastewater measured in different dates in first test run. There was an average(including both blank and metal) 52 %, 38%, 57%, and 55% of TN removal from wastewater in algae bottle set A, B, C and D respectively. We can see that blank samples without metals have done pretty well in removing TN from wastewater .Blue green species in set D seem to be affected more with presence of metal in wastewater as there is a significant difference in removal percent of TN in blank and metal sample in set D. The same ‘blue green algae species’ in set D have also the highest TN removal if we compare the blank sample bottle with other sets.

TABLE 8: TN Concentration(mg/l) in different PBR algae bottle with its removal percent in second test run.

SET A	Initial reading	After 2nd day	After 6th day	After 9th day	% Removal
Blank(mg/l)	90,00	75,53	70,33	65,83	26,85
Metal(mg/l)	90,00	77,80	79,03	63,60	29,33
SET B					
Blank(mg/l)	54,80	34,53	35,90	36,77	32,91
Metal(mg/l)	54,80	43,77	42,63	40,97	25,24
SET C					
Blank(mg/l)	48,70	35,97	39,27	36,50	25,05
Metal(mg/l)	48,70	36,97	24,83	35,23	27,65
SET D					
Blank(mg/l)	45,50	35,83	37,83	34,87	23,37
Metal(mg/l)	45,50	40,73	30,40	44,27	2,71

The table 8 above shows the TN concentration in wastewater measured in different days in second test run. There was an average(including both blank and metal) 28 %, 29%, 26%, and 13% of TN removal from wastewater in algae bottle set A, B, C and D respectively.

By having a close look at the table above we can see again that TN removal was better in ‘blank’ sample than in the ‘metal’ sample. TN removal is not that much higher than it was seen in the first test run. But overall, absorption of nutrient nitrogen was seen in all algae bottles.

4.4 TN/TP ratio

TABLE 9: Nitrogen to phosphorus mass ratio in First test run.

SET A	Initial N/P ratio	After 4th day	After 6th day	After 13th day	After 17th day
Blank	14,25	6,14	9,61	8,60	4,11
Metal	14,25	5,17	11,36	8,37	4,23
SET B					
Blank	15,99	24,50	32,28	27,97	24,23
Metal	15,99	23,80	22,06	18,60	15,11
SET C					
Blank	40,89	75,56	46,76	27,49	32,20
Metal	40,89	60,92	138,69	46,44	42,49
SET D					
Blank	15,94	86,15	89,72	25,62	26,50
Metal	15,94	66,84	117,17	82,55	78,00

The table 9 above is the representation of nitrogen to phosphorous mass ratio in each set of wastewater in different days in first test run.

TN/TP ratio is helpful in determining which of the nutrient(nitrogen or phosphorous) is the limiting factor for algae growth in water. Study suggest that when TN/TP ratio is less than 10, a lake is nitrogen limited, when TN/TP ratio is between 10-17 each of the nutrient either nitrogen or phosphorous might be limited and if TN/TP ratio is greater than 17 phosphorous is the limiting factor (Florida, 2015). In many cases phosphorous threshold values also help to determine which nutrient is limited. In lakes where TP concentrations is above 0,1mg/L there is a chance that nitrogen might be the limiting factor rather than phosphorous and their TN/TP ratio is generally less than 17. In lakes where TP concentration is less than 0,05mg/ , phosphorous is the limiting factor (Florida, 2015)

As we can see from the table that ,TN/TP ratio for algae bottle sets B,C and D is usually greater than 17 which shows that phosphorous was the limiting nutrient in algae growth while in set A TN/TP ratio is below 10 (excluding initial ratio TN/TP>10) , nitrogen was the limiting nutrient.

TN/TP ratio is not only helpful to know which of this nutrient is the limiting factor in growth of algae or any phytoplankton in waterbody but also helpful in predicting if blue green algae are growing more and dominating the green algae or vice versa. At lower

TN/TP ratio usually below 22 or 30, blue green algae also known as cyanobacteria start to increase in number by absorbing most of the nutrients .Although in this situations nitrogen can be limited but some cyanobacteria are able to utilize dissolve nitrogen gas .They have competitive advantage when nitrogen is limited and phosphorous is not limiting. Nitrogen fixing cyanobacteria such as *Anabeca spp* and *Aphanzomenon flos-aquae* are found to be more radially present in lakes with TN/TP ratio less than 30:1 or 22:1. (Schaedel, 2011).

In set 'A' which is a mixture of green algae and blue green algae TN/TP ratio is far below 22 which shows that blue green algae might have increased in number and grown well than green algae species. It could be assumed that the competition between these two algae species might be the reason for no PO_4^{3-} removal in set A. Likewise, TN/TP ratio for blank sample in set D on the 13th and 17th day of measurement is below 30 so this might have caused the increase in blue green algae population. In the same set D when we see the metal sample ,TN/TP ratio is greater than 30 which might have affected the blue green algae to grow well. This might be the reason why we saw more nutrients removal in blank sample than in metal sample of set D.

TABLE 10 : Nitrogen to Phosphorus ratio in Second test run.

