MICROALGAE CULTURE DEVELOPMENT IN BIOLOGICALLY TREATED WATER OF POULTRY SLAUGHTERHOUS

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ABSTRACT

In this study biological treatment wastewater of poultry slaughterhouse was used to produce microalgae culture for biodiesel production. The culture media was collected as 24 hours composite after the aerobic biologically treated of poultry slaughterhouse wastewater at Şen Piliç – Turkiye activated sludge treatment plant. Chlorella vulgaris Beijerick was prepared using sterilized BG11 medium. The cultures were performed at room temperature with continuous white light illumination and were carried out on a shaker at 120 rpm. Periodically pH were changed to overcome algae-consuming predator problem. Microalgae culture was successfully developed in biologically treated wastewater. Optimum microalgae growth were observed in sample C10,3. Chlorophyll-a results for C10,3 were reach from 1.82 to 4.46 mg/L. Total lipid estimated as 0.1533 g and FAME composition was C18:2, C20:3, C20:4 and C23:0 respectively for C10,3. The maximum total lipid amount observed in C5,1 as 0.3388 g and sample C5,2 was contained highest FAME quantities.

1. INTRODUCTION

Fossil fuel consumption caused enhancement of carbon dioxide level at the atmosphere and consequently, researches on renewable and sustainable energy sources such as biodiesel was increased [1]. Unlike fossil fuels, microalgae reduce carbon dioxide level via photosynthesis. Microalgae are promising biodiesel source because of their rapid grow rate and less land requirement compared to other vegetable biodiesel sources such as corn, soybean, and canola etc. [1,2]. While dry biomass of Chlorella vulgaris contains 28-32 % lipids and have high grow rate, some microalgae types have higher lipid amount but less grow rate [3,4]

The wastewater contains essential nutrients for algae growth. Studies with different microalgae species performed with various domestic and industrial wastewater types. In the first place, integrating microalgae growth to the wastewater treatment study for an alternative treatment method applied on open ponds [5-8]. Recent years, bioreactors were started to use instead of open ponds to increase biomass efficiency. Some studies open ponds and bioreactors compared. In terms of productivity, laboratory type bioreactors have more microalgae yield. Recent years, bioreactors were started to use instead of open ponds to increase biomass efficiency [9-12]. In our study, only erlenmeyer flasks were used as experiment environment. As a nutrient medium, biologically treated water of poultry slaughterhouse was used.

Wastewater treatment and algae growth were integrated in many studies However, studies about algae consuming predator problem are almost absent. Kim et al. [13] studied removal of rotifers in microalgae cultivation using hydrodynamic cavitation and reached high efficiency as %96. However there is nonexistence of study about using biological wastewater of poultry slaughterhouse and pH change to overcome algae-consuming predator problem.

2. METHODS

2.1. Microalgae Cultivation

Chlorella vulgaris Beijerick 1890 (IBL-C105) which were kept on the Microalgae Bank of Sakarya University, Faculty of Arts and

Sciences, Biology Department, Turkiye, were prepared using sterilized BG11 medium [14]. The cultures were performed at room temperature with continuous white light illumination of 100 μmol photons m−2 s−1 in 1L erlenmeyer flasks. Flask cultivation was carried out on a shaker at 120 rpm. The culture media was collected as 24 hours composite after the aerobic biologically treated of poultry slaughterhouse wastewater at Şen Piliç – Turkiye activated sludge treatment plant.

Nutrients were used by microalgae as a feedstock. But the predators (protozoa and rotifers) were consumed microalgae biomass and were existed at high rates in aerobic biologically treated water. For inhibiting predators without damaging the microalgae cells, pH of culture medium was changed at certain times and periods; pH was maintained at 2.5 in 5 and 10 minutes with 1, 2 and 3 days interval, subsequently pH was adjusted to 8. The samples were named as "C5,1", "C5,2", "C5,3", "C10,1", "C10,2" and "C10,3". While 5 and 10 indicates pH retention time, 1, 2 and 3 indicates pH change periods. For instance, the sample, which pH was maintained to 2.5 for 5 minutes and repeated every second days, was named as C5,2,. With total suspended solids and chlorophyll-a analysis, increase of microalgae quantity was observed in every 5 days.

