

## Phycoremediation and the Potential of Sustainable Algal Biofuel Production using Wastewater

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### ABSTRACT

Wastewater remediation by microalgae is an eco-friendly process with no secondary pollution as long as the biomass produced is reused and allows efficient nutrient recycling. This study was undertaken to evaluate the remediation of wastewater by the green alga *Chlorella vulgaris* and the potential of these alga (biomass) to produce sustainable biofuel. The results shows that the cultivation of green alga *Chlorella vulgaris* on wastewater has a positive effect on removal the major inorganic elements form the wastewater. Biodiesel can be produced from *Chlorella vulgaris*. The *Chlorella vulgaris* can be used for remediation of wastewater and the producing biomass can be used as source of renewable energy.

**Keywords:** Phycoremediation, Water, Algae, Biofuel

### 1. INTRODUCTION

Algae are important bioremediation agents and are already being used by many wastewater facilities. The potential for algae in wastewater remediation is however much wider in scope than its current role. The release of wastewater poses serious environmental challenges to the receiving water bodies (De-Bashan and Bashan, 2010). The major effect of releasing wastewater rich in organic compounds and inorganic chemicals such as phosphates and nitrates is mainly eutrophication (Pizarro *et al.*, 2006; Munoz and Guieysse, 2008; Mulbry *et al.*, 2008; Godos *et al.*, 2009). This is a global problem that can be solved by the use of microalgae whereby the wastewater is used as feed for microalgal growth. The advantage is that while the microalgae will be removing excess nutrients in the wastewater, there will be concomitant accumulation of biomass for downstream processing (Chinnasamy *et al.*, 2010). The use of a wide range of

microalgae such as *Chlorella*, *Scenedesmus*, *Phormidium*, *Botryococcus*, *Chlamydomonas* and *Spirulina* for treating wastewater has been reported and efficacy of this method is promising (Olguin, 2003; Chinnasamy *et al.*, 2010; Kong *et al.*, 2010; Pittman *et al.*, 2010; Stephens *et al.*, 2010).

With global shortages of fossil fuels, especially oil and natural gas, a major focus has developed worldwide on renewable biofuel production. Algae, particularly green unicellular microalgae have been proposed for a long time as a potential renewable fuel source (Oswald and Golueke, 1960). Microalgae have been estimated to have higher biomass productivity than plant crops in terms of land area required for cultivation, are predicted to have lower cost per yield and have the potential to reduce green house emissions through the replacement of fossil fuels (Brune *et al.*, 2009; Chisti, 2008; Dismukes *et al.*, 2008).

The use of microalgae is desirable since they are able to serve a dual role of bioremediation of wastewater as well as generating biomass for biofuel production with concomitant

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carbon dioxide sequestration (Olgun *et al.*, 2003; Mulbry *et al.*, 2008; Rawat *et al.*, 2011). In addition, wastewater remediation by microalgae is an eco-friendly process with no secondary pollution as long as the biomass produced is reused and allows efficient nutrient recycling (Godos *et al.*, 2009). This study was undertaken with the aim to evaluate the remediation of waste water by the green alga *Chlorella vulgaris* and the potential of these alga (biomass) to produced sustainable biofuel.

## 2. MATERIALS AND METHODS

### 2.1. Wastewater Collection

The wastewaters used in the study, were collected (in April 2010) from four different sources of wastewater on Al-Hassa area, Saudi Arabia.

### 2.2. Physico-Chemical Characteristics of Wastewaters

pH, conductivity, total dissolved salts and Dissolved oxygen of the wastewater were measured in location. pH was measured using a pH meter (370 pH meter Jenway, UK), conductivity and total dissolved salts using a calibrated Conductivity Meter (470 Conductivity meter, Jenway, UK). Dissolved oxygen was measured according to the Winkler method (Strickland and Parsons, 1972). Total alkalinity, phosphate-P, nitrate-N, chloride, COD and major cations were measured according to Adams (1991). Sodium and potassium concentrations were determined photometrically by flame emission according to Golterman and Clymo (1971). The results were calculated as mean values of triplicate measurements made on each water sample from each of the four sampling stations.

