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Influence of culture media and carbon sources on biomass productivity and oil content of the algae *Sirogonium sticticum, Temnogyra reflexa, Uronema elongatum,* and *Chroococcus turgidus*

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Abstract: The aim of the present study was to investigate the effect of nutritional formulations, viz. Bristol's medium (BM), water extract of dairy waste (DW), commercial fertilizers mix (FM), and carbon sources such as $NaHCO_3$, $CaCO_3$, and wheat flour hydrolysate on the biomass productivity and oil content of the algal species *Sirogonium sticticum*, *Temnogyra reflexa*, *Uronema elongatum*, and *Chroococcus turgidus*. Among the nutritional formulations tested, BM showed the highest average effect, followed by DW, on the biomass and oil contents of the species. As for carbon sources, $CaCO_3$ was more efficient in enhancing algal biomass, while $NaHCO_3$ was proficient in increasing the oil content of the species. *S. sticticum* produced the highest average biomass and oil content regardless of the media and carbon sources. It was concluded that in terms of cost effectiveness and commercial availability, DW was a suitable medium for commercial cultivation of microalgae on a large scale.

Key words: Biomass, carbon sources, media, *Sirogonium sticticum*, *Temnogyra reflexa*, *Uronema elongatum*, *Chroococcus turgidus*

1. Introduction

Due to rapidly growing urbanization and industrialization, energy requirements are increasing day by day. It has been predicted that the world's oil requirements will increase by 50% in the upcoming third decade of the 21st century, while the present petroleum reserves are diminishing very rapidly (Khan and Dessouky, 2009; Rhodes, 2009). These resources of fossil fuels will not be available in the same amount and at the same price in future. In addition, the burning of fossil fuels causes severe environmental and economic damage due to the release of greenhouse gases (Mullner et al., 2007; Sialve et al., 2009). Therefore, the world's economists are searching for energy resources alternative to fossil fuels that are environmentally friendly, sustainable, and available in an affordable price range compared to the current petroleum price (Oguchi et al., 1989; de Vries et al., 2010; Johnson et al., 2010). Photosynthetic autotrophs are considered as one of the substitute sources of energy, especially for biofuel production. However, plant resources have certain limitations, i.e. competition with food, land utilization, long cultivation time, low yield, seed toxicity, and only seeds contain oil in extractable quantities. Therefore, algae appear to be the cheapest source among all the renewable sources for biodiesel production. Green microalgae contain 20% to 70% lipid and exhibit extraordinary potential for cultivation as energy crops (Xu et al., 2006; de Vries et al., 2010). They do not need arable land for cultivation and can be grown in industrial, municipal, and agricultural effluents, and fresh- and seawater (Chinnasamy et al., 2010). The growth and oil producing efficiency of algae is much higher than that of conventional and oil seed crops such as corn and soybean (Li et al., 2008). Due to its high oil content, various countries like the UK, USA, China, Belgium, Denmark, India, and Singapore have started utilizing algae as a source of biofuels on a commercial scale.

Cultivation modes and nutritional management affect the growth rate and biochemical composition of algae (Hsieh and Wu, 2009). Previous studies have demonstrated that the biomass and lipid content in some microalgae were affected by various cultivation conditions such as nitrogen supply and its sources (Li et al., 2008; Arumugam et al., 2013), salt concentration (Takagi et al., 2006), level of CO_2

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(Chinnasamy et al., 2009), light intensity, and temperature (Renaud et al., 2002; Khotimchenko and Yakovleva, 2005; Juneja et al., 2013). These observations allowed us to hypothesize that there might be a profound effect of media type and carbon sources on the biomass and oil content of the microalgae *S. sticticum*, *T. reflexa*, *U. elongatum*, and *C. turgidus*. Therefore, this study was conducted to evaluate the effect of nutritional formulations, viz. Bristol's solution, fertilizer mix, and water extract of farmyard manure, and carbon sources such as $NaHCO_3$, $CaCO_3$, and wheat flour hydrolysate on the biomass and oil content of the aforementioned algal species. First, the effect of media on the biomass and oil content of the selected species was investigated. Next, the basal nutritional medium (Bristol's solution) was modified with the carbon sources and their effect was studied on the biomass productivity and oil contents of the selected algal species.

