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THE POTENTIAL OF *CHLORELLA VULGARIS* FOR WASTEWATER TREATMENT AND BIODIESEL PRODUCTION

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Abstract

The release of municipal wastewater from various sources can cause contamination of water bodies and algal blooms. In this study isolated strain of *chlorella vulgaris* was used for treatment of municipal wastewater and biodiesel production. Initially Chlorella culture was prepared in artificial media then it was inoculated in transparent and covered, glass ponds containing wastewater to investigate its treatment efficiency. A lab scale biological wastewater treatment design was also made using *chlorella vulgaris*. Harvested biomass was transesterified to biodiesel using sodium metal as a catalyst. Percent reduction of COD (chemical oxygen demand), BOD (biochemical oxygen demand), NO_3^-, PO_4^{2-} and TC (total coliforms) was almost similar in all types of transparent and covered ponds after treatment with *chlorella vulgaris*. Analysis of selected parameters was carried out at each step of biological treatment design and maximum reduction percentage of COD (99.9%), BOD (100%), NO_3^- (99.98%), PO_4^{2-} (99.96%) and TC (100%), was observed by applying *chlorella vulgaris*. Biodiesel produced by direct transesterification of dried algal biomass was analyzed and compared with ASTM (American Standard Test Method) standards. Results showed that biodiesel produced was of good quality and it can be used as a fuel in vehicles.

Introduction

With global shortage of fossil fuels, especially oil and natural gas, a major focus was shifted towards renewable biofuels (Barbara, 2007). Among the oilseed crops, algae has got much attention due to higher yield per unit area as compared to other sources of biofuel as algae produces 10 to 100 times more energy than other second generation biodiesel crops (Greenwell *et al*., 2010; Ahmad *et al*., 2012). Currently biodiesel produced from algae is costly but it can be made cheaper by growing algae in a wastewater as it contains all the necessary nutrients required for algal growth. Algae can also be grown on land unsuitable for food crops. It was estimated that microalgae can produce 5000 to 20,000 gallons of oil per acre per year (Chisti, 2008).

Cultivation of algae was started as early as in 1970s but initially it was grown in wastewater pond to treat secondary effluent in order to prevent eutrophication. This treatment removed nutrients very efficiently. Hence it was suggested to apply the algal system as secondary rather than tertiary treatment (Tam & Wong, 1989). Chlorella was widely used to treat wastewater as it removes nitrogen, phosphorus, BOD and COD very efficiently with different retention time ranging from 10 h to 42 days (Wang *et al.,* 2010).

The biotechnology of growing microalgae in waste water is getting importance as biomass of these algae can be used as food and many other valuable products including biodiesel. It is covering more than 10% of world's primary energy demand. In the current scenario of increasing oil prices, depletion of resources and environmental challenges, only algal biomass has the potential to supply alternative energy to energy hungry civilization (Antoni *et al.,* 2007). There are many studies on the potential of algal biomass for biofuel production as it has high productivity at a cheaper cost (Mohan *et al.,* 2009).

There are number of benefits of growing algae in wastewater as it absorbs nutrient thus reducing the treatment cost of wastewater. Secondly it assimilate large amount of organic carbon to produce its biomass which can further be processed to biodiesel production. Growing algae in wastewater is the most feasible way to reduce the economic and environmental cost of biodiesel production. Microalgae have been used for wastewater treatment for more than 50 years (Min *et al.,* 2011). The oxygen produced by algae in wastewater is utilized by heterotrophic bacteria for the conversion of wastewater nutrients to biomass (Gracia *et al.,* 2006).

Biodegradation of pollutants present in wastewater can be resulted by oxygen produced as a result of photosynthetic activity of algae. Algal treatment methods are commonly used for the removal of nutrients, pathogens and other type of contaminants (Munoz & Guieysse, 2006). Other mechanisms for the reduction of pollutants in algal based system can be nutrient competition, increase in pH due to CO_2 consumption, toxins produced by algae and adhesion or attachment to the algal cells.

Microalgae grown in wastewater can accumulate valuable lipids and fatty acids which can be extracted from the dried biomass of algae and can be used for biodiesel production by transesterification process (Miao *et al.,* 2004; Spolaore *et al.,* 2006). The residue obtained from this reaction can be used as aquaculture feed, animal food supplement or a source of pharmaceuticals (Chen, 1996).

