WASTEWATER TREATMENT USING MICROALGAE AND SUCCESSIVE BIOETHANOL GENERATION

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Abstract - *Global threats of fuel shortage in the near future and climate change due to green house gas emission and posing serious challenges and hence it is imperative to explore means for suitable ways of averting the consequences. The dual applications of microalgae for wastewater treatment and biomass production for sustainable biofuels production is a feasible option. It is mainly characterized by high levels of ammonia-nitrogen, chemical oxygen demand (COD), biological oxygen demand (BOD) and heavy metals. Microalgae biomass is considered as one of the value added feedstock as this has good potential for biofuel production. The aim of this study was to determine the ability of microalgae spirulina and chlorella vulgaris in removing the pollutants from municipal wastewater and to evaluate their potential in bioethanol generation. Study was conducted in 50%and 30% diluted wastewater. The algal biomass after treatment was harvested and tested for their usefulness in bioethanol production.*

Key Words: **Wastewater, Microalgae, Biomass, Biofuel, Bioethanol**

1. INTRODUCTION

This document is template. We ask that authors follow some simple guidelines. Industrialization and urbanization of the society in recent years have led to a great increase in generation of Wastewater. Microalgae cultivation in wastewater for wastewater treatment, pollution control and production of energy from biomass. Microalgae cultivation is one of good solutions to solving all the environmental problems in our environment such as global warming, the increase of ozone hole and climate changed because it consumes high quantity of carbon dioxide in Photosynthesis process to produce oxygen and glucose. Algae can adapt in any environment and under all the conditions Biological treatment that involves the utilization of micro-organisms to ameliorate wastewater streams has become a necessary practice. Currently, there is much focus on the use of microalgae for the phycoremediation of effluent due to their metabolism. Phycoremediation is a process permitting the removal or biotransformation of inorganic and organic pollutants by algae during their growth in wastewaters.. It also plays a dual role as microalgal biomass is an eligible feedstock for biofuel generation.

1.1 Objective of study

(1) To evaluate the ability of microalgae to remove pollutants in Municipal wastewater

(2) To compare the pollutant removal efficiency of spirulina and chlorella vulgaris

(3) To evaluate the potential of microalgae for bioethanol production.

2. METHODOLOGY

2.1 Sample collection

In this study, Wastewater sample were taken from the two collection points situated at, Kozhikode Oxidation ditch of medical college Calicut and The apartment of Hi-lite. Fig 1 shows the raw sample collected from Kozhikode.

Fig-1 : Raw Sample

2.2 Collection of microalgae

Microalgae species selected for study are Spirulina and Chlorella vulgaris, which was purchased from CMFRI, Kozhikode. Cultures collected are shown in fig 2. Spirulina is a planktonic photosynthetic filamentous cyanobacterium (blue green algae) that forms massive populations in tropical and subtropical bodies of water which have high levels of carbonate and bicarbonate and alkaline pH up to 11. It is also used as a feed supplement in the aquaculture and poultry industries. The first photosynthetic microbe to be isolated and grown in pure culture was the freshwater microalgae, chlorella vulgaris. It is a spherical unicellular eukaryotic green algae, which presents a thick cell wall as the main characteristic. Chlorella species was maintained in BG11 medium and spirulina in zarrouks medium and compositions of mediums.

Fig-2 : Spirulina and Chlorella vulgaris cultures

2.3 Treatment using microalgae

Both species of microalgae, spirulina and chlorella were added to the prepared diluted wastewater. Both species which were maintained in medium was allowed to multiple their numbers. 500 ml of species was added to eight litres of wastewater taken in a plastic tub. Then these plastic tubs were kept at sunlight allowing microalgae to grow. Water was stirred 4-5 times a day in order to ensure proper sunlight is obtained to the microalgae. Samples were tested for COD, BOD, ammonia nitrogen, phosphate, nitrate and chloride in alternate days. Since wastewater collected showed very high amount of COD, BOD and ammonia nitrogen, samples have to be diluted to reduce the toxicity which is adaptable for microalgae. As nutrients are the key factor for the growth of microalgae, studies conducted so far has shown that microalgae could survive up to 760 mg/l of ammonia nitrogen. So in this study, 50% and 30% dilutions of wastewater were selected which can be adaptable for the growth of microalgae.

