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Mixotrophic Cultivation of *Chlorella vulgaris* on Banana Waste for Biodiesel Production

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ABSTRACT

Environmentally friendly biofuels are currently produced in large quantities using algal lipids. **Objective:** To perform mixotrophic cultivation of *Chlorella vulgaris* on Banana Waste for Biodiesel Production. **Methods:** Banana waste was treated with acid/alkaline, ground, and sun dried. The resultant hydrolysate was used into studies comparing photoautotrophic and mixotrophic conditions in microalgae culture. When biomass productivity and lipid content were measured. For mono-unsaturated, poly-unsaturated, and saturated forms, the lipid content differed. The research used analytical methods for fatty acid methyl ester analysis, such as GC-MS. **Results:** Mixotrophic cultivation exhibited a much higher biomass productivity (135 mg L-1 d-1) than photoautotrophic cultivation (115 mg L-1 d-1). Additionally, mixotrophically raised biomass had a much larger (w/w) lipid content (45%) than photo-autotrophically raised biomass (30 %). Higher amount of polyunsaturated fatty acids (palmitic and oleic acids) was shown by Lipidome. **Conclusions:** High-quality biofuel might be made possible by the regular availability of polyunsaturated fatty acids (64 mg g-1 of dry biomass) in the lipid contents of mixotrophically produced algal biomass

INTRODUCTION

Fossil fuels are the primary source of energy in the entire world. Furthermore, there is an uneven distribution of fossil fuels among nations and they are all non-renewable. Fossil fuels are decreased day by day and we totally depend upon these fossil fuels for our needs so it is very compulsory to develop other eco-friendly, sustainable and low-cost energy resources [1]. This issue has grown increasingly serious as a result of Pakistan's growing reliance on fossil fuels for transportation and production, which has a detrimental effect on the economy of the nation [2]. With the rapid growth of the world's population and the need for human energy, the scientific community needs to look at different energy sources [3]. Other fuels, especially biodiesel, are now considered to be some of the most effective ways to support our economic growth. Microalgae produces many lipids, which can be collected and used to make biodiesel [4]. The Chlorella genus of unicellular freshwater microalgae exhibit the greatest potential due to their heterotrophic production capability, high productivity, and protein content [5, 6]. Although mass production is required to achieve economically sustainable production of algal fuel and value-added goods, the exorbitant price of nutrients required for microalgae growth is a necessary to restraint [7]. Microalgae are found

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all over the world. Microalgae do not need to grow in arable land, can grow in seawater or residual nutrients [8]. Due to the high cost of the methods, the commercial production of microalgae biomass is very limited. In the face of massive disease growth and commercial production, the price of moderate growth is a major problem [9]. Microalgae are microscopic and common photosynthetic molecules. Basically, they supply 50% of the oxygen in the air and store CO_2. Microalgae with high growth rates and high productivity are essential for the production of biofuel. Dry waste from 37 to 55 percent of *C. vulgaris* contains starch in the chloroplasts, cellulose, and hemicellulose on the cell walls [10]. Through this research people will be able to produce energy through banana peels which automatically reduce the use of natural resources by production of biofuels and help to minimize the environmental pollution by utilizing waste Furthermore, microalgae, which have a high growth rate, high lipid content, and the capacity to survive in adverse conditions, are the most practical source of biofuel. This is the first study to cultivate *C. vulgaris* on banana waste for the manufacture of biofuels in order to determine the most effective method for producing biomass as well as lipid and fatty acids.