SET A	Initial N/P ratio	After 2nd day	After 6th day	After 9th day
Blank	14,25	52,85	42,68	53,51
Metal	14,25	34,30	37,37	46,74
SET B				
Blank	15,99	71,50	61,12	88,03
Metal	15,99	44,71	88,27	69,75
SET C				
Blank	40,89	62,99	94,76	164,49
Metal	40,89	61,91	72,47	83,05
SET D				
Blank	15,94	67,36	133,25	184,24
Metal	15,94	66,75	109,60	138,43

The table above is the representation of nitrogen to phosphorous ratio in each set of wastewater in second test run in different days of measurement. We can see that TN/TP ratio is above 17 in all sets of wastewater which shows that phosphorous was the limiting factor for algae growth. It is hard to relate TN/TP ratio with nutrient removal results in

second test run. TN/TP ratio suggest that phosphorous was the limiting nutrient and nitrogen was readily available but there has been no nitrate nitrogen (NO_{3}- N)removal and not much TN removal but much of the orthophosphate was removed from wastewater.

4.5 pH, Temperature and Conductivity

FIGURE 2: Variation of pH during the Nickel test run.

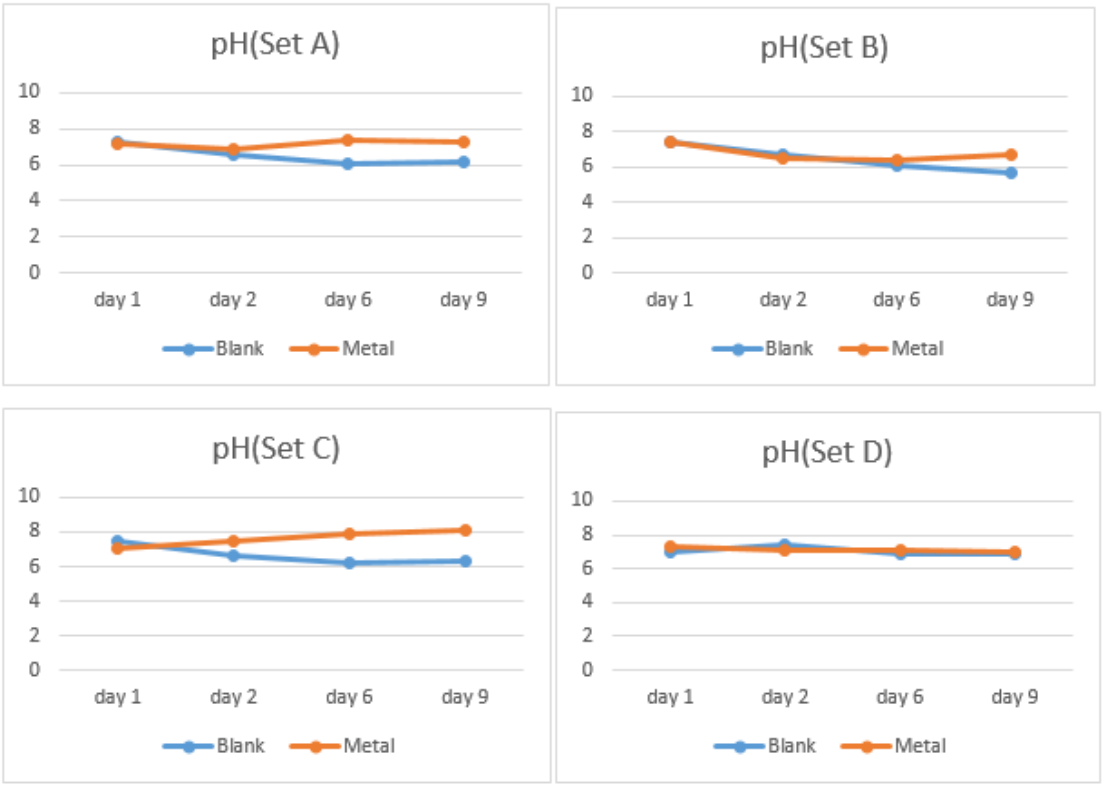

FIGURE 3: Variation of pH during the Copper test run.

We can see from the above figure '2' and '3' that pH remained on the range of 6-8 which has favoured the growth of algae.

Temperature on the other hand was within the range of (18-22)ºC in first test run and in the second test run within the range of (15-22)ºC.

pH and temperature play a very important role in the growth of phytoplankton in any water body. The information provided by FAO (Food and Agriculture Organization of the United Nations) says that the optimum pH for the growth of phytoplankton is believed to be from 8,2 - 8,7 when cultured while most of the cultured algae species would do fine or grow in pH between 7-9 ((FAO,Fisheries and aquaculture department). Some studies have revealed that at pH 7 ,algae growth is increased and maximum amount of heavy metal and nutrients(phosphorous and nitrogen) are removed from wastewater. In studies done with marine macro-algae *Caulerpa taxifolia* and red algae *Kappaphycus alvarezii* to test the heavy metal and nutrient absorption in wastewater , it has been found that at pH 7 maximum amount of heavy metal zinc and nutrients phosphorous and nitrogen were removed from wastewater (R.Mithra, 2012).