### 2.3. Organism and Culture Condition

*Chlorella vulgaris* Beyerinck was isolated from Al-Asfar Lake, Al-Hassa, Saudi Arabia. Isolation and purification were made by dilution and plating technique method. The alga was grown in 250 mL flasks containing 100 mL Kuhl (1962) medium and incubated in an illuminated incubator (Precision, USA) at 22°C and irradiance at 150  $\mu\text{mol m}^{-2} \text{sec}^{-1}$ , provided by cool white fluorescent lamps set on 14:10 h photoperiod. All cultures were shaken twice daily to prevent cells from clumping. Sterile technique was used at all times.

### 2.4. Determination of Algal Growth

Growth was measured in terms of cell number using a Haematocytometer, which was used for calculation of growth rate.

### 2.5. Treatment of Wastewater Samples

Wastewater was injected with *Chlorella* sp. suspension (each flask contain two liters of wastewater) with aeration and illumination for 7 days until odorless and the culture became clear and green colour appeared. The physic-chemical characteristics of wastewater before and after treatment with alga were determined as follows in previously in case of the determination of physic-chemical characteristics of wastewater. Nutrient removal rates were calculated by dividing the difference between the first day and final day concentrations by the first day concentration, then multiplied by 100 and expressed as percentage.

### 2.6. Biochemical Analysis

The anthrone method (Roe, 1955) was applied for total carbohydrate estimation using fresh material and glucose as a standard. Total lipids content was determined according to Moore and Stein (1948). Total protein was measured according to Lowry *et al.* (1951).

### 2.7. Oil Extraction

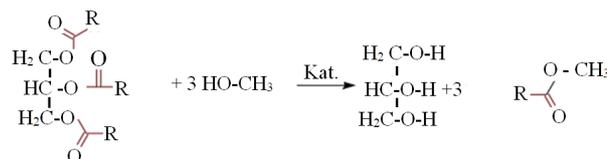
Algae were ground with motor and pestle as much as possible. The ground algae were dried for 20 min at 80°C in a incubator for releasing water. Hexane and ether solution (20 and 20 mL) were mixed with the dried ground algae to extract oil. Then the mixture was kept for 24 h for settling. The extracted oil was evaporated in vacu to release hexane and ether solutions using rotary evaporator. Mixing of catalyst and methanol: 0.25 g NaOH was mixed with 24 mL methanol and stirred properly for 20 min.

### 2.8. Biodiesel Production

The mixture of catalyst and methanol was poured into the algal oil in a conical flask. The following reaction and steps were followed National Biodiesel Board.

### 2.9. Transesterification

The reaction process is called transesterification. The conical flask containing solution was shaken for 3 h by electric shaker at 300 rpm. After shaking the solution was kept for 16 h to settle the biodiesel and sediment layers clearly (**Fig. 1**).



**Fig. 1.** The simplified transesterification reaction.

## 2.10. Separation of Biodiesel

The biodiesel was separated from sedimentation by flask separator carefully. Quantity sediment (glycerine) was measured. Biodiesel was washed by 5% water until it was become clean. Biodiesel was dried by using dryer and finally kept under the running fan for 12 h. Biodiesel production was measured by using measuring cylinder, pH was measured and stored for analysis.

## 3. RESULTS AND DISCUSSION

The chemical compositions of the four wastewaters are listed in **Table 1**. The data show that the pH values of wastewater samples I and II were in the acidic side whereas in samples III and IV in alkaline side. Samples III and IV were found to be characterized by highest total dissolved salts in comparison to other samples. It is worthy to mention that the nitrate and phosphate concentrations on sample IV were found to be high than other samples. The data also reported that the of divalent (calcium and magnesium) and monovalent cations (sodium and potassium) were relatively high at all samples, irrespective of some minor fluctuations. COD was taken in the present study as a measure of the oxygenated state and additionally the amount of organic matter in water as well. The data of this study show that COD tended to be higher in samples III and IV (26.10 and 22.8 mg L<sup>-1</sup>, respectively).