2. Materials and methods

2.1. Culture collection, isolation, and identification

Algal strains *S. sticticum*, *T. reflexa*, *U. elongatum*, and *C. turgidus* were collected from freshwater sources in Khyber Pakhtunkhwa Province of Pakistan. All the samples were collected in Falcon tubes containing freshwater and identified at the Department of Agricultural Chemistry, the University of Agriculture Peshawar, Pakistan (Prescott, 1951; Guiry and Guiry, 2014). After identification, the selected algal species were cultured in 250-mL Erlenmeyer's flasks containing Bristol's basal medium for subsequent cultivation in open ponds.

2.2. Media formulation and carbon sources

The experiment was carried out with three nutritional media: Britol's medium (250 mg of $NaNO_3$, 75 mg of K_2HPO_4, 175 mg of KH_2PO_4, 25 mg of $CaCl_2$, 25 mg of NaCl, 75 mg of $MgSO_4.7H_2O$, 0.3 mg of $FeCl_3$, 0.3 mg of $MnSO_4.4H_2O$, 0.2 mg of $ZnSO_4.7H_2O$, 0.2 mg of H_3BO_3, and 0.06 mg of $CuSO_4.5H_2O$ per 1000 mL of distilled water), fertilizer mix (4 g of diammonium phosphate, 5 g of single super phosphate, 3 g of calcium ammonium nitrate, 3 g of NPK, 2 g of urea, and 2.5 g of nitrophos per 1000 mL of distilled water), and water extract of dairy wastes [farmyard manure:water (1:5)]. The carbon sources used in the present study were sodium bicarbonate (baking soda), calcium carbonate (chalk), and wheat flour hydrolyzed in boiled water. Five grams of each carbon source were separately dissolved in 1000 mL of Bristol's basal medium solution and 10 mL of each was applied to the algal strain in each open pond.

2.3. Experimental design and application of media and carbon sources to the algal strains

The study was completed in two sets of experiments. The first experiment was conducted in a complete randomized design with four algal species and three media each with three replications. The control treatments were used without any media. The same design was followed for the second set of experiments; however, in this case the media were replaced with carbon sources. A total of 48 open ponds each with dimensions of 61 × 61 × 45.72 cm were constructed of concrete and filled with 50 L of water. The ponds were divided into four groups, each consisting of 12 ponds. Each group was assigned to a single species. Three grams of wet biomass of each species was diluted into 300 mL of distilled water, shaken vigorously, and transferred in equal amounts (25 mL) to the twelve ponds. Ten milliliters each of the three media (BM, FM, and DW) was randomly applied to triplicate ponds in each group. The control ponds were kept without the addition of any media. Fresh media were added to each pond at a regular interval of 5 days for 15 days. At the end of the experimental period, samples were collected for the determination of biomass and oil contents. After the completion of the first experiment, all the ponds were evacuated, washed with clean water, and air-dried. The same design was used for the application of carbon sources as discussed previously for the media. The carbon sources, viz. $NaHCO_3$, $CaCO_3$, and flour hydrolysate (10 mL per pond), were applied to individual species in triplicate ponds keeping the Bristol's medium as the basal medium for all species. The control ponds were kept without the addition of any carbon source. Fresh solutions of the carbon sources (10 mL per pond) were added to each pond at a regular interval of 5 days for 15 days. Samples were taken on the 15th day of the experiment and tested for biomass and oil content.

2.4. Biomass estimation

The biomass was determined spectrophotometrically. At the end of the experimental period (15 days), the cell growth in each treatment was measured by examining the optical density of the algal suspension at 686 nm. The absorbance reading was transformed to biomass concentration (g/L) using a regression equation (Xu et al., 2006).