Rationale of the study: Anthropogenic activities resulted in the production of more wastewater thus becoming matter of serious concern. Conventional wastewater treatment methods were not only expensive but those needed more energy which could not be fulfilled due to crises of energy. Algal based systems are not only helpful in pollutant reduction in wastewater but the biomass produced can be harvested for biodiesel production. The current study was designed to get double benefit from microalgae (*Chlorella vulgaris).*

Objectives of the study: In the current study *Chlorella vulgaris* was grown in wastewater to absorb nutrients and reducing pollution load in terms of BOD and COD. Algal culture was prepared in artificial medium to inoculate in wastewater for measuring its treatment efficiency. Growth rate in terms of OD_{680} was also measured in synthetic medium and wastewater. A lab scale biological wastewater treatment design was made using *Chlorella vulgaris*. Dried biomass of chlorella was transesterified to biodiesel and its quality was assessed.

Materials and Methods

Algae sampling: Samples of *Chlorella vulgaris* were collected from fish forms of Department of Fisheries near Manawa police station Lahore, Pakistan. It was taken from Mr. Tariq Rashid working in the same department on utilization of *Chlorella sp.* as fish feed. Algal samples were identified by using methods described by Ali *et al.*, (2010) and Zarina *et al*., (2005a, b).

Pollutants Analysis: Wastewater samples were collected from municipal drain and analyzed for BOD, COD, NO_3^-, PO_4^{2-} and TC by Anon., (2005) standards methods and then put into selected ponds for the growth of *Chlorella vulgaris*. Reduction in these parameters was determined by inoculating equal amount of *C. vulgaris* in transparent and covered ponds (having black sides and transparent upper cover) having dimensions 0.3 x 0.3 x 0.15m with same water quantity, light intensity, temperature and other climatic condition.

Artificially synthesized medium: In order to compare growth of *C. vulgaris* in synthetic medium and wastewater, an artificial medium was synthesized in the following way, six stock solutions were prepared in a distilled water as follows; (a) 30g sodium nitrate per liter, (b) 2g potassium phosphate per liter, (c) 3g iron sulfate, 3g citric acid, 1.5g boric acid and 1g manganese chloride per liter, (d) 0.22g zinc sulphate, 0.79g copper sulphate, 1.5g ammonium molybdate, 0.23g ammonium vanadate, 2.5g ethylene diamine tatra acetic acid and 0.12 g cobalt chloride per liter, (e) 0.007g vitamin B_{12} per liter and (f) 3g ethylene diamine tatra acetic acid, disodium salt per liter. Synthetic medium for algal growth was prepared by mixing 10, 5, 1, 0.1, 20 and 20 ml per liter of each stock solution from serial (a) to (f) respectively (Hur, 2008).

Measurement of algal growth: For the comparison of growth of *C. vulgaris* in synthetic medium and wastewater, equal quantity of algal culture was inoculated in measured quantity of synthetic medium and wastewater (5 L) under similar climatic condition and pond size. Optical density of samples, taken every day from both media, was measured by spectrophotometer at 680 nm of wavelength. The growth rate (GR) was determined by fitting the optical density (OD_{680}) in the following formula:

$$GR = (\ln ODt - \ln OD_0)/t$$

where OD_0 is the optical density at the initial day, ODt is the optical density measured on day t. Each recorded ODt was corrected by taking away that of the corresponding blank sample (Wang *et al.,* 2010).

Laboratory scale biological treatment design: A lab scale treatment design consisting of screening, sedimentation, filtration and chlorella treatment unit was made to measure the reduction in BOD, COD, NO_3^-, PO_4^{2-} and TC at each step of the treatment design.

Analysis of dried biomass of microalgae: Protein, lipid and carbohydrate contents of air dried biomass of *C. vulgaris* were determined to check its feasibility for biodiesel production. Block digestion method was used for protein determination and lipid contents were measured by solvent extraction method (Boccard *et al.*, 1981).