Algae represent the best biological treatment for wastewater because they increase oxygen content of waters *via* photosynthesis. In this study, waste water was injected with an active green alga *Chlorella vulgaris* culture. After the incubation period the pH value was changed to the alkaline side. This general tendency to the alkaline side may be due to the increased photosynthetic activity of planktonic algae, or to the chemicals nature of water (Fathi *et al.*, 2001).

*Chlorella vulgaris* growth in terms of cell number in the four wastewaters sample was plotted in **Fig. 2**. No lag phases were observed in all of the four curves, indicating that this wild-isolated algae *Chlorella* sp. could adapt well in all of the wastewater samples. Similar growth patterns, with exponential phases in the first 3 days followed by stationary phases in the next 6 days were present for all wastewaters, except the sample no. IV, in which the exponential phase lasted 1 day more before entering into a stationary phase. Moreover, it can be found that the algal growth was significantly enhanced in the sample no. IV. It could be due to its much higher levels of nitrogen, phosphorus and COD than the other

three wastewaters (**Table 1**). Therefore, the results show that the sample no. IV is the best media for algal growth.

The data of **Table 2** performed that the cultivation of algae removed almost the measured parameters specially the major inorganic nutrient (phosphate and nitrate). On the other hand, oxygen content, alkalinity and COD were found to increased in compare to other tested parameters. Dissolved oxygen is an important parameter for identification of different water masses. The relatively high concentrations of dissolved oxygen recorded after algal treatment could be mainly attributed to photosynthetic activity of algae (Fathi *et al.*, 2001). However, the highest concentrations of alkalinity may be due to the bacterial decomposition of organic substrates coming with the re-use drainage water receiving by this site (Abdel-Satar and Elewa, 2001). When the organic substrate is not available, autotrophic growth uses CO<sub>2</sub> as the carbon source, excreting small molecular organic substances such as glycolic acid to the environment as a product of photosynthetic carbon reduction cycle (Wang *et al.*, 2010) which is the reason why COD in effluent increased after algal cultivation. Generally the data shows that the green alga *Chlorella vulgaris* can be for removed the major inorganic elements form the wastewater.

In recent years, biofuel production from algae has attracted the most attention among other possible products. The recent soaring and crashing of oil prices and diminishing world oil reserves, coupled with enhanced greenhouse gases and the predicted threat of climate change, have generated renewed interest in using algae as alternative and renewable feedstock for energy production (Ramachandra *et al.*, 2009). Algae are of great interest in the production of biofuels due to the fact that a number of species of freshwater and marine algae contain large amounts of high quality polyunsaturated fatty acids which can be produced for aquaculture operations. Algae can grow heterotrophically on cheap organic substrates, without light and under well-controlled cultivation conditions. The characteristics of algal oil are similar to those of fish and vegetable oils and can thus be considered as potential substitutes for the product of fossil oil (Ramachandra *et al.*, 2009).

The data of **Table 3** performed the chemical composition of tested alga. The data show that *Chlorella vulgaris* contain highest amount of protein and lipids in compared to the other tested biochemical parameters. Microalgae which include algal strains, diatoms and cyanobacteria have been found to contain high levels of lipids-over 30%. Due to the high lipid content, these microalgal strains are of great interest in the search for sustainable sources for the production of biodiesel (Hossain and Salleh, 2008).