2.5. Lipid extraction

Lipid content of the algal species was determined by the method of Bligh and Dyer (1959). Briefly, 2 g of dried algae biomass was successively extracted with 7.5 mL of chloroform, methanol (1:2) mixture, 2 mL of chloroform, and 2 mL of water each for 10 min in a pre-weighed glass tube. The solid particulates of algae were removed by filtration through Whatman No. 1 filter paper. The filtrate was centrifuged at 1000 rpm for 5 min to obtain a biphasic system of water and organic solvents. The water phase was discarded and the organic phase was recovered for the estimation of oil content. The oil content was calculated gravimetrically by using the formula

Oil content (%) = (weight of the oil extracted/fresh weight of sample) × 100.

2.6. Statistical analysis

The data were subjected to analysis of variance (ANOVA) by using a completely randomized design (Steel et al., 1996). All the analyses were carried out with the statistical software package Statistix 8.1. Means were separated by least significant difference (LSD) test at P = 0.05. Each mean was calculated from triplicate values.

3. Results and discussion

3.1. Effect of media on algal biomass production

The effect of media, i.e. BM, FM, and DW, on the biomass production of *S. sticticum*, *T. reflexa*, *U. elongatum*, and *C. turgidus* is shown in Figure 1. Significant ($P < 0.05$) variation existed between the species with respect to biomass production on different media. Out of the three media tested in the experiment, BM exhibited the best results on algal growth followed by DW, whereas the lowest amount of biomass was produced in control ponds. The interactive response of algal species and media indicated that *S. sticticum* and *U. elongatum* produced higher biomass (0.87 g/L each) on BM, whereas the lowest biomass (0.11 g/L) was produced by *C. turgidus* in the control treatment. Regarding species, *S. sticticum* produced the highest average biomass (0.67 g/L) followed by *T. reflexa* (0.66 g/L), whereas the lowest average value (0.20 g/L) was recorded for *C. turgidus*. Our results were supported by the work done by Mohan et al. (2009), who demonstrated a remarkable increase in the growth of *C. vulgaris* cultured on CFTRI medium as compared to the control. In another study, Mohan et al. (2010) reported that *C. turgidus* and *Oscillatoria* spp. showed a steady increase in biomass after 5 days of culturing on CFTRI medium. Similarly, Woertz et al. (2009) used anaerobically pretreated dairy wastewater in different dilutions as a growth medium for biomass production of algae and concluded that the maximum biomass production was due to higher nutrient concentrations available for algal growth. It was seen in the present study that DW showed promising results in enhancing algal biomass, making it an economical nutritional media for large-scale cultivation of algae. This may be due to the fact that dairy waste is a rich source of all micro- and macro-elements needed for plant growth (Negassa et al., 2005). Since the ecological and nutrient need may affect the biomass production of different algal species differently (Munn et al., 2010), it could be assumed that physical habitat in the present study was not favorable for enhanced biomass production of *C. turgidus* despite the similar feeding regime.

3.2. Effect of media on oil content

The amount of oil produced by *S. sticticum*, *T. reflexa*, *U. elongatum*, and *C. turgidus* on different media was determined by the interaction of algal species and nutritional media, and their effects on oil content are shown in Figure 2. Analysis of variance indicated that the individual effects of species and media were significant ($P < 0.05$), whereas the interactive effect was nonsignificant ($P > 0.05$). Among the individual effects, species had a higher impact than media on oil content. In the present study, the highest amount of oil (8.37%) was produced by *S. sticticum* on BM, whereas the lowest amount (6.25%)