Regarding temperature, the optimum temperature for many cultured species of algae ranges from 20 to 24 ºC. It is also believed that many cultured species of micro algae will tolerate the temperature from (16 -27)ºC. Below 16 ºC the growth rate slows down and above 35 ºC many algae species may start to die (FAO,Fisheries and aquaculture department) .

In another case study done on blue green algae in Lake Mendota, Wisconsin it was found that the optimum temperature for photosynthesis by blue green algae was usually between 20 and 30 degree Celsius (Allan Konopka, 1978). Recent news published in 'Biotechniques ,the international Journal of life Science methods' , suggest that some red algae species like *Galidieria sulphuraria* could be able to survive in extreme environment like hot sulphur springs. They are able to tolerate extreme pH and temperature. These algae species are able to survive this extreme conditions by borrowing genes from bacteria and archaea that protect them from unbearable heat and toxic conditions (Chi, 2013).

No extreme temperature or pH was observed that could have harmed the growth of algae in both the test run. Somewhere in second test run the temperature of wastewater in different algae bottles have also dropped below 16 ºC when outside temperature was also very low but it has remained usually above 18 ºC in many measurements.

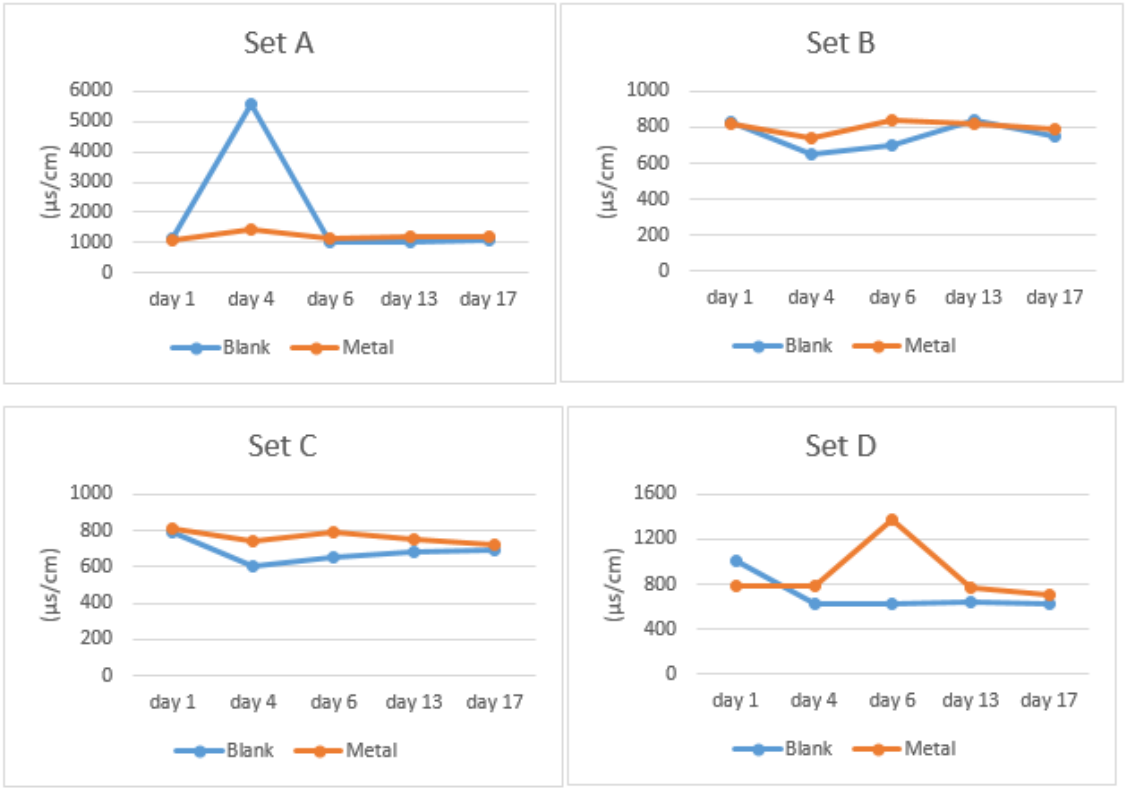

FIGURE 4: Variation of conductivity during the Nickel test run.