**Table 1.** The physical and chemical characters of the collected four wastewater samples

| Parameters                                    | Sample I   | Sample II | Sample III | Sample IV |
|---|------------|-----------|------------|-----------|
| pH  | 6.65       | 6.75      | 7.72       | 7.74      |
| Conductivity (mS)                             | 2.96±0.01  | 2.15±0.01 | 4.56±0.01  | 4.55±0.01 |
| T.D.S. (g L <sup>-1</sup> )                   | 1.77±0.01  | 1.41±0.01 | 3.78±0.02  | 2.76±0.01 |
| Dissolved O <sub>2</sub> (g L <sup>-1</sup> ) | 2.50±0.02  | 4.64±0.15 | 2.7±0.15   | 4.00±0.02 |
| Alkalinity (mg L <sup>-1</sup> )              | 52.21±0.15 | 110±0.01  | 99.2±0.02  | 129±0.01  |
| Chloride (mg L <sup>-1</sup> )                | 212±0.02   | 644±0.01  | 742±0.01   | 538±0.15  |
| Nitrate-N (mg L <sup>-1</sup> )               | 0.70±0.01  | 0.3±0.01  | 0.40±0.01  | 3.74±0.02 |
| Phosphate -P (mg L <sup>-1</sup> )            | 0.33±0.01  | 0.02±0.01 | 0.15±0.01  | 2.75±0.15 |
| Sodium (mg L <sup>-1</sup> )                  | 154±0.00   | 234±0.00  | 402±0.00   | 315±0.00  |
| Potassium (mg L <sup>-1</sup> )               | 28.4±0.00  | 22.0±0.00 | 27.4±0.01  | 21.0±0.00 |
| Calcium (mg L <sup>-1</sup> )                 | 176±0.00   | 157±0.00  | 161±0.00   | 158±0.02  |
| Magnesium (mg L <sup>-1</sup> )               | 70.0±0.00  | 68.7±0.00 | 71.0±0.00  | 68.0±0.05 |
| COD (mg L <sup>-1</sup> )                     | 11.2±0.00  | 11.8±0.00 | 26.1±0.00  | 22.8±0.05 |

**Table 2.** The physical and chemical characters of wastewater sample before and after cultivation of *Chlorella* sp

| Parameters                                    | Before cultivation of <i>Chlorella</i> | After cultivation of <i>Chlorella</i> | Removal rates (%) |
|---|--|---------------------------------------|-------------------|
| pH  | 7.74                                   | 8.10                                  |                   |
| Conductivity (mS)                             | 4.55±0.01                              | 2.21±0.02                             | 51.43             |
| T.D.S. (g L <sup>-1</sup> )                   | 2.76±0.01                              | 1.55±0.00                             | 43.84             |
| Dissolved O <sub>2</sub> (g L <sup>-1</sup> ) | 4.00±0.02                              | 6.00±0.05                             | -50.00            |
| Alkalinity (mg L <sup>-1</sup> )              | 129±0.01                               | 225±0.05                              | -74.41            |
| Chloride (mg L <sup>-1</sup> )                | 538±0.15                               | 500±0.02                              | 7.06              |
| Nitrate-N (mg L <sup>-1</sup> )               | 3.74±0.02                              | 1.84±0.02                             | 50.80             |
| Phosphate-P (mg L <sup>-1</sup> )             | 2.75±0.15                              | 1.06±0.02                             | 61.45             |
| Sodium (mg L <sup>-1</sup> )                  | 315±0.00                               | 288±0.00                              | 8.57              |
| Potassium (mg L <sup>-1</sup> )               | 21.0±0.00                              | 12.0±0.00                             | 42.86             |
| Calcium (mg L <sup>-1</sup> )                 | 158±0.02                               | 55.0±0.15                             | 65.19             |
| Magnesium (mg L <sup>-1</sup> )               | 68.0±0.05                              | 35.0±0.02                             | 48.52             |
| COD (mg L <sup>-1</sup> )                     | 22.8±0.05                              | 30.2±0.12                             | -32.46            |