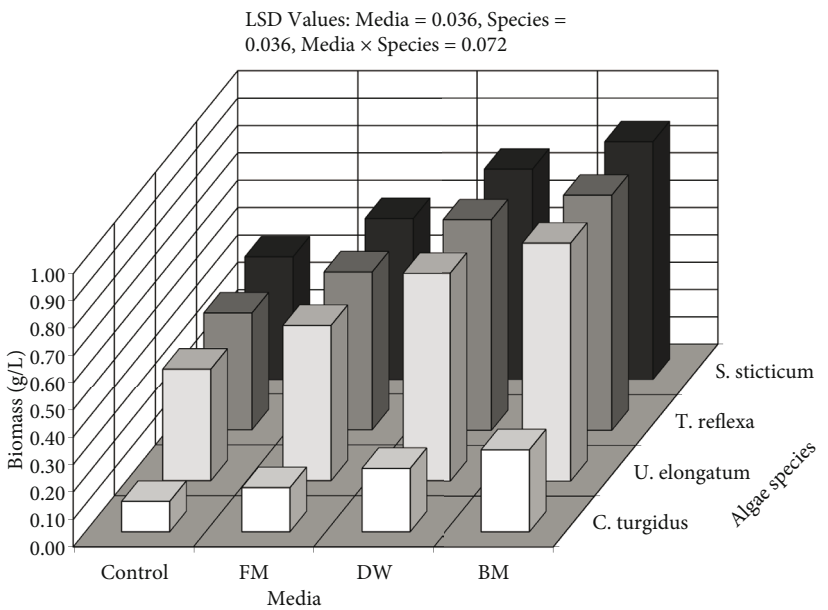

Figure 1. Effect of different media (FM = fertilizer composite, DW = dairy wastes, BM = Bristol's medium) on biomass production of selected algal species.

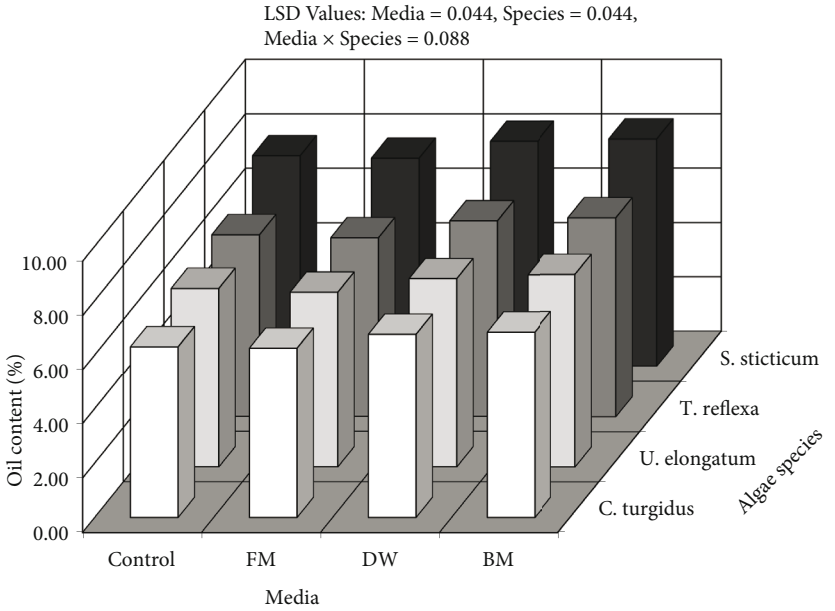

Figure 2. Effect of different media (FM = fertilizer composite, DW = dairy wastes, BM = Bristol's medium) on oil content (%) of selected algal species.

was seen for *C. turgidus* grown on FM. When the average oil contents of all the species on different media were compared, the oil production was maximum on BM. This indicated that BM not only enhanced the biomass (Figure 1) but also positively affected the oil content of the species. This showed an interaction of balanced nutrient formulation with the oil production efficiency of algal species. The high oil production potential of all four algae grown in BM medium might be due to the availability of sufficient nutrients for high biomass production and eventually high lipid content. The findings of the present study coincided fairly well with the work done by other researchers. Woertz et al. (2009) reported high lipid productivity of algal species under nutrient sufficient conditions when dairy wastewater was used for biomass production. In the present study, the lower amount of lipid production by algae species cultured on FM might have been due to high concentrations of nitrogen and phosphorus, because excess of these nutrients caused a decrease in lipid synthesis in algae cells (Mutlu et al., 2011). Kurt et al. (2010) investigated the lipid biosynthesis of *C. vulgaris* grown in high and low nitrogen-containing Bold's media, and reported that lipid production increased in nitrogen-deficient media.