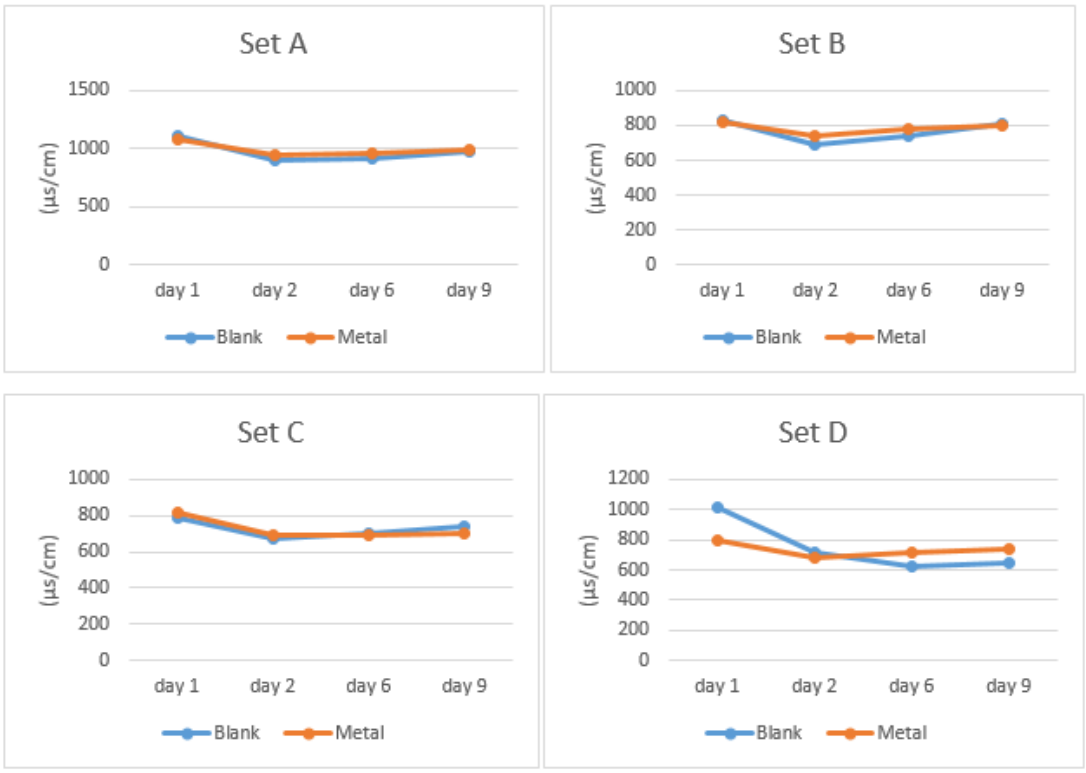

FIGURE 5: Variation of conductivity during the Copper test run.

The above figure ‘4’ and ‘5’ represent the variation of conductivity in wastewater during nickel and copper test run respectively. We can see that wastewater which has metal have higher conductivity than the wastewater in blank sample in most of the sets. A decrease in conductivity can also be seen in many algae bottle sets in both the test run which also indicates that with absorption of nutrients by algae from water, conductivity of water has also decreased. In set ‘A’ conductivity values were higher because it had more algae species than other sets so more nutrients were available in the wastewater from the beginning that gave up higher conductivity values.

Conductivity is one another important parameters that measures water quality. Conductivity is the early indicator that show the change in water system . (Christine, 2014). It measures the dissolved ions in the water through which water can conduct electricity. Dissolved ions in water come from various sources like dissolve salts, inorganic ions like chlorides, sulphides, alkalis, carbonate compounds, metal ions like zinc, copper, nickel, lead ,mercury, silica, inorganic nitrate ,phosphate and many more . A typical freshwater body might have conductivity from 100-2000(µs/cm), industrial wastewater (10000 µs/cm), sea water (55000 µs/cm), distilled water (0,5-3 µs/cm) (Christine, 2014) .Conductivity and temperature are directly related. With the increase in temperature conductivity of water also increases as mobility of ions increases as well as dissolving capacity of many salts and minerals increases. Conductivity of any waterbody can go up in the day time when temperature is warmer due to sunlight and go low at night when temperature is cool (Christine, 2014).

5 Conclusions

From the results of both test run, it can be seen that ‘Set D’ which is a mixture of blue green algae have proved to work better in removing most of the nutrients from wastewater. This also tells us that if blue green algae are grown under controlled conditions (monitoring their growth and toxicity) to purify wastewater they can be very useful. Similarly, presence of metal in wastewater have also affected the nutrient absorbing capacity of different algae. Unfortunately, due to limited time we were not able to do more research on how metal binding on algae cells affect their nutrient absorption property. Further research on how metal binding on algae affect their ability to absorb nutrients is necessary.

Parameters like pH(6-8) and temperature(16-22)ºC in this project were in favour of algae growth. TN/TP ratio results on the other hand was helpful in predicting which nutrient was limiting factor for algae but did not provide sufficient information on how it affected growth of several mixtures of algae. More research is needed to exactly know how TN/TP ratio affects the growth of several algae species .

Nutrient absorption in algae biomass was not analysed. This could have provided more detailed information on how much nutrients was absorbed by different algae species in different sets of wastewater bottles.

Overall, this project has revealed that algae are definitely able to absorb nutrients from wastewater and blue green algae were able to perform better than green algae .It can be concluded that further research would be useful to test several blue green algae species in treatment of wastewater.

