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# Uptake of Nutrients from Municipal Wastewater and Biodiesel Production by Mixed Algae Culture

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**Abstract:** Eutrophication of water bodies is matter of concern for the last few decades because wastewater without treatment can cause serious health effects and disturb functioning of ecosystem. The objective of the current study was to utilize nutrient present in wastewater for algal growth and biodiesel production from harvested biomass of algae. Mixed algae culture (*Microspora sp.*, *Diatoms*, *Lyngbya sp.*, *Cladophora sp.*, *Spirogyra sp.* and *Rhizoclonium sp.*) collected from Botanic Garden of Government College University Lahore was grown in artificial ponds of 13.5 L capacity. Algal growth was monitored for six days by measuring its fresh and dry weight which showed almost similar results of 3.34 g/day and 3 g/day respectively. Uptake of total Kjeldahl nitrogen (55%), total phosphorous (61%), nitrate (97%), phosphate (93%), sulphate (34%) and chloride (41%) was carried by mixed algae culture from wastewater. Seasonal variation in the absorption of nutrients in alternate months of the year showed higher uptake in March, September and November due to favorable conditions for algal growth. Yield of biodiesel produced by transesterification of dried algal biomass was calculated to be 15.13% on an average. Quality of biodiesel was analyzed for kinematic viscosity (4.5 $mm^2/s$), flash point (167°C), specific gravity (0.895 g/ml), iodine value (80 mg/g), acid number (0.65 mg. KOH/g) and water contents (32 mg/kg). It was found that the quality of biodiesel was according to the ASTM standards for biodiesel.

**Key words:** Wastewater, algae, nutrients, biodiesel, growth, transesterification

## INTRODUCTION

Composition of wastewater varies depending upon its source of production but normally it contains pathogens (bacteria, viruses, parasitic worms etc.), organic particles (Urea, proteins, sugars, lipids, feces, humus etc.) and inorganic constituents (ammonia, nitrates, phosphates, sulphates, chlorides etc.). These nutrients cause pollution of water bodies if not properly managed (McGriff and McKinney, 1972; Tam and Wong, 1989). Algae cultivation in wastewater as tertiary treatment process was started in 1970s and it was observed that algae uptake nutrients from domestic wastewater efficiently than any other biological process suggesting that it would be more economical to use it at secondary level (Oswald *et al*., 1978).

Eutrophication of lakes, ponds, drains and streams is normally caused nutrients coming from human activities. Nitrogen and phosphorous are the major cause of eutrophication and these can efficiently be taken up by algae for its growth but excessive growth of algae can affect water quality resulting in decreased transparency and oxygen depletion (Carpenter *et al*., 1998).

Global crises of fossil fuel in the last few years compelled to focus on renewable biofuels (Barbara, 2007). Algae in this respect got attraction due to its higher oil yield per unit area than any other oilseed crop. It is claimed that algae can yield 10 to 100 times more energy per unit area than other second generation biodiesel crops (Sheehan *et al*., 1998; Greenwell *et al*., 2010). Another issue with fossil fuel is increasing $CO_2$ emission which deteriorate the global environment soon if renewable and environment friendly energy source replacement could not found (Marland *et al*., 2007). Currently biofuel produced from algae is expensive but cost can be reduced by growing it on cheap medium as algae require few nutrients for its growth which can be derived from wastewater and even it can be grown on land unsuitable for other crops thus eliminating the competition with food crops. Monoculture of algae is difficult to maintain therefore mixed algae cultures can be used for nutrient removal from wastewater and biodiesel production. The oil yield of algae was estimated to be 5000 to 20,000 gallons per acre per year (Chisti, 2008).

Currently most of the research work on algal biofuel has been carried out in private sector and it is observed from small scale experiments that production of biodiesel from algae can be the only viable method to overcome the current energy crises. It was estimated that to replace the global production of 1.1 billion ton of conventional diesel, a land area of 57.3 million hectares would be required (Shirvani *et al*., 2011).