Fig-3: Treatment using spirulina and chlorella vulgaris in 50% diluted sample

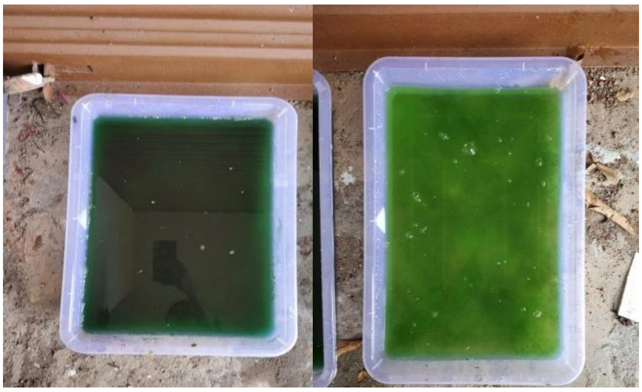

Fig-4: Treatment using spirulina and chlorella vulgaris in 30% diluted sample

2.4 Biomass preparation

After the treatment process, the very next step was to harvest the biomass. Water was filtered through filter cloth mesh and biomass was collected. Obtained biomass is sundried for 3 days and pretreatment was given. Pretreatment involves drying and grinding the biomass into powdered form. Pretreatment is done for making the raw materials susceptible for quick hydrolysis. Biomass was grinded well into powdered form. Pretreatment increases the surface area, unveiling more active site for the action of acid in the hydrolysis process. Fig 5 and 6 shows the pretreated dried biomass of spirulina and chlorella vulgaris.

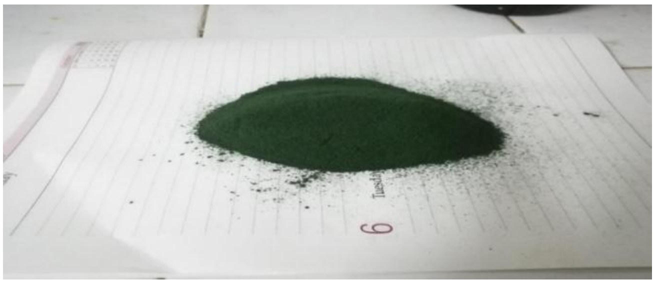

Fig-5: Spirulina dried biomass

Fig-6: Chlorella vulgaris dried biomass

2.5 Hydrolysis

Conversion of starch into simple sugars takes place in this step. Acid helps to coverts the cleavage of long dextrin chain in the starch of biomass to simple sugars. It helps in faster fermentation and higher ethanol yields. Dilute sulfuric acid (2N) is used for converting starch into sugars. Firstly powdered mass is mixed with distilled water and dil. sulfuric acid in the ratio 1:3:2. This liquid mass boiled for 30 min at a temperature of 100^0C with continuous stirring using a glass rod . Sugar filtrate is obtained after centrifugation.

2.6 Fermentation

Ethanol formation takes place in the fermentation step by anaerobic respiration of saccharomyces cerevisiae, a species of yeast. An air tight vessel was set up which ensured anaerobic condition. The extraction of ethanol is achieved by distillation. 20 g of overnight grown yeast was added to the sugar filtrate and was allowed for an anaerobic fermentation of 48 hours. Carbon dioxide formed as a byproduct of fermentation process should be released to the atmosphere to reduce pressure inside the vessel. But at the same time atmospheric contact of the fermenting solution should be avoided. So outlet is dipped in water. The sugar is then converted into ethanol and carbon dioxide. Bubbles are formed due to the production of carbon dioxide. The fermentation is complete when the carbon dioxide production stops. When the bubble formation stops, the process is stopped and fermented mash taken out for distillation.