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7 Appendices

Appendix 1: Initial Wastewater Nutrients and Heavy Metal (Ni) Readings (first test run)

Date	PH	conductivity (µs/cm)	Temperature ºC	NO_3^--N (mg/L)	PO_4^{3-} (mg/L)	TN (mg/L)	Nickel (mg/L)
6.2.2015	7,2	1073	22,1	21	3,2	60,76	0

Appendix 2: Mesurement on 10.2.2015(first test run)

Sample	PO_4^{3-} (mg/l)	Av. PO_4^{3-} (mg/l)	NO_3^- N(mg/l)	Av. NO_3^- N(mg/l)	TN(mg/l)	pH	Conductivity (µs/cm)	Temp°C
	31,1		60					
A1a	32,2	31,65	59	59,5	28,4	7,86	5580	22
	19,6		88					
A1b	20,8	20,2	86	87	53,5	6,76	5581	22,3
	38,4		78					
A1c	32,2	35,3	73	75,5	92,8	7,19	5582	23,3
	42,7		85					
A2a	42,7	42,7	83	84	86	7,59	2360	21,9
	50		89					
A2b	50	50	67	78	49,3	7,87	1030	21,4
	21,5		42					
A2C	21,5	21,5	41	41,5	57,3	7,4	940	22,1
	4,5		19					
B1a	4,5	4,5	18	18,5	37,9	7,51	641	21,6
	5,2		13					
B1b	5	5,1	12	12,5	53,4	7,59	706	21,3
	7,6		33					
B1c	7,8	7,7	31	32	47,1	7,29	609	23,6
	10		24					
B2a	10,9	10,45	22	23	66,9	7,55	853	21,7
	9,8		24					
B2b	10,1	9,95	21	22,5	82,3	8,09	705	21,1
	4,9		20					
B2c	4,9	4,9	20	20	47,2	8,04	647	21,2
	0,7		16					
C1a	0,6	0,65	17	16,5	35,6	7,97	624	22
	1,9		18					
C1b	2,1	2	16	17	31,3	7,93	620	21,7
	1,3		29					
C1c	1,1	1,2	28	28,5	27,8	8	560	21,4
	2,7		28					
C2a	2,6	2,65	27	27,5	47,1	7,56	736	22,2
	0,7		10					
C2b	0,5	0,6	10	10	38,8	7,7	712	21,4
C2c	1,4	1,45	13	12,5	8,34	7,82	779	22,2

	1,5		12					
	1		19					
D1a	1,1	1,05	18	18,5	24,6	7,83	634	21,9
	1		19					
D1b	0,9	0,95	17	18	33,3	7,76	611	21,2
	0,7		18					
D1c	0,7	0,7	17	17,5	18	7,79	614	21,4
	1,5		12					
D2a	1,4	1,45	11	11,5	35,8	7,81	801	22,3
	1,4		14					
D2b	1,4	1,4	13	13,5	32,3		788	21
	1,7		15					
D2c	1,7	1,7	14	14,5	32		779	20,5

Appendix 3: Measurement on 12.02.2015 (first test run)

Samples	PO_4^{3-} (mg/l)	Av. PO_4^{3-} (mg/l)	(NO_3^- N)(mg/l)	Av. (mg/l)(NO_3^- N)	TN(mg/l)	pH	Conduc-tivity(µs/cm)	Temp° C
	34,2		51					
A1a	33,1	33,7	53	52	97,2	6,47	982	20,6
	16,6		48					
A1b	16,4	16,5	53	50,5	74,4	6,39	966	20,8
	35,6		50					
A1c	34,8	35,2	68	59	96,2	6,33	1032	21
	40,3		61					
A2a	39,9	40,1	72	66,5	141	8,02	1310	20,3
	24		59					
A2b	24,1	24,1	69	64	117	7,94	1140	20
	37		59					
A2C	37	37	45	52	117	8,01	1030	19,9
	2,5		27					
B1a	2,2	2,4	32	29,5	37,5	7,74	693	20,4
	1,4		34					
B1b	1,4	1,4	27	30,5	38,5	7,69	689	20,4
	7,6		31					
B1c	8	7,8	39	35	46,3	6,92	724	20,6
	9,6		27					
B2a	9,7	9,7	29	28	72,6	8,17	914	20,3
	10,9		26					
B2b	10,8	10,9	28	27	68,3	8,23	903	20
	4,2		27					
B2c	4,1	4,2	28	27,5	37,7	7,99	694	20,4
	3		19					
C1a	5	4	20	19,5	29,3	7,57	642	20,8
	0,5		24					
C1b	0,7	0,6	22	23	29,7	7,59	635	20,4
	0,6		23					
C1c	0,5	0,6	23	23	20,2	7,44	677	21
	1,2		28					
C2a	1,4	1,3	30	29	50,2	7,83	769	20,6
	0,3		15					
C2b	0,5	0,4	15	15	42,1	8,02	771	20,3
	1		21					
C2c	1,3	1,2	20	20,5	39,4	7,98	844	20,8
	0,9		21					
D1a	0,8	0,9	19	20	24,5	7,76	631	20,7
	0,8		20					
D1b	0,7	0,8	26	23	24,9	7,78	618	21
	0,9		28					
D1c	0,6	0,8	21	24,5	23,5	7,65	613	21