10 ml of sample was taken from each erlenmeyer flask for total suspended solid and chlorophyll-a analysis. Samples were filtered through pre-weighted filter papers, were dried in oven at 103^{O}C, were weighted and TSS was calculated. For chlorophyll-a analysis same filter papers were cut into small strips and were placed into the test tubes. 5 ml acetone was added on to them and waited for 5 days. Samples were measured by spectrophotometer at 664, 647 and 630 nm wavelengths to calculate the chlorophyll-a quantity with equation 1 and 2 [15].

$$\mathrm{CE, a} = 11.85\ (\mathrm{Abs}\ 664) - 1.54\ (\mathrm{Abs}\ 647) - 0.08\ (\mathrm{Abs}\ 630) \quad (1)$$

$$\mathrm{Cs} = \frac{\mathrm{CE, a}\ \times \text{extract volume (L)}\ \times \mathrm{DF}}{\text{CE sample volume (L)}\ \times \text{cell length (cm)}} \quad (2)$$

Since the feedstock was limited, the microalgae biomass was approached the highest yield after 30 days. The experiment was ended to avoid microalgae death. Samples were filtered for harvest and biomass dried at room temperature.

2.2. Cell Distruption

To increase lipid extraction efficiency, different cell disruption methods [16-19] such as microwave, osmotic shock, ultrasonic, bead beating and electroflocculation used on different microalgae species. In this study autoclave with nitric acid solution was used as a cell disruption method modified from Lee et al. [17]. Total biomass for each sample was autoclaved with %5 nitric acid solution at 121^{o}C for 30 minutes. After cell disruption completed, disrupted biomass dried at room temperature to be prepared for lipid extraction.

2.3. Lipid Extraction

Organic solvent extraction method modified from Soares et al. and Folch et al. [20-21] were used to extract total microalgal lipids in this study. Disrupted biomasses for each sample were placed into capped bottles and 20 ml chloroform-methanol solvent mixture (1:1 v/v) was added on to the tube. Samples were extracted for 2 hours on orbital shaker. The liquid part was separated to a beaker. The disrupted biomass was extracted again with 20 ml solvent mixture for an hour. Solvent part was transferred on to the same beaker and was evaporated under the fume hood until approximately 10 ml liquid left. The aliquot was centrifuged at 4000 rpm for 15 minutes and was transferred in to pre-weighted beaker. The aliquot were evaporated under the fume hood until the dryness. Total lipids were calculated after the samples weighted again.

2.4. Transesterification and Fatty Acid Methyl Esters Analysis

After total lipid extraction, transesterification process was carried out. The same method were used as Soares et al. [20] 17.5 mg total lipid for each sample was weighed into a tube. 0.25 ml 0.5 molar sodium hydroxide added to methanol was placed into the tube and heated on the water bath for 10 minutes. Tubes were cooled with the ice bath. 0.75 ml esterification mixture was added on to the each tube. The esterification mixture was prepared with 2 g NH_4Cl, 60 ml methanol and 3 ml H_2SO_4 and was refluxed for 15 minutes. Tubes were heated on the water bath for 10 minutes and were cooled with ice bath again. 2.5 ml n-heptan and 5 ml distilled water was added on the tubes and shaken for a while. Tubes had two phases. The upper part contained FAME (fatty acid methyl esters). The upper liquid phase was taken from the tubes and was analysed by GC-MS to identify FAME composition and their concentrations.

3. RESULTS AND DISCUSSION

3.1. Microalgae growth

To determine microalgae growth, chlorophyll-a analysis and total suspended solids analysis were done. When chlorophyll-a amounts indicate alive microalgae cells, total suspended solids contains dead and alive microalgae and predator cells. Microalgae were more resisted to pH changes than predators however they were also affected. Microalgae amount was decreased slightly after each pH changes. As seen in Fig. 1, sample C5,1 and C10,1, microalgae biomass were firstly increased. Due to pH changes in every day for 5 and 10 minutes, these samples' growth rates were slowed down at towards the end of the experiment. Therefore, chlorophyll-a results of C10,3 and C5,3 indicated higher microalgae growth. Optimum microalgae growth were observed in sample C10,3. Chlorophyll-a results were reach from 1.82 to 4.46 mg/L (Fig. 1).