2.7 Distillation

Distillation is done for separating ethanol from fermented mesh. The distillation temperature of ethanol is 78^0C. Simple distillation was carried out for both 2 samples to extract ethanol. The distillation is done consecutively two times to increase the ethanol concentration. First stage distillation was performed at 90-100^0C and the distilled liquid collected completely in a beaker was poured again into the distilling flask. Then second stage of distillation was performed at 78 to 90oC. Finally, ethanol is collected from both microalgal biomass.

2.8 Test for ethanol

Two tests were performed for the identification of ethanol

(1). Potassium dichromate test

(2). Flame test

Potassium dichromate test performed by adding ethanol to a beaker containing potassium dichromate acidified with dilute sulphuric acid and boiled for at least 10 to 15 minute time. If the orange colour changes to dark green it indicates the presence of ethanol.

Flame test is a conventional test performed by introducing a flame into a cotton ball that dipped in the distilled liquid. If the ball catch fire immediately indicates an ethanol concentration of above 30%.

3. RESULTS AND DISCUSSION

The aim of the study was to investigate the potential of microalgae on the treatment of municipal wastewater and to produce bioethanol successively. For that certain parameters were tested regularly during the treatment period. The characteristics studied were COD, BOD, ammonia nitrogen, nitrate, phosphate and chloride. From the characteristic study conducted on raw sample collected from Kozhikode , it was found to be highly polluted since it has a very high value of COD, BOD and ammonia nitrogen. So considering the survival of microalgae first treatment was carried out in 50% diluted wastewater sample. After 50% diluted sample treatment, 30% dilution was also tried to ensure better removal efficiencies of microalgae. A control without microalgae was also analyzed in both dilutions. The biomass obtained after both treatment was used to produce bioethanol. In this study a comparison is made with the potential of each microalgae to remove pollutants and with their ability to produce bioethanol

Table -1: Characteristics of Municipal wastewater before treatment

Parameters	Before treatment
pH	5.4
COD (mg/l)	20100
BOD (mg/l)	16000
TDS (mg/l)	10100
Phosphate (mg/l)	32
Ammonia nitrogen mg/l	1150
Nitrate mg/l	90
Chloride (mg/l)	1500

3.1 Analysis of parameters

The characteristics studied were COD, BOD, ammonia nitrogen, nitrate, phosphate and chloride. The percent removal of each parameter is shown in below charts.

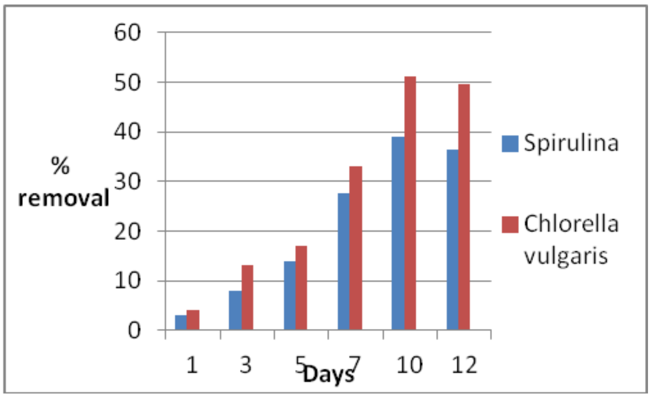

Chart -1: COD (50%Dilution)

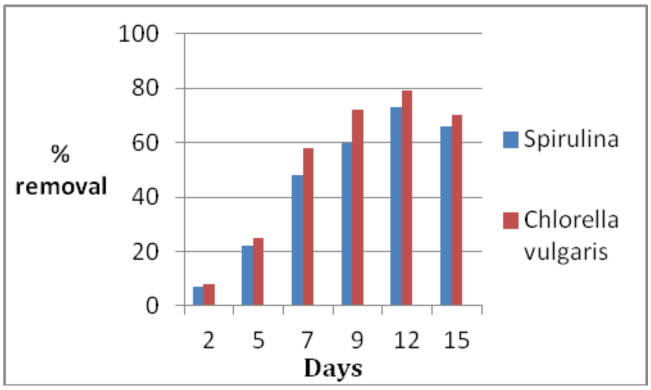

Chart -2: COD (30% Dilution)