METHODS

The Applied and Environmental Microbiology Laboratory at the Department of Wildlife and Ecology at the University of Veterinary and Animal Sciences, Lahore, Pakistan, provided the pure culture of the microalgal strain. In Lahore, trash like banana peels was gathered from milk or fruit chat shop. The banana waste was taken to laboratory for further process. The banana waste was sun dried for few days and then dried a t60°C in an electric oven that was followed by grinding to a size of 2 cm [11, 12]. Acidic and alkaline treatments was applied. Prior to BP treatment (banana peels treatment) the banana waste was subjected to three separate sulfuric acid treatments in 500 ml flasks (0, 0.5, and 1% v/v), then alternatively in an autoclave at 121℃ for 15 minutes [13]. Both lines are same. 10 g from bananas were soaked in 5% of sulfuric acid and then burned for between 15 and 20 mints at 121 °C. Alkaline treatment was done with 5% sulfuric acid dissolved in 10 grams of banana peel and autoclaved at 121 °C for 15-20 minutes [14]. The organic matter was subjected to treatment with 0.5% sulphuric acid. It was then autoclaved at 121°C for 15 minutes. Banana waste was mixed in 10g sulfuric acid (5%). Banana peels were heated first. It was cooled and dried in an oven at 78°C before being treated with sulphuric acid (95 ml water and 5 ml sulphuric acid). 10 g of banana waste were added to the sulfuric acid (5%). After mixing, banana waste was autoclaved at 121°C for 60 and 90 minutes. The banana waste hydrolysate was obtained after filtration using Whatman filter paper that included carbon (sugars). Both photoautotrophic and mixotrophic techniques were used to develop the microalgal strain [15, 16]. Using two integrated batch experiments, the algal biomass was maximized. In the original experimental configuration, microalgae strains were grown in BG-11 medium under photoautotrophic conditions (using Co_2 from the atmosphere as the only carbon source). In the second investigation, BG-11 was supplemented with 2% hydrolysate of banana peels as a carbon source. Therefore, by combining banana peel hydrolysate and natural Co_2 as both carbon sources, the second experimental configuration allows microalgal cells to multiply effectively. For every experiment requiring the growth of microalgae for various trophic conditions, sterilized clear, autoclavable plastic vessels with an average of 10 L volume were used in triplicate. To promote the development of microalgae, a twelve-hour light/dark cycle and aeration with a filter (11 L min-1) are employed. After 24 hours of incubation, the microalgal rates of growth and nutritional deficiencies (nitrates and phosphates) had been assessed. Aluminum sulphate was used to precipitate micro-algal growth after a successful 20-day incubation period. By putting the banana biomass into an electric oven set at 60℃ for two days, the precipitated micro-algal cell were completely dehydrated and the following formula was used to compute the biomass productivity:

P= DX/Dt

P equals biomass productivity (mg L-1 d-1), DX equals variation in biomass concentration (mg L-1), and Dt is change in cultivation time (day).The calculation of lipid content (percent) is done using the following formula: Lipid content (percent) is calculated as The weight of recovered lipid (g)/Weight of microalgal biomass (g)= 100.

Lipid content (%) was calculated by the following formula:

$$\text{Lipid content (\%)} = \frac{\text{Weight of extracted lipid (g)}}{\text{Weight og micro - algal biomass (g)}} \times 100$$

Lipids were extracted from dried microalgal biomass by combining 1 g of dry biomass and powder with 10 cc of heinicosanoic acid; the mixture was then mixed with 200 mL of a 0.9 % (w/v) sodium chloride, 200 mL of hexane, and 5% acetyl chloride in methanol. After that, it is cooked in a boiling water bath at 90°C for an hour. After that, the mixture is crushed and spun for five minutes at 2000 rpm to ensure for layer separation. Hexane layer (100 ml) was combined with heptadecanoic acid (10 mL) prior to the analysis of the acid methyl esters. Microalgal FAME testing was performed using GC-MS. FAME was separated using a polar capillary column (diameter 0.25 mm; length 30 m; thickness of film 0.25 lm). An injector is placed after the column has been heated to a temperature of 265 to 250°C

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[17]. The technique was used to inject a heptane mixture (3-5 mg in 1 mL). A 150:1 dividing ratio is required. Comparing the results to the norms for polyunsaturated fatty acids (PUFA), saturated fatty acids were found in all forms [18]. The acquired data were displayed as mean the standard error of the three replicates. One way ANOVA Duncan test was used to assess lipid content and biomass production under photoautotrophic and mixotrophic conditions. The lipid content there are different values for mono-unsaturated and poly-unsaturated and also for unsaturated. In mono-unsaturated mixotrophic and photo-autotrophic cultivation produced the 55 and 38 mg g-1 and in poly-unsaturated 62 and 37mg g-1 respectively and in saturated 42 and 32mg g-1. In biomass productivity mg L-1 day-1 mixotrophic cultivation produced 135 and photo-autotrophic cultivation produced 115 mgL-1 day-1. Its means the mixotrophic condition was more significant.