In the current study mixed algae culture was grown in wastewater to measure its ability to uptake nutrients and

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growth rate. Harvesting of algae was carried out after six days and it was dried, ground and transesterified to check its efficiency for biodiesel production. Quality of biodiesel was analyzed by comparing it with international standards.

## MATERIALS AND METHODS

**Sampling and inoculation of algae:** Algal samples were collected from botanic garden Government College University Lahore and various species present in this mixed algae culture were identified by using methods described by Zarina *et al*. (2005a,b). This algal culture was kept under local outdoor conditions and inoculated into artificial ponds containing municipal wastewater, having dimensions 0.3 x 0.3 x 0.15 m with final capacity of 13.5 L.

**Nutrient uptake analysis:** Wastewater collected from municipal drains of Lahore city was analyzed for Total Kjeldahl Nitrogen (TKN), Total Phosphorous (TP), nitrate ($NO_3$-N), phosphate ($PO_4^{2-}$), sulphate ($SO_4^{2-}$) and chloride ($Cl^-$) by APHA (2005) standards methods and then put into selected ponds to act as medium of growth for algae. Nutrient concentrations were analyzed every second day to measure their decrease in percentage in the wastewater or uptake by algae. Seasonal variations in the uptake of these nutrients were also analyzed in alternate months of the year with different climatic conditions.

**Growth rate and algal biomass analysis:** The rate of growth of these algal species was measured by the estimation of both fresh and dry weight. These algal species were harvested every second day to measure its fresh weight then these samples were dried in an oven at 60°C for 48 h to calculate its dry weight. Algal biomass was analyzed for protein, carbohydrates and lipids. Protein content was determined by the block digestion method and lipid contents by solvent extraction (Boccard *et al*., 1981).

**Transesterification process:** The transesterification process was conducted simultaneously with the extraction in order to avoid the additional step of oil extraction and purification of obtained oil. Hundred grams of dried algae was mixed with 300 mL of hexane and introduced in a thermostated reactor. The mixture will be heated at 62°C and after that; methanol (10 wt% dry basis) having sodium metal (0.1 wt% dry basis) previously dissolved was added to the reactor. Reaction was conducted at the same temperature for four hours with constant stirring at 110 rpm. The reaction mixture, after the reaction, was cooled to room temperature. Then, the solid phase was separated by filtration using a separating funnel under vacuum condition. Finally, the bottom layer of glycerin was separated from the mixture biodiesel and hexane layer (top layer), which was then washed with water to remove excessive methanol and the traces of catalyst (Karaosmanoglu *et al*., 1996; Lang *et al*., 2001).

**Quality of biodiesel:** Biodiesel was analyzed for its various properties (kinematic viscosity, flash point, specific gravity, iodine value, acid number and water contents) and these were compared with ASTM standards to estimate the quality of obtained biodiesel.

## RESULTS AND DISCUSSION

**Mixed algae culture:** Identification of algal samples revealed that it contained various species of algae which were:

- *Microspora* sp.
- *Diatoms*
- *Lyngbya* sp.
- *Cladophora* sp.
- *Spirogyra* sp.
- *Rhizoclonium* sp.

**Nutrient uptake in time:** Retention time of algae in wastewater has profound effect on nutrient uptake efficiency because algal bimass increased and required more nutrient for its stable growth. In the current study wastewater was exposed to algae for six days and nutrient analysis was carried out every second day. A control setup without algae was run to estimate the nutrient reduction by oxidation or other natural sources, which was deducted from the experimental results to minimize error in nutrient uptake. It was found that maximum uptake of nitrate (97%) and phosphate (93%) was carried out by algae as these were directly absorbed while a significant uptake of other nutrient was also observed (Fig. 1). Nitrogen and phosphorous removal ability of chlorella species was also assessed by Wang *et al*. (2010) with different retention times ranging from 10 hrs to 42 days.