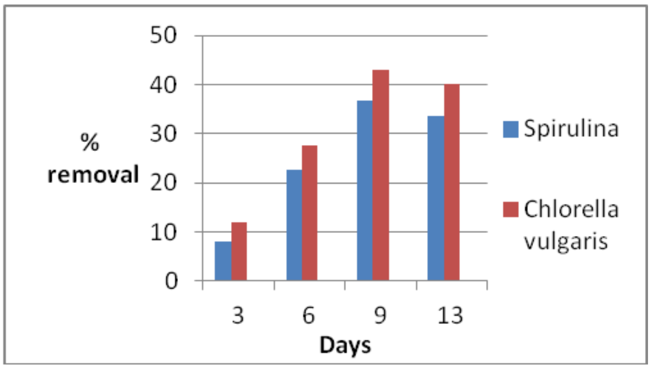

Chart -3: BOD(50% Dilution)

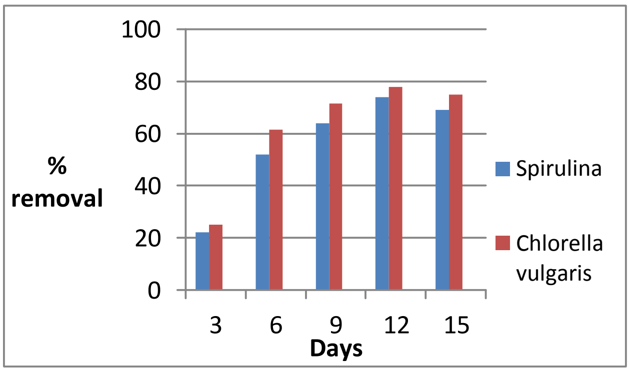

Chart -4: BOD (30% Dilution)

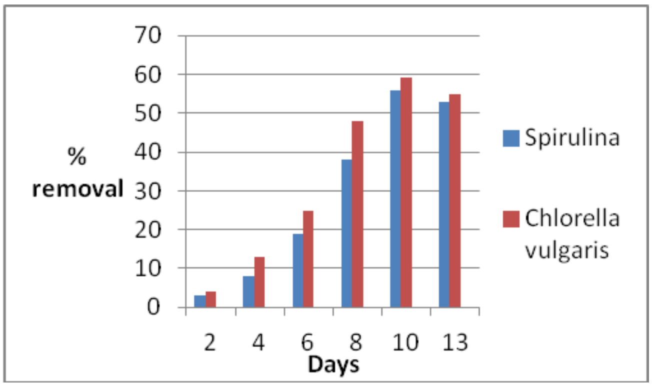

Chart -5: Ammonia Nitrogen (50% Dilution)

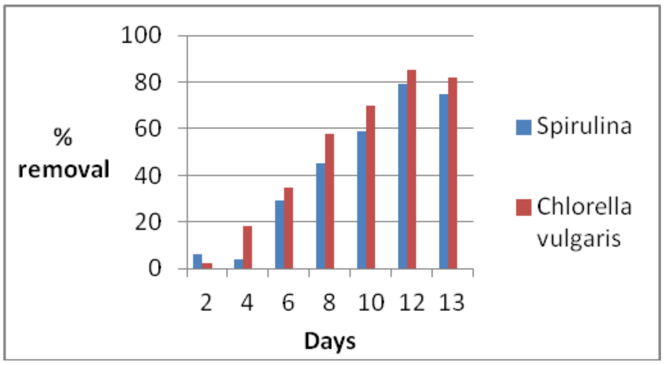

Chart -6: Ammonia Nitrogen (30% Dilution)