3.3. Effect of carbon sources on biomass production

Figure 3 shows the effect of carbon sources, viz. $NaHCO_3$, $CaCO_3$, and wheat flour hydrolysate, on the biomass productivity of the four algal species. The highest amount of biomass (1.62 g/L) was recorded for *U. elongatum* when its basal medium was supplemented with $CaCO_3$. The lowest biomass (0.35 g/L) was produced by *C. turgidus* in the control treatment. Analysis of variance indicated that carbon sources had a greater impact on biomass productivity than the type of species. Comparing the average values, CO_3^{-2} carbon produced better results than HCO_3^{-1} and flour hydrolysate. Regarding the species, *S. sticticum* and *U. elongatum* produced the highest average biomass (1.22 g/L), whereas the lowest average value (0.74 g/L) was recorded for *C. turgidus*.

Most of the algae strains were able to utilize carbonates and bicarbonates but the carbon source preferred most for higher biomass production was CO_3^{-2}. The fast biomass production of these algae was due to high availability of dissolved CO_2 dehydrated by carbonic anhydrase enzyme of algal chloroplast from the carbon sources in which carbonate was the readily ionizable substance. It was suggested that at elevated levels of CO_2 algae showed sharp increases in biomass production due to increased photosynthetic rates as shown in the experiment conducted by Chinnasamy et al. (2009), who investigated the growth responses of *C. vulgaris* under different CO_2 concentrations and concluded that algae boosted their growth superiorly at high CO_2 concentrations. Spalding et al. (1983) studied the activity of carbonic anhydrase in wild-type *Chlamydomonas reinhardii* and concluded that this enzyme converted bicarbonate to CO_2, thus stimulating photosynthesis in algae. Kim et al. (2009) studied the growth kinetics of *C. vulgaris* under different pH conditions in Na_2CO_3 medium and reported that at pH 7 to pH 9 bicarbonate ions served as an inorganic

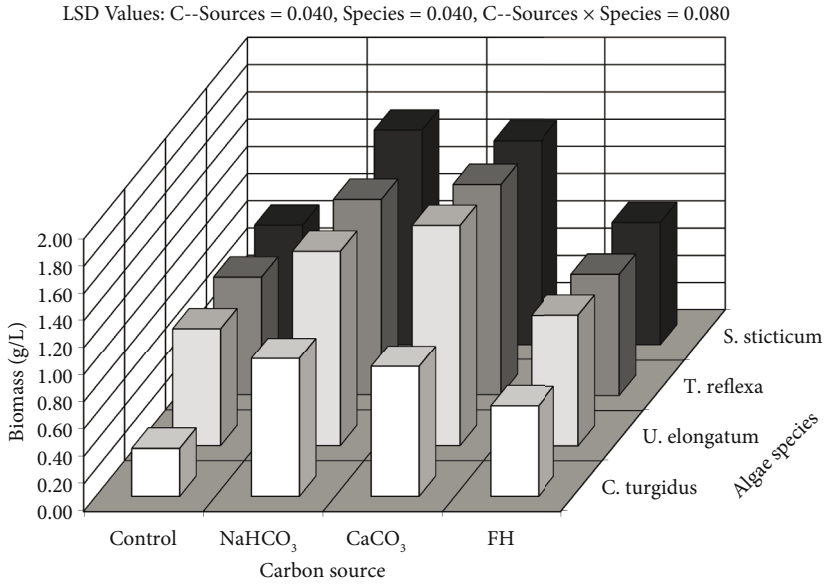

Figure 3. Effect of carbon sources ($NaHCO_3$ = sodium bicarbonate, $CaCO_3$ = calcium carbonate, FH = flour hydrolysate) on biomass production of selected algal species.