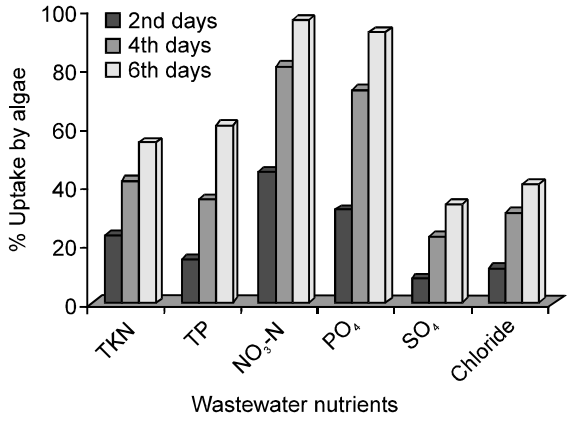


Fig. 1: Uptake of nutrients from medium by algae with change in time period

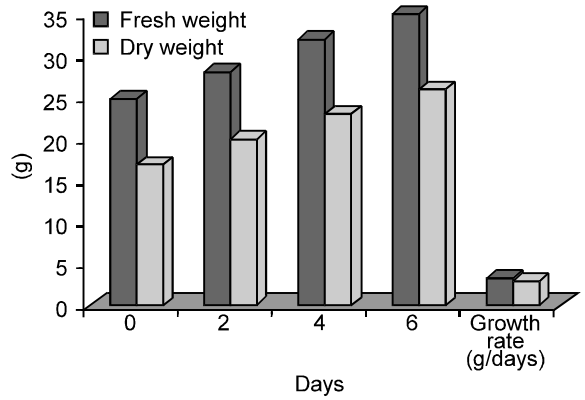


Fig. 2: Growth rate of algae cultured in artificial ponds with change in time period

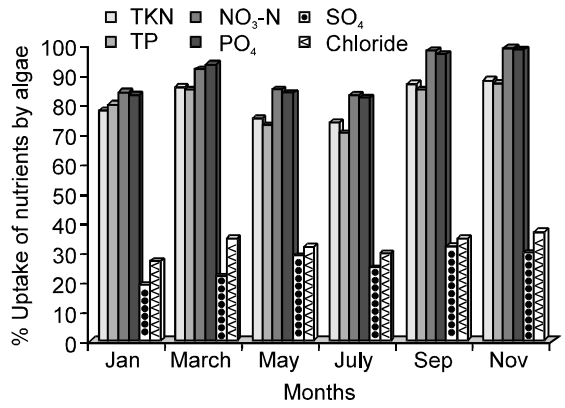


Fig. 3: Seasonal variation of absorption of nutrients from wastewater

**Increase in fresh and dry weight:** Growth rate of mixed algae culture was assessed by measuring its fresh and dry weight every second day from 0-6. Algal biomass was increased with increase in retention time but on average similar increase was observed both in fresh (3.34 g/day) and dry weight (3 g/day) after six days (Fig. 2). Ruiz-Marin *et al*. (2010) performed many experiment under batch culture condition in which microalgae showed high growth rates in initial days but growth and chlorophyll contents were decreased after four cycles of culture indicating collapse of the culture due to nutrient deficiency.

**Effect of seasonal variations on nutrient uptake:** Nutrient uptake in alternate months of the years was observed and it was found that maximum nutrients were absorbed by algae in months of march, September and November because weather conditions were favorable for the growth of algae. It was also observed that uptake of sulphate and chloride were constantly absorbed in low percentage throughout the year so wastewater having $SO_4^{2-}$ and $Cl^-$ can not be efficiently treated by algae (Fig. 3). Algae belonging to chlorophyta absorbed more nitrogen and phosphorous and absorption further increased if phosphorous contents remained high (Li *et al*., 2011).