Extraction of oil and its transesterification: Oil was extracted from dried biomass of *C. vulgaris* by using n-hexane as a solvent. For 50 g of algal biomass 300 mL of hexane was used and extraction was carried out in Soxhlet extractor (UNE-EN 734-1, 2006) (Khola & Ghazala, 2010) for 4 hours. The resultant oil was transesterified to biodiesel by using sodium metal as a catalyst. The mixture of oil and methanol having sodium metal was heated for 1 hour at 62°C with constant stirring at 110 rpm. After 1 hour 2 layers, a liquid phase (biodiesel) at the top and solid phase (glycerin) at the bottom were obtained which were separated by separating funnel. Biodiesel obtained was washed with water to remove methanol and catalyst while the access of solvent was removed by distillation (Lang *et al.,* 2001).

Biodiesel quality assessment: Biodiesel obtained was analyzed for its different properties like kinematic viscosity, flash point, density, water content and clorific value using ASTM standards methods and were compared with international standards of biodiesel.

Results and Discussion

Analysis of raw wastewater: Raw waste water was collected from municipal drains of Lahore city at specific time interval then these samples were mixed to form composit sample. The composite sample was divided into three replicates, these replicated were tested for various parameters with following mean results; BOD (366.6 mg/l), COD (981.6 mg/l), NO_3^{2-} (18 mg/l), PO_4^{2-} (28.1 mg/l) and TC (1.5 x 10^3 MPN/ml) (Table 1). Results showed that amount of biochemical oxygen demand and chemical oxygen demand were well above than national environmental quality standards (Anon., 2000) value for BOD (80 mg/l) and COD (150 mg/l).

Table 1. Analysis of characteristics of raw wastewater before treatment.

Wastewater characteristics	Pollutants quantity
COD (mg/l)	981.6 ± 10.31
BOD (mg/l)	366.6 ± 11.15
NO_3 (mg/l)	18 ± 1.94
PO_4 (mg/l)	28.1 ± 2.97
TC (MPN/ml)	1.5 x 10^3 ± 1x10^2

Growth curve of *C. vulgaris* in different media: Growth curve of *C. vulgaris* in terms of optical density (OD_{680}) in both nutrient media is shown in Fig. 1. As this species of algae was well adapted in both media hence lag phase was absent. *C. vulgaris* showed similar growth in both media in first two days but after that growth rate was noted to be higher in synthetic medium as compared to wastewater. Growth became stationary on 6th and 7th day in synthetic medium and wastewater respectively. Wang *et al.*, (2010) measured the growth of chlorella sp. for ten days in four types of wastewaters and observed the stationary phase after 3rd day of cultivation time and lasted for next six days.

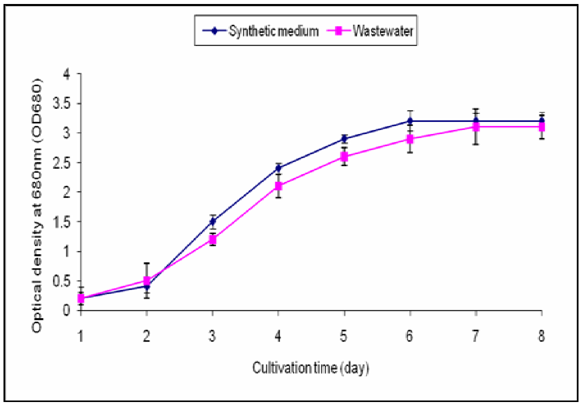

Fig. 1. Growth curve of *C. vulgaris* in wastewater and synthetic medium.

Comparison of transparent and covered ponds in terms of pollutants reduction: Reduction in wastewater characteristics was analyzed in transparent pond and ponds covered with black sheet from all four sides but having transparent glass on upper side. As the light penetration for photosynthesis was higher in transparent ponds as it trapped light from all sides hence there was more reduction in these ponds except in case of TC whose reduction was observed to be hundred percent in both types of ponds. Though the percent reduction of COD (95.6%), BOD (96.8%), NO_3 (97%), and PO_4 (95.5%) was higher in transparent ponds than percent reduction of COD (94.4%), BOD (95.2%), NO_3 (96.3%), and PO_4 (94.7%) in covered ponds (Fig. 2) but there was not much difference in both of them. Gracia *et al.*, (2006) also observed the reduction of nitrogen (43%) and phosphorous (73%) in wastewater by *chlorella species.*

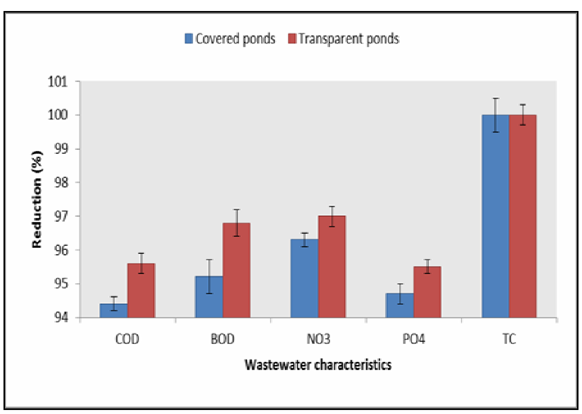

Fig. 2. Comparison of covered and transparent ponds in terms of percentage reduction in wastewater characteristics.