	1		9					
D2a	1,5	1,3	10	9,5	46,9	8,22	2190	19,5
	1,1		10					
D2b	1,3	1,2	9	9,5	45,7	8,22	821	19,8
	1,6		13					
D2c	0,8	1,2	14	13,5	48,5	8,12	1118	19,6

Appendix 4: Measurement on 16.02.2015(first test run)

Samples	**pH**	**Conductivity(µs/cm)**	**Temp°C**
A1a	6,28	10004	18,5
A1b	6,81	964	18,3
A1c	5,92	1294	18,5
A2a	7,25	1295	18,5
A2b	6,99	1131	18,4
A2C	7,73	983	18,5
B1a	7,47	717	18,5
B1b	7,61	656	17,9
B1c	7,57	752	18,4
B2a	8,12	905	18,7
B2b	7,96	864	17,9
B2c	7,53	690	18,4
C1a	7,67	665	18,7
C1b	7,7	634	18,1
C1c	7,56	699	19,3
C2a	7,9	753	18,7
C2b	8,12	737	18,1
C2c	7,99	793	18,9
D1a	7,87	638	18,4
D1b	7,98	613	17,3
D1c	7,83	623	18,6
D2a	8,1	786	18,6
D2b	8,13	775	17,8
D2c	8,06	774	18,4

Appendix 5: Measurement on 19.2.2015(first test run)

Sample	PO_4^{3-} (mg/l)	Av. PO_4^{3-} (mg/l)	NO_3^- N(mg/l)	Av. NO_3^- N(mg/l)	TN(mg/l)	pH	Conduc-tivity(µs/cm)	Temp °C
	30,4		50					
A1a	30,5	30,5	45	47,5	63	5,95	1022	20,9
	11,5		40					
A1b	12,1	11,8	50	45	64,3	6,91	972	20,9
	32,5		51					
A1c	33,1	32,8	53	52	83,5	6,01	1060	21,4
	40,8		74					
A2a	41,1	41	65	69,5	80,8	7,74	1302	21,2
	26,2		106					
A2b	26,2	26,2	70	88	89,3	6,83	1209	20,6
	26,4		61					
A2C	26	26,2	64	62,5	85	6,94	985	21
	3,6		33					
B1a	3,3	3,5	28	30,5	40,3	7,93	901	20,8
	2,6		26					
B1b	2,8	2,7	27	26,5	34	7,97	864	20,4
	5,4		25					
B1c	5,5	5,5	25	25	32,5	7,86	748	20,8
	10,2		23					
B2a	10,2	10,2	22	22,5	51,6	8,04	900	20,9
	9,9		32					
B2b	10,1	10	40	36	48,3	8	868	20,5
	1,8		24					
B2c	1,7	1,8	26	25	33,6	8,01	693	20,9
	1,5		26					
C1a	1,7	1,6	26	26	25,7	8,04	671	21,1
	1,8		20					
C1b	1,6	1,7	21	20,5	13,6	8,1	642	20,8
	3,4		26					
C1c	3,6	3,5	30	28	21,8	7,9	734	21,3
	0,9		49					
C2a	0,9	0,9	52	50,5	28,1	8,27	732	21
	2,3		11					
C2b	2,2	2,3	9	10	26,3	8,1	764	20,9
	1,8		11					
C2c	1,9	1,9	11	11	22,9	7,83	775	21,2
	1,3		17					
D1a	1,1	1,2	18	17,5	12,5	8,08	643	21,2
	1,9		16					
D1b	2,1	2	16	16	16,6	8,06	626	20,9
	2		14					
D1c	2,1	2,1	12	13	15,3	8,07	629	21,3
	1,8		9					
D2a	1,8	1,8	13	10,5	39,7	7,97	789	21
D2b	1,2	1,2	9	8	46,3	7,93	787	21

	1,2		7					
	1,4		8					
D2c	1,3	1,4	8	8	32,8	7,99	750	21,2

Appendix 6: Measurement on 23.2.2015(first test run)

Sample	(mg/l) PO_4^{3-}	(mg/l) Av. PO_4^{3-}	(mg/l) (NO_3^- N	Av.(mg/l) NO_3^-N	(mg/l) TN	pH	Conduc-tivity(µs7cm)	Temp °C
	32,8		24					
A1a	34,6	33,7	14	19	43	6,14	1133	20,9
	15,2		20					
A1b	15,2	15,2	14	17	28,4	7,16	989	21,3
	37,2		28					
A1c	35,6	36,4	28	28	43	6,25	1087	22,1
	46,2		36					
A2a	54,2	50,2	30	33	52	7,55	1320	20,8
	30		50					
A2b	29,2	29,6	36	43	51,2	6,34	1270	20,9
	23,6		36					
A2C	23,6	23,6	34	35	39,7	6,5	1019	21,6
	4		9					
B1a	3,7	3,85	9	9	28,9	7,68	731	20,8
	4		1,1					
B1b	3,8	3,9	7	4,05	30,1	7,64	729	20,9
	3,9		9					
B1c	4	3,95	7	8	33,5	8,16	799	21,9
	10,5		7					
B2a	10,4	10,45	7	7	38,2	8,18	862	20,9
	8		34					
B2b	8,1	8,05	30	32	54,4	8,26	820	20,8
	3,9		7					
B2c	3,7	3,8	6	6,5	17,3	8,4	698	21,7
	2,4		3					
C1a	2,2	2,3	6	4,5	19	8,08	678	20,9
	2		4					
C1b	1,9	1,95	3	3,5	21	8,19	634	20,8
	1,6		5					
C1c	1,7	1,65	4	4,5	22,1	8,05	767	21,6
	1,3		3,4					
C2a	1,3	1,3	3,7	3,55	18	8,1	735	21,1
	2,3		2,6					
C2b	2,3	2,3	2,6	2,6	22,3	8,09	751	21
	1		9					
C2c	0,8	0,9	8,9	8,95	22,1	8,29	671	21,5
	1		3,9					
D1a	1	1	3,7	3,8	10,9	8,4	629	20,9
	1,5		7,2					
D1b	1,5	1,5	7,4	7,3	10,4	8,38	610	20,8