RESULTS

The aim of this work was to assess the biomass output, lipid content, and *C. vulgaris* cultivated under photoautotrophic and mixotrophic conditions. The increase of microalgal biomass revealed intriguing results under various trophic circumstances. The biomass's productivity under mixotrophic conditions, C. *vulgaris* seems to be substantially more numerous than under photoautotrophic ones. Mixotrophic culture generated 140.37 mg L-1 d-1 of algal biomass (Figure 1).

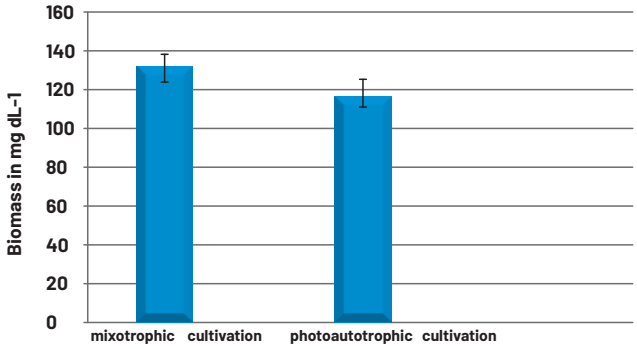

Figure 1: Biomass production in photoautotrophic and mixotrophic environments

The mixotrophic cultivation of *C. vulgaris* has been observed for 5 to 15 days. In photoautotrophic and mixotrophic cultivation, the lag rate is seen to be up to 4 and 7 days, respectively. The amounts of monosaturated, polyunsaturated, and saturated fatty acids were much higher. Comparisons of fatty acid concentrations showed that cultures that had been grown photo autotrophically had lower quantities of each fatty acid. The most abundant fatty acid types in cultures grown in a mixotrophic environment were linoleic acid, linolenic acid, oleic acid, palmitic acid, palmitoleic acid, and stearic acid. All of these fatty acid types fell inside the C16 to C18 range, cultivation of *C. vulgaris* (Figure 2).

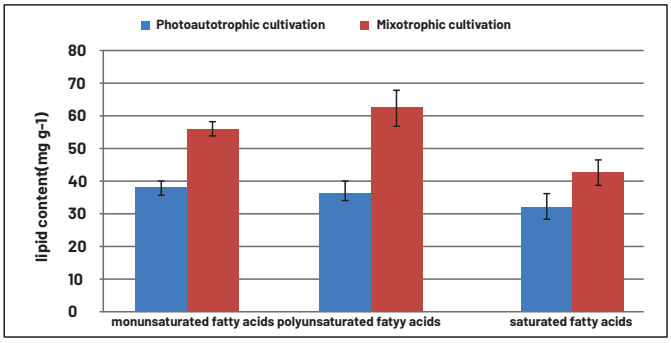

Figure 2: Concentration of lipids in biomass produced mixotrophically and phototrophically

Compared to photoautotrophic cultivation, which produced only 91.57 + 7.9 mg L-1 day-1 of biomass, mixotrophic farming produced 135.43 + 13.3 mL-1 day-1. When compared to photoautotrophic cultivation, mixotrophic cultivation had a much higher biomass lipid content (39 percent) (Table 1 and Figure 3)

Table 1: Statistical analysis of the data for growth parameters of micro-algae

Day Number	Mixotrophic cultivation	Photoautotrophic cultivation
Day 1	1.6a ± 0.3	1.6a ± 0.3
Day 2	1.8a ± 0.2	67.9b ± 0.2
Day 3	3.4a ± 0.4	400.7b ± 0.4
Day 4	5.6a ± 1.1	789.8b ± 1.2
Day 5	15.6a ± 1.4	1456.7b ± 1.4
Day 6	77a ± 6.7	3878.8b ± 6.5
Day 7	407.8a ± 64	6754b ± 543
Day 8	1789.5a ± 789	9765b ± 784
Day 9	4567.4a ± 657	11234b ± 665
Day 10	8768.9a ± 987	12456b ± 56
Day 11	15678a ± 982	13567b ± 32
Day 12	18765a ± 567	13567b ± 32
Day 13	20876a ± 321	14987b ± 43
Day 14	21367.8a ± 923	15124b ± 764
Day 15	21987.7a ± 777	15234b ± 554