Pollutant Reduction at different steps of a treatment design: A biological treatment design consisting of screening, sedimentation, filtration and chlorella treatment pond was made at laboratory scale to analyze pollutant reduction efficiency at each stage of the design. Considerable reduction in the concentration of pollutants was not observed in screening process as it removed only coarse particles. After this wastewater was allowed to stay in sedimentation pond for the settlement of particles at the bottom, as a result, some reduction was observed in this step. Wastewater was filtered through column containing gravel and sand particles, where it showed more reduction in pollutant quantity. Maximum reduction in COD (99.9%), BOD (100%), NO_3 (99.98%), PO_4 (99.96%) and TC (100%) was observed in pond containing *C. vulgaris* (Fig. 3).

Analysis of dried biomass of *C. vulgaris*: Biomass harvested from the algal culture was dried in an oven at 80 ^{o}C and tested for protein, carbohydrate and lipid contents. It was observed that lipid percentage (42.53 %) of dried biomass was higher than protein (38.56 %) and carbohydrate percentage (15.33%) (Table 2). On the basis of high lipid contents, it was proved that *C. vulgaris* is a potential organism for biodiesel production. Li *et al.*, (2007) reported lipid contents 46.1%, 48.7%, and 44.3% of cell dry weight of heterotrophic *Chlorella species* from 5, 750 and 11,000L bioreactors, respectively.

Table 2. Protein, carbohydrates and lipid contents of *C. vulgaris* on dry matter basis.

Composition	Percentage of biomass *of C. vulgaris*
Protein	38.56 ± 2.56
Carbohydrates	15.33 ± 1.15
Lipids	42.53 ± 2.29
Others	3.56 ± 2.17

Fatty acid composition of oil extracted from *C. vulgaris*: Fatty acids profile (14:0, 16:0, 16:1, 16:2, 16:3, 16:4, 18:0, 18:1, 18:2, 18:3, 18:4, 20:0, 20:1, 20:2, 20:3 and 20:4) of oil extracted from biomass of *C. vulgaris* was analyzed by Gas Chromatography (GC). It was found that the extracted oil contained both saturated and unsaturated fatty acids but the percentage of unsaturated fatty acids (77.85%) was much higher than its saturated fatty acids percentage (21.15%) (Table 3). Chinnasamy *et al*., (2010) also observed similar fatty acid profile in crude algal oil using GC. Gouveia & Oliveira, (2009) reported that microalgal lipids derived from *Chlorella vulgaris, Scenedesmus maxima, Nannochloropsis oleabundans, Scenedesmus obliquus and Dunaliella tertiolecta* were mainly composed of unsaturated fatty acids (50-65%).

Products and by products of biomass processing: Three replicates of hundred grams of dried biomass of *C. vulgaris* were used for oil extraction and biodiesel production. Results indicated that 42.66 g of oil was extracted from algal biomass using hexane as a solvent. The extracted oil was converted to biodiesel by transesterification reaction using sodium metal as a catalyst. Yield of biodiesel was obtained by this reaction was 93.52% while rest was converted to byproducts (Table 4). Li *et al.*, (2007) obtained 98.15% yield of biodiesel from algal oil in 12 hours of transesterification using immobilized lipase as a catalyst.