	1,4		5,7					
D1c	1,3	1,35	5,7	5,7	11,9	8,42	625	21,7
	1,1		8,1					
D2a	1,3	1,2	8	8,05	32,5	8,27	722	20,6
	1		4,8					
D2b	1	1	4,5	4,65	30,7	8,24	727	21
	1,2		24,4					
D2c	1,3	1,25	24,4	24,4	24,6	8,64	651	21,7

Appendix 7: Measurement on 17.3.2015 (initial readings for second test run)

Samples	**PO_4^{3-} (mg/l)**	**Av. PO_4^{3-} (mg/l)**	**NO_3^- N(mg/l)**	**Av. NO_3^-N (mg/l)**	**TN(mg/l)**	**pH**	**Conduc-tivity (µs/cm)**	**Temp °C**
A1 Group	19,8	19,35	35	36,5	90	7,25	1116	22,3
A2 Group	18,9		36			7,12	1083	21,2
B1 Group	10,2	10,5	23	22	54,8	7,4	826	21,4
B2 Group	10,8		21			7,38	818	21
C1 Group	3,6	3,65	19	18,5	48,7	7,48	790	22,1
C2 Group	3,7		18			7	813	22,2
D1 Group	8,9	8,75	17	17	45,5	7,03	1014	21
D2 Group	8,6		17			7,27	791	22,5

Appendix 8: Measurement on 19.3.2015(second test run)

Sample	(mg/l) PO_4^{3-}	(mg/l) Av. PO_4^{3-}	(mg/l) (NO_3^-N)	Av. (mg/l) NO_3^-N	TN(mg/l)	pH	Conduc-tivity(µs/cm)	Temp°C
	5		38					
A1a	3,3	4,15	44	41	65	6,68	919	15,1
	5,1		46					
A1b	4,9	5	47	46,5	78	6,46	929	14,6
	4		39					
A1c	4	4	40	39,5	83,6	6,43	857	15,2
	10,9		53					
A2a	11,1	11	51	52	75,1	6,46	1045	14,8
	4,4		49					
A2b	4,2	4,3	52	50,5	76,4	7,29	856	14,5
	5,6		43					
A2C	5,5	5,55	41	42	81,9	6,94	951	16,1
	1,7		26					
B1a	1,9	1,8	25	25,5	32	6,83	681	16,2
	1,3		34					
B1b	1,3	1,3	34	34	39,7	6,79	708	15,6
	1,5		26					
B1c	1,2	1,35	27	26,5	31,9	6,43	678	15,9
	2,9		30					
B2a	3,6	3,25	32	31	43,3	6,43	764	15,7
	2,7		50					
B2b	2,9	2,8	51	50,5	46	6,57	703	14,9
	2,9		35					
B2c	3	2,95	37	36	42	6,4	747	16,3
	1,7		40					
C1a	1,8	1,75	41	40,5	49,3	6,47	653	16,5
	1,9		36					
C1b	1,9	1,9	36	36	33,3	6,36	682	15,7
	1,3		12					
C1c	1,9	1,6	14	13	25,3	7,06	692	16,8
	1,7		52					
C2a	1,9	1,8	53	52,5	40,9	7,13	683	17,4
	2,1		17					
C2b	1,8	1,95	16	16,5	40,2	7,9	727	16,3
	1,8		57					
C2c	1,7	1,75	57	57	29,8	7,52	675	18,2
	1,8		11					
D1a	2,1	1,95	10	10,5	30,7	7,52	794	18,2
	1,4		10					
D1b	1,4	1,4	12	11	41,9	7,87	705	17,3
	1,2		27					
D1c	1,9	1,55	30	28,5	34,9	6,71	656	17,9
	2,2		49					
D2a	1,9	2,05	48	48,5	44,9	7,43	680	18,4
D2b	2	1,95	40	40	41,7	6,9	687	17,9

	1,9		40					
	1,5		55					
D2c	1,7	1,6	53	54	35,6	6,82	682	18,5

Appendix 9: Measurement on 23.3.2015(second test run)