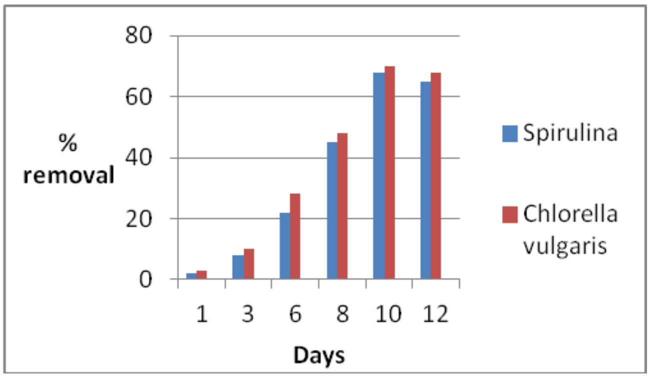

Chart -7: Nitrate(50% Dilution)

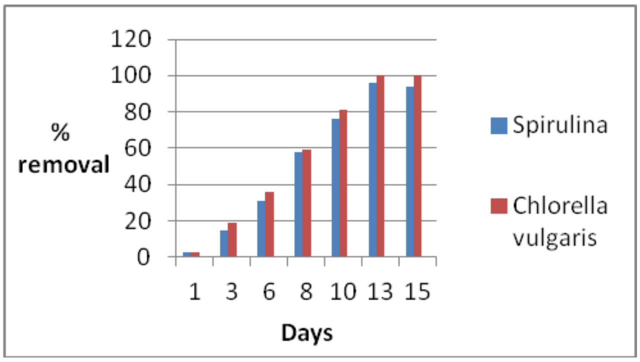

Chart -8: Nitrate(30% Dilution)

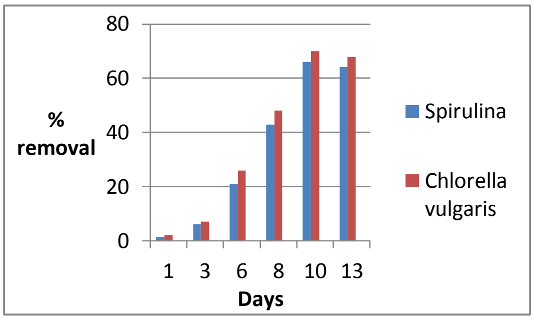

Chart -9: Phosphate (50% Dilution)

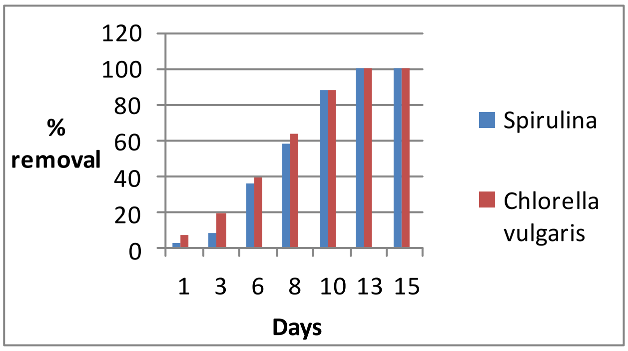

Chart -10: Phosphate (30%Dilution)

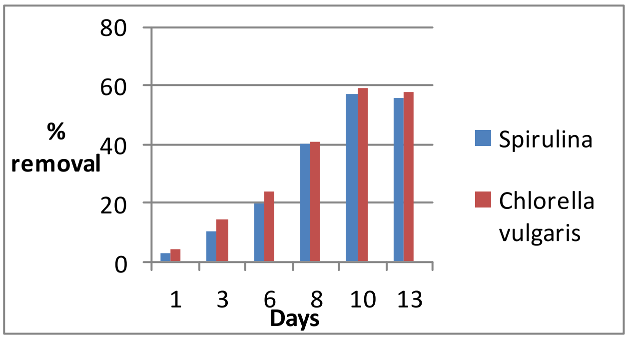

Chart -11: Chloride(50% Dilution)