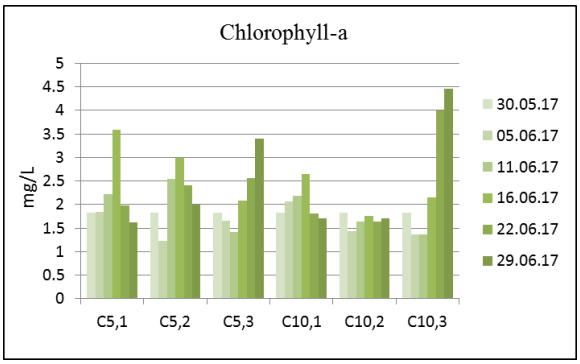

Fig. 1 Chlorophyll-a concentrations of samples

As a consequence predators were exploded in time and microalgae cells were increased. With total suspended solids (TSS) and chlorophyll-a analysis, increase of microalgae quantity was observed. Total suspended solids indicate all dead and alive cells (Fig. 2). Beginning of experiment TSS were measured as 26 mg/L and sample C10,1 reached to the highest total suspended solids amount as 1370 mg/L.

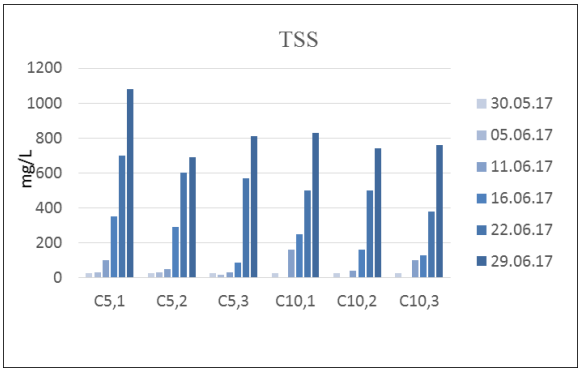

Fig. 2 Total Suspended Solid concentrations of samples

BG11 was used as nutrient medium for Chlorella vulgaris in various studies [14]. In this study, biologically treated water of poultry slaughterhouse was used as a nutrient medium. Microalgae cells could increase in biologically treated water despite the predator existence.

3.2. Total lipids and Fatty Acid Composition

Despite the slowing growth rates due to the pH changes in every day, highest total lipid amount was found in C5,1. (table 1.)

Table 1. Total lipid amounts extracted from Microalgae biomass

	Total lipid (g)
C5,1	0,3388
C5,2	0,2209
C5,3	0,1908
C10,1	0,2061
C10,2	0,1151
C10,3	0,1533

Fatty acid methyl esters were determined by GC-MS analysis. C18:2, C20:3, C20:4 and C23:0 were appeared to be main fatty acids. These FAME are unsaturated fatty acids and considered as suitable for production of biodiesel [17,22,23]

Table 2. Amount of Fatty Acids methyl ester

Fatty acid	Amount of Fatty Acids (mg L^{-1})					
	C5,1	C5,2	C5,3	C10,1	C10,2	C10,3
C18:2	41,71	110,49	55,00	84,11	63,30	41,92
C21:0	ND	27,08	ND	ND	ND	ND
C22:0	ND	34,78	ND	24,27	13,23	ND
C20:3	58,13	132,56	67,26	95,80	57,89	47,49
C20:4	56,17	128,09	64,99	92,57	55,94	45,89
C23:0	14,92	35,37	13,91	21,76	13,03	10,91
C24:0	ND	28,13	ND	ND	ND	ND

ND: not detected

The variances between samples were originated from pH changes. Although C10,3 were showed highest microalgae growth, according to fatty acid methyl ester analysis, C5,2 were produced most suitable microalgae biomass for producing biodiesel.

4. CONCLUSION

Wastewater treatment and algae growth were integrated in many studies. However there is nonexistence of study about using biological wastewater of poultry slaughterhouse and pH change to overcome algae-consuming predator problem.

Microalgae culture was successfully developed in biologically treated wastewater. Optimum microalgae growth were observed in sample C10,3. Chlorophyll-a results for C10,3 were reach from 1.82 to 4.46 mg/L. Total lipid estimated as 0.1533 g and fatty acid methyl ester composition was C18:2 , C20:3, C20:4 and C23:0 respectively for C10,3.

Although C10,3 had highest microalgae growth, maximum total lipid amount measured in C5,1 as 0.3388 g and highest fatty acid methyl ester quantities were measured in C5,2. Fatty acids named C21:0 and C24:0 was found only in sample C5,2.

Nomenclature

$C_{E,a}$ Concentration (mg/L) of chlorophyll a in the extraction solution analyzed,

C_s concentration (mg/L) of chlorophyll pigment in the whole water s sample

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