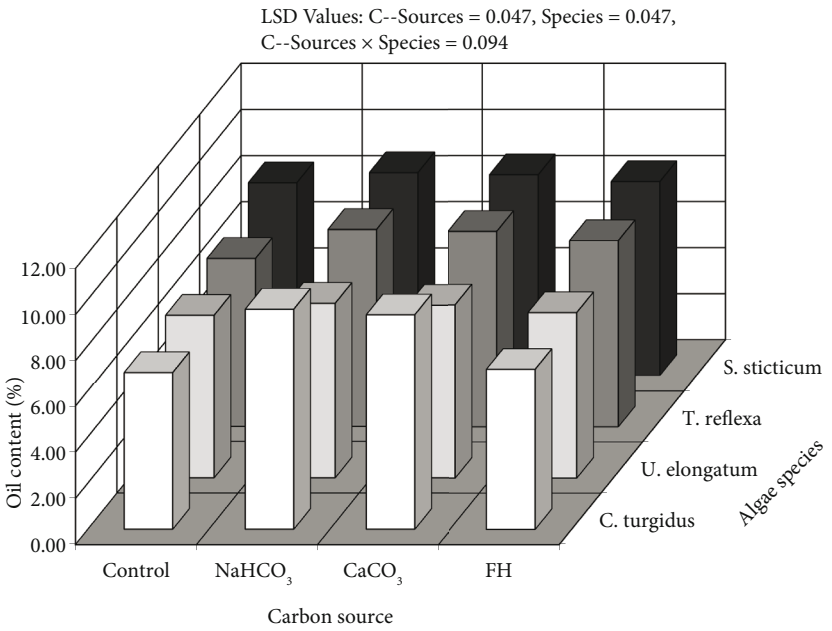

Figure 4. Effect of carbon sources ($NaHCO_3$ = sodium bicarbonate, $CaCO_3$ = calcium carbonate, FH = flour hydrolysate) on oil content (%) of selected algal species.

carbon source for rapid growth of algae. Similar results were observed by Widjaja (2009), who demonstrated that biomass productivity of *C. vulgaris* could be increased by increasing CO_2 concentration.

3.4. Effect of carbon sources on oil content

The highest amount of oil (9.57%) was recorded for *C. turgidus* grown on $NaHCO_3$, whereas the lowest value (6.82%) was seen for the same species grown on the control treatment (Figure 4). Comparing the average individual effects of carbon sources, $NaHCO_3$ was found to be the best carbon source for enhanced production of oil by the selected algal species. Regarding the species, the highest average value of oil content (8.59%) was recorded for *S. sticticum*, followed by *C. turgidus* (8.17%), whereas the lowest average value (7.35%) was seen for *U. elongatum*. The present study was supported

by the work done by Abdo et al. (2013), who reported 10% lipids for *C. turgidus*. The enhanced production of lipid at optimum level of C-source may be explained by the fact that algae require a steady supply of carbon-containing precursors, particularly acetyl-CoA for lipid biosynthesis (Rodolfi et al., 2009). Ohlrogge and Browse (1995) assumed that acetyl-CoA might provide all of the carbon atoms for fatty acid synthesis in plastid, which in turn was produced from plant photosynthate through the action of pyruvate dehydrogenase. In the present study, lipid production was enhanced when there was an optimum level of available carbon. These propositions strongly support our findings.

It was concluded from the present study that both the media types and carbon sources significantly influenced the biomass productivity and oil content of the microalgae *S. sticticum*, *T. reflexa*, *U. elongatum*, and *C. turgidus*. All the species produced the highest biomass and oil content on Bristol's medium followed by DW. Amongst the carbon sources, $CaCO_3$ was best for higher biomass production, whereas $NaHCO_3$ application resulted in higher oil content in the selected species. However, the effect of $CaCO_3$ and $NaHCO_3$ on biomass production nonsignificantly varied between each other. Regarding the algal species, *S. sticticum* showed the overall best results for biomass production and oil contents. Keeping in view the cost effectiveness and commercial availability, DW was recommended for mass cultivation of microalgae. However, it is suggested that further modification in nutrient media should be carried out and genetically modified species may be adapted on cost effective nutritional formulations to increase oil production for biodiesel technology.

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