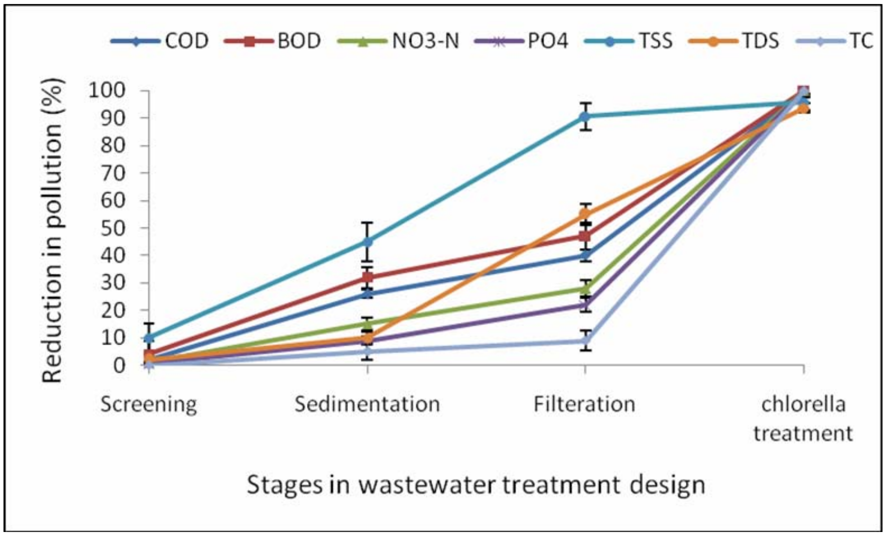

Fig. 3. Percentage reduction in wastewater characteristics at different stages of a biological treatment design.

Table 3. Percentage composition of fatty acids of oil extracted from *C. vulgaris*.

Fatty acid profile	Fatty acid names	Amount of fatty acids in *C. vulgaris* oil (%)
C14:0	Tetradecanoic acid	2.16
C16:0	Hexadecanoic acid	19.0
C16:1	Hexadecenoic acid	9.40
C16:2	Hexadecadienoic acid	0.07
C16:3	Hexadecatrienoic acid	6.34
C16:4	Hexadecatetraenoic acid	7.67
C18:0	Octadecanoic acid	0.69
C18:1	Octadecenoic acid	19.62
C18:2	Octadecadienoic acid	11.2
C18:3	Octadecatrienoic acid	22.12
C18:4	Octadecatetraenoic acid	*n.d.
C20:0	Eicosanoic	0.3
C20:1	Eicosenoic	0.89
C20:2	Eicosadienoic acid	0.75
C20:3	Eicosatrienoic acid	*n.d.
C20:4	Eicosatetraenoic acid	0.02
Saturated	------	21.15
Unsaturated	------	77.85

*n.d. = not detected

Table 4. Quantification of yield of biodiesel and by products synthesized from algal biomass.

Products and by products	*C. Vulgaris* oil samples
Total biomass used (g)	100 ± 0.00
Residual biomass (g)	61.96 ± 6.90
Quantity of oil extracted (g)	42.66 ± 1.88
Yield of biodiesel from extracted oil (%)	93.52 ± 0.78
Glycerin and other by products (%)	6.48 ± 0.78

Quality of biodiesel: Analysis of biodiesel was carried out after its washing by water to remove excess of catalyst and alcohol. Kinematics viscosity, flash point, density, water contents and calorific value of biodiesel was determined in laboratories of Southern Electric Power Company Limited, Raiwind Road Lahore. Results showed that all the parameters were within the limits of ASTM standards (Table 5) hence the biodiesel produced was of good quality and it can be used as vehicular fuel in pure form or blending with diesel fuel.

Table 5. Analysis of biodiesel produced from C. vulgaris ant its comparison with international standards.

Properties	Units	Biodiesel produced	ASTM D-6751~02 Standards
Kinematics Viscosity at 37.8°C	cSt	4.8	1.9-6.0
Flash point	°C	163	>130
Density at 15°C	g/cm^3	0.89	0.85-0.90
Water contents	% Vol.	0.03	0.05% vol.max
Calorific value	MJ/kg	34.5	-------------

Conclusions

Sewage water discharged from household activities contains many inorganic and organic pollutants which can cause serious impacts on human health and aquatic life. Treatment of this wastewater by algal species can be useful method as biomass produced from these algae can be used for biodiesel production. In the current study *C. vulgaris* was used for wastewater treatment and biodiesel production. Results indicated that wastewater treated by this species can be used for irrigation purpose as BOD and COD were recorded to be within national environmental quality standard's limits. Oil extracted from *C. vulgaris* was used for biodiesel production and its quality was compared with ASTM standards for biodiesel. It was observed that properties of biodiesel were within standards limits therefore it can be used as fuel in vehicles.

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