Samples	(mg/l) PO_4^{3-}	(mg/l) Av. PO_4^{3-}	(mg/l) (NO_3^-N)	Av.(mg/l) NO_3^- N	TN(mg/l)	pH	Conduc-tivity(µs/cm)	Temp °C
	6,5		72					
A1a	6,6	6,55	74	73	69,8	6	913	20,4
	4,9		61					
A1b	4,9	4,9	72	66,5	93,6	5,9	981	20,7
	3,7		51					
A1c	3,7	3,7	51	51	47,6	6,2	871	20,5
	8,5		66					
A2a	8,3	8,4	63	64	100	7,8	1029	20,2
	3,9		48					
A2b	3,7	3,8	49	48,5	43,7	8,2	846	20,2
	7,2		67					
A2C	7,3	7,25	55	61	93,4	6	1004	20,9
	1,7		46					
B1a	1,6	1,65	47	46,5	34,7	5,9	717	20,2
	2,2		47					
B1b	2,2	2,2	48	47,5	46,9	5,6	792	20
	1,6		25					
B1c	1,5	1,55	25	25	26,1	6,8	701	20,9
	1,6		29					
B2a	1,6	1,6	30	29,5	39	5,8	793	20
	1,5		69					
B2b	1,4	1,45	54	61,5	31,9	7,4	736	19,8
	1,4		36					
B2c	1,4	1,4	36	36	57	6,1	796	20,7
	1,2		52					
C1a	1,2	1,2	55	53,5	33,5	5,6	701	20,2
	1,2		45					
C1b	1,4	1,3	45	45	63,9	5,5	776	20,4
	1,3		14					
C1c	1,3	1,3	13	13,5	20,4	7,5	638	20,8
	1,2		39					
C2a	1,1	1,15	41	40	23,3	7,6	654	20,1
	0,8		15					
C2b	0,8	0,8	15	15	29	8,3	729	20
	1,2		38					
C2c	1,2	1,2	36	37	22,2	7,7	681	20,6
	0,8		33					
D1a	0,7	0,75	32	32,5	27,6	7,6	598	20,1
	1,2		36					
D1b	1,4	1,3	36	36	50,4	7,5	585	19,8

	0,6		38					
D1c	0,5	0,55	35	36,5	35,5	5,5	700	20,6
	1,2		36					
D2a	1,4	1,3	34	35	32,6	7	697	20,2
	0,6		25					
D2b	0,5	0,55	25	25	23	7	728	20,1
	0,6		37					
D2c	0,8	0,7	36	36,5	35,6	7,2	707	20,8

Appendix 10: Measurement on 26.3.2015(second test run)

Sample	(mg/l) PO_4^{3-}	(mg/l) Av. PO_4^{3-}	(mg/l) (NO_3^-N)	Av. (mg/l) NO_3^-N	TN(mg/l)	pH	Conduc-tivity(µs/cm)	Temp °C
	4,2		37					
A1a	5,6	4,9	41	39	57,2	6	957	20,1
	4,5		46					
A1b	4,3	4,4	43	44,5	74,9	6	1057	20
	2		29					
A1c	2	2	29	29	65,4	7	906	19,8
	4,8		45					
A2a	4,5	4,65	47	46	75,5	8	1060	20,3
	1,9		37					
A2b	2	1,95	40	38,5	42,7	8	844	19,9
	5,8		69					
A2C	6	5,9	67	68	72,6	5	1073	20,2
	1,2		37					
B1a	1,3	1,25	38	37,5	39,4	5	800	20,1
	1,5		43					
B1b	1,5	1,5	39	41	43,8	6	875	19,7
	1,1		46					
B1c	1,1	1,1	52	49	27,1	6	743	20,5
	1		25					
B2a	1,2	1,1	24	24,5	41,8	6	820	20,1
	4		39					
B2b	3	3,5	37	38	31,6	8	753	20
	0,9		45					
B2c	0,7	0,8	45	45	49,5	6	824	20,3
	0,7		35					
C1a	0,7	0,7	38	36,5	41,4	6	741	20,5
	0,8		45					
C1b	0,9	0,85	49	47	47,1	6	872	20,2
	0,6		23					
C1c	0,4	0,5	22	22,5	21	7	615	20,6
	1,1		47					
C2a	1	1,05	47	47	30	8	673	19,9
	1,8		23					
C2b	2,1	1,95	21	22	41,3	8	766	19,8
	0,9		15					
C2c	0,9	0,9	16	15,5	34,4	8	675	20,5

	0,5		18					
D1a	0,5	0,5	18	18	25,7	8	590	19,9
	0,7		13					
D1b	0,7	0,7	15	14	26,3	8	572	19,6
	0,6		31					
D1c	0,5	0,55	30	30,5	52,6	5	762	20,4
	1		49					
D2a	1	1	47	48	38	7	737	20,2
	1		35					
D2b	0,8	0,9	35	35	56,9	7	771	19,8
	1,1		34					
D2c	1	1,05	39	36,5	37,9	7	723	20,6