**Analysis of dried algae:** Three samples of dried algal biomass were analyzed for proteins, carbohydrates and lipids to minimize error. It was found that algae were rich in protein (46.4%), carbohydrates (26.17%) and lipid (17.53%) contents (Table 1). It was deduced from the results that this mix algae culture can be used for biodiesel production because it is difficult to maintain monoculture of algae due to problem of contamination with other species. Li *et al*. (2007) calculated lipid content of heterotrophic *Chlorella protothecoides* up to 46.1%, 48.7% and 44.3% of cell dry weight in samples from 5 L, 750 L and 11,000 L bioreactors, respectively.

**Quantity and yield of biodiesel:** Fifty gram of dried algal biomass was used for the production of biodiesel in triplicate and it was found that quantity and yield of biodiesel was almost comparable in all three samples. Mean value showed that yield of biodiesel was (15.13%) and left was residual biomass (39.23%). A deficiency of 7.31% of experimental material was stuck with glassware or lost during transesterification process (Table 2). The residual biomass can be used as an organic fertilizer.

Table 1: Chemical composition of mixed culture algae on dry matter basis

| Algal samples | Protein (%) | Carbohydrates (%) | Lipids (%) | Others (%) |
|---|---|---|---|---|
| Sample 1 | 46.4 | 24.10 | 17.30 | 12.2 |
| Sample 2 | 44.5 | 29.20 | 16.00 | 10.3 |
| Sample 3 | 48.3 | 25.20 | 19.30 | 7.2 |
| Mean value | 46.4 | 26.17 | 17.53 | 9.9 |

Table 2: Quantification of yield of biodiesel synthesized from algal biomass

| Parameters | Sample 1 | Sample 2 | Sample 3 | Mean value |
|---|---|---|---|---|
| Dry weight (g) | 50.0 | 50.0 | 50.0 | 50.00 |
| Residual biomass (g) | 38.2 | 40.0 | 39.5 | 39.23 |
| Quantity of biodiesel produced (g) | 8.5 | 6.9 | 7.3 | 7.57 |
| Yield of biodiesel (%) | 17.0 | 13.8 | 14.6 | 15.13 |

Table 3: Analysis of biodiesel produced from mixed culture algae and its comparison with international standards

| Properties | Units | Biodiesel produced | ASTM D-6751~02 Standards |
|---|---|---|---|
| Kinematic viscosity at 40°C | $mm^2/s$ | 4.5 | 1.9-6.0 |
| Flash point | °C | 167 | >130 |
| Specific gravity at 28°C | g/ml | 0.895 | 0.88 |
| Iodine value | (mg/g) | 80 | <120 |
| Acid number | mg. KOH/g | 0.65 | 0.8 max |
| Water contents | mg/kg | 32 | <300 |

**Biodiesel analysis:** Biodiesel produced as a result of transesterification of direct algal biomass was analyzed for kinematic viscosity, flash point, specific gravity, iodine value, acid number and water contents (Table 3). On comparison with ASTM D-6751~02 standards it was found that biodiesel of this study was of good quality and can be used directly or blending with conventional diesel and petrol. Similar results with chlorella were found by Li *et al*. (2007).

**Conclusion:** Wastewater contains many inorganic and organic nutrients, which come into water stream by anthropogenic activities. Eutrophication occurs when these nutrients are present in excess thus causing damage to ecosystem. Utilization of these nutrients for useful purposes was carried out in this study. Mixed algae culture was grown in wastewater as monoculture of algae required more precautionary measure to avoid contamination with other species. Results indicated that mixed algae culture absorbed nutrients from wastewater efficiently. Harvested algal biomass after 6th day of inoculation was dried, ground and transesterified to biodiesel by mixing it with methanol and sodium metal as a catalyst. About fifteen percent of the used algal biomass was converted into biodiesel, whose quality was assessed and compared with ASTM standards. It was proved from the results that algae can play an efficient role in removing nutrients from municipal wastewater and production of good quality biodiesel.

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