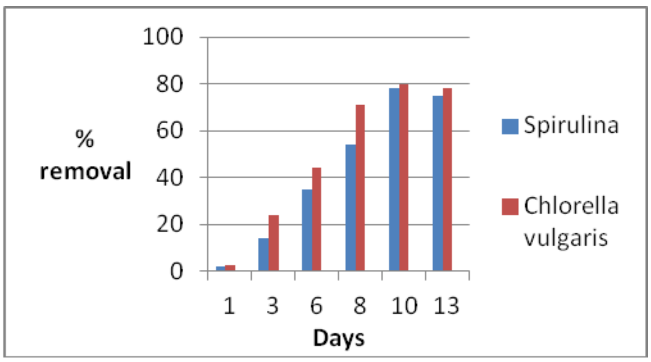

Chart -12: Chloride (30% Dilution)

3.2 Biomass production

Microalgae after treatment of 13 days of treatment in 50% dilution and 15 days of treatment in 30% dilution biomass was harvested and were sundried for 3 days and biomass of both Spirulina and Chlorella vulgaris was collected. Biomass collected was monitored by taking their weights. Table 4.1 shows the amount of biomass obtained from Spirulina and Chlorella vulgaris. Even though Chlorella vulgaris showed a better efficiency in removing the pollutants, the amount of biomass produced was more by Spirulina than by Chlorella vulgaris. Spirulina produced 1.9 g/ l biomass in 50% diluted sample and 2.8 g/l in 30% diluted sample. Whereas Chlorella vulgaris produced 1.6g/l in 50% diluted sample and 2.5 g/l in 30% diluted sample. From these results we could understand that microalgae showed a good growth in 30 % diluted sample, which indicates better growth of microalgae in more diluted samples.

Table -2: Amount of biomass obtained from Spirulina and Chlorella vulgaris obtained from 8 litres of wastewater

Microalgae	50% dilution	30% dilution
Spirulina	15.2 g	22.4 g
Chlorella vulgaris	12.8 g	20 g

3.3 Bioethanol yield from microalgae

Obtained biomass after giving pretreatment, acid hydrolysis, fermentation and distillation , bio ethanol was generated. The ethanol yield from Spirulina and Chlorella vulgaris was monitored. Test for bio ethanol was conducted to ensure the presence of ethanol in the distilled liquid. Table 3 shows the yield from each microalgal biomass.

Table -3: Yield from microalgal biomass

Feedstock	Mass of feedstock	Volume of ethanol generated	Bioethanol yield in ml/g of biomass
Spirulina	52g	24ml	0.4615ml/g
Chlorella vulgaris	45g	19ml	0.4222ml/g

The yield from microalgal biomass is several orders of magnitude larger than yields obtained for other feedstocks Ethanol was produced with microalgal photosynthesis and intracellular anaerobic fermentation. Microalgae like Chlorella, Spirulina are known to contain a large amount of starch, cellulose and glycogen. Species like Spirulina have the ability of producing high levels of carbohydrates instead of lipids. So, the result indicates that microalgae can be used for the production of ethanol biofuel and are definitely a promising feedstock.

4. CONCLUSIONS

From the study, it can be concluded that microalgae has the potential to treat wastewater and to produce bioethanol. Both microalgae Spirulina and Chlorella vulgaris grown in 50% and 30% dilution have showed good growth. But nutrient removal efficiencies were found to be higher in 30% diluted samples, indicating higher removal efficiencies in more diluted samples. Chlorella vulgaris was found to have more potential than Spirulina in removing pollutants as they could remove 79% COD, 78% BOD and 85% ammonia nitrogen. Even though greater removal efficiencies of COD, BOD and other nutrients were noticed, parameters were not bought into desirable limits. This could be because of the high values of pollutants. So treatment with microalgae could be suggested as a post treatment for Wastewater that is highly polluted. As a post treatment process microalgae could show best results as the treated wastewater is less concentrated than the raw wastewater. It was clearly seen in 30% diluted synthetic sample, that it could remove nitrate and phosphate completely and other parameters also at a high removal rate. After the treatment, biomass could be efficiently converted into useful products. From this study, 24 ml of bioethanol was extracted from 52g of Spirulina biomass and 19 ml of bioethanol was extracted from 45 g of Chlorella vulgaris biomass.

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BIOGRAPHIES

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