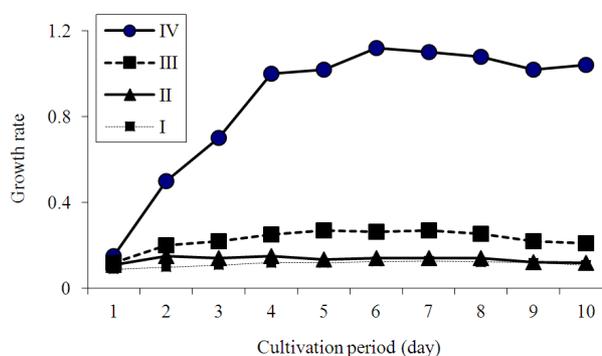
**Table 3.** The chemical composition of *Chlorella vulgaris* expressed on a dry weight (%)

| Parameters          | Concentrations (%) |
|---------------------|--------------------|
| Total protein       | 54.0               |
| Total carbohydrates | 18.6               |
| Total lipids        | 30.5               |
| Nucleic acid        | 3.5                |

**Table 4.** The Biodiesel production (% of dry weight) and Sediments (after algal biodiesel extraction) in *Chlorella vulgaris*

| Parameters   | Concentrations (%) |
|--|--------------------|
| Biodiesel production (% of dry weight)                   | 72.54              |
| Sediments (glycerine) (after algal biodiesel extraction) | 32.52              |

Regarding to the biodiesel production from the tested alga the data of this investigation performed that the extracted oil seemed to be higher in compare to the other investigation (Ramachandra *et al.*, 2009; Hossain and Salleh, 2008), **Table 4.** It seems biodiesel can be produced from macro algae though it contains lower lipid content than micro algae (Sharif *et al.*, 2008).



**Fig. 2.** Growth curves of the green alga *Chlorella vulgaris* grown in waste water

On other hand, sediment were also higher in *Chlorella vulgaris* than its recorded on other algal species (Ramachandra *et al.*, 2009; Sharif and Salleh, 2008; Hossain and Salleh, 2008). Sharif *et al.* (2008) reported that algal oil and biodiesel (ester) production was higher in *Oedogonium* than *Spirogyra* sp. However, biomass (after oil extraction) was higher in *Spirogyra* than *Oedogonium* sp.

Biodiesel can be produced from macro algae because of lipid contents. Sijtsma and Swaaf (2004) stated that Docosaehaenoic Acid (DHA) was a polyunsaturated fatty acid composed of 22 carbon atoms and six double bonds that belonged to the so-called w-3 group. Vincenate (2006) suggested that seaweeds contain about 5.5% oil. Heterotrophic growth of certain marine eukaryotes, such as the microalgae, is enhanced in this medium. The samples harvested from these examples produce lipid fractions containing Omega-3 fatty acids. After extraction and esterification to form the methyl esters, gas chromatographic analyses show that the w-3 fatty acids may constitute as much as 10-50% of the total fatty acid fraction. Spolaore *et al.* (2006) evaluated that

microalgae can provide several different types of renewable biofuels. Many researchers reported that biodiesel was derived from microalgal oil (Thomas, 2006; Sharif *et al.*, 2008). Our results indicate that biodiesel can be produced from *Chlorella vulgaris*. In this way algae can be used as renewable energy.

#### 4. CONCLUSION

Microalgal cultures offer an interesting alternative for waste water treatment because they provide a tertiary biotreatment coupled with the production of potentially valuable biomass, which can be used for several purposes. Microalgae have the potential to generate significant quantities of biomass and oil suitable for conversion to biodiesel. This study was undertaken to evaluate the remediation of wastewater by the green alga *Chlorella vulgaris* and the potential of these alga (biomass) to produce sustainable biofuel. The data indicated that cultivation of green alga *Chlorella vulgaris* on wastewater has a positive effect on removal of the major inorganic elements from the wastewater. Biodiesel can be produced from *Chlorella vulgaris*. The *Chlorella vulgaris* can be used for remediation of wastewater and the producing biomass can be used as source of renewable energy.

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