Here a and b shows statistical significance and CFU denoted the colony forming units

Micro algal growth/cell number (CFU ml-1x10^6)

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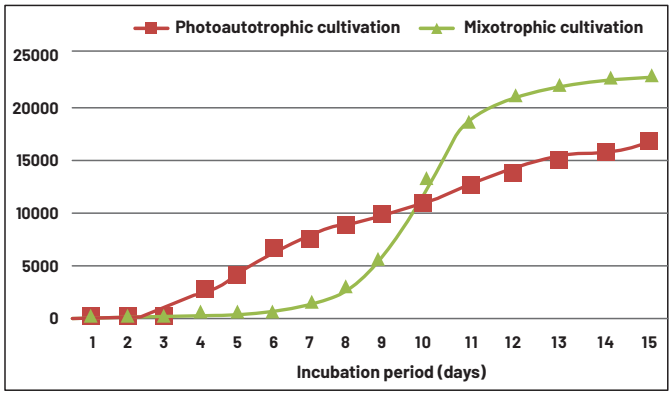

Figure 3: Growth of *C. vulgaris* under mixotrophic and photoautotrophic conditions

Cultivation showing extended lag phase of mixotrophic cultivation (in mixotrophic the growth rate was much higher than the photo-autotrophic condition). Monounsaturated, polyunsaturated, and saturated fatty acid contents were greater in the culture grown in mixotrophic conditions. However, the corresponding fatty acids were significantly lower in mixotrophic and photoautotrophic cultures that were fed an adequate amount of nutrients. The main fatty acids in the mixotrophically raised biomass varied from C16:0 to C18:0; the best fatty acids for biodiesel synthesis were palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1n9), linoleic acid (C18:2n6), and linolenic acid(C18:1).

DISCUSSION

Fossil fuels are depleting day by day and also a source of pollution resulting in global warming. This study deals with alternate method to fulfil the deficiency and provide environment friendly source. In this study microalgae *C. vulgaris* was exploited to screen its ability to grow in banana waste. A high seed content (41 percent) was also recovered from the biomass grown under mixotrophic conditions, in addition to the high biomass production. Mixotrophic conditions also lead to greater biomass product. Enough biomass produced by mixotrophic and photoautotrophic processes together with their corresponding rates of growth. According to the results of the previous study, the highest biomass value for cultivation with molasses added as an additional source of carbon was 140 mg dL-1 [19]. Declared noticeably higher biomass (nearly twice as high) than that produced by photoautotrophic growth or when microalgae was cultured without the banana peel hydrolysate. Mixotrophic cell cultivation employing an organic carbon supply and a light source is thought to be the most efficient way to produce micro algal biomass [20]. Mixotrophic cultivation has a higher energetic efficiency than other forms of cultivation since the majority of the energy spent in it is used to assimilate carbon. Examining the lipidome of microalgae biomass for practical rearing was the goal of the current work. Two concurrent series of experiments were performed to increase the biomass of *C. vulgaris*. *In* the initial research, molasses and banana peels hydrolysate were used as a supplementary source of carbon and energy in a mixotrophic culture environment. The second set of assays was carried out in photoautotrophic culture conditions as a control group. Some intriguing findings were obtained from the generation of microalgae biomass under two different growing conditions. Monounsaturated, polyunsaturated, and saturated fatty acid contents were greater in the culture grown in mixotrophic conditions.

CONCLUSIONS

The findings of this study indicate that producing microalgal biomass from agro-industrial wastes can concurrently provide two advantages: producing high-quality microalgal biomass at a reasonable cost, and simultaneously utilizing or remediating the wastes involved. The results of this study will be helpful in the development of cost-effective and ecologically friendly biofuels. Microalgal mixotrophy is advancing on several front. Ultimately, mixotrophically produced microalgal biomass yields significant amounts of superior fatty acids that can be used to create superior biofuels. The utilization of agro-industrial waste is advised for high quality microalgal biomass productivity and lipid content, together with concurrent treatment of the relevant wastes.

Authors Contribution

Conceptualization: AA
Methodology: AUMF
Formal analysis: RB, FA
Writing-review and editing: AA, RB, HF, AR

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The author declares no conflict of interest.

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