

RESEARCH ARTICLE

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Feasibility of microalgae culturing on dairy waste for biodiesel production

ABSTRACT:

Recently Biodiesel has a lot of concerns by number of researchers, due to its environmental benefits, a renewable energy resource, could replace fossil fuels, and the fact that lack of fossil fuels has become imminent. The cost of biodiesel production is the main barrier. The objectives of the present study were economic mass production of microalgae growing on dairy waste (sweet whey) for lipid accumulation. The obtained data revealed that diluted waste (50%), when compared with control (BBM) medium was a promising alternative medium for cultivating of *Anabaena oryzae*, *Chlorella vulgaris* and mixture of both microalgae. While the difference in (chlorophyll a and dry weight) obtained from 50% waste and BBM cultures of all tested organisms was insignificant, lipid percentage increased by (29.3, 13.4, and 16.5%) in *Anabaena*, *Chlorella* and their mixture, respectively, compared with corresponding control cultures. Maximum lipid productivity of *Anabaena*, *Chlorella* and their mixture was (2.03, 5.78, and 4.88 mg/L/day). Role of microalgae in biological treatment of waste was noticeable, whereas reduction in all tested parameters (Total suspended solids (TSS), total dissolved solids (TDS), chemical oxygen demand (COD), total nitrogen (TN), ammonia (NH₃), Nitrate (NO₃) and Phosphorus(P) were recorded. Extracted oil was esterified and analysed using GC-MS analysis. The predicted biodiesel properties using obtained fatty acid profile promised with good clean biodiesel meet international qualities of biodiesel.

KEY WORDS:

Biodiesel, Cultivation, Phycoremediation, Production, microalgae, Whey.

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INTRODUCTION:

Nowadays dependence on fossil fuel-based energy resources has become unsustainable and costly, because the rapid increase in populations and increased demand of energy depletion of fossil fuels became imminent Narendrakumara *et al.* (2018). Microalgae are very small aquatic photosynthetic microorganisms with simple growing requirements (light, sugars, CO₂, N, P, and K) that can produce lipids, proteins and carbohydrates in large amounts over short periods of time, which can be processed into both biofuels and valuable products (Brennan and Owende, 2010). Microalgae are currently being promoted as an ideal third generation biofuel feedstock because of their rapid growth rate, CO₂ fixation ability and high production capacity of lipids; they also do not compete with food or feed crops and can be produced on non-arable land. Microalgae have broad bioenergy potential as they can be used to produce liquid transportation and heating fuels, such as biodiesel and bioethanol (Dragone *et al.*, 2010). Microalgae could not only produce large amounts of biodiesel feedstock, but also recycle waste CO₂ emissions thereby reducing their build-up in the atmosphere (Gallagher, 2011).

Because of its easy availability and cost effective for large biomass production

capability, microalgae have become a viable option as biodiesel feedstock (Calderón *et al.*, 2018). Making biodiesel from microalgae oil is similar to the process of making biodiesel oil from any other oilseed, so the same conversion processes to produce biodiesel from microalgae are quite possible (Mofijur *et al.*, 2019). Algal biofuel production is still not economically feasible due to several limitations related to algal culture. Cheese whey, the liquid by-product remaining from the cheese manufacturing process constitutes a serious environmental problem of dairy industries due to its high organic matter content (Panesar and Kennedy, 2012). Among the major components of whey, the disaccharide lactose, which on hydrolysis yields glucose and galactose, is greatly responsible for its high Biochemical Oxygen Demand (BOD, 30000: 50000) mg/L and Chemical Oxygen Demand (COD, 60000: 80000) mg/L. In addition to this carbohydrate, cheese whey contains proteins, lipids, water-soluble vitamins and minerals (Siso, 1996). Cheese whey permeate has been investigated as a potential DOC (dissolved organic carbon) source for microalgae cultivation for biodiesel production (Girard *et al.*, 2014). Cultivation of microalgae in dairy waste has received increased attention, since they can grow well in these effluents because of its high nutrition content. Dairy industry wastewater was good nutrient supplement for algal growth in comparable with BG-11 growth medium (Kothari *et al.*, 2013). Many workers have demonstrated that most of the algal species such as *Chlorella vulgaris* (Feng *et al.*, 2011), and *Chlorella pyrenoidosa* (Kothari *et al.*,

2012) can be effective tool for wastewater treatment. Use of algae can remove major inorganic contaminants like nitrogen (50%) and phosphorus (90%) from the wastewater (Wang *et al.*, 2013). The addition of whey, an industrial dairy waste from cheese making, enhances growth of *Euglena* cells on undiluted waste (Waygood, 1976).

Oscillatoria sp. showed relatively high growth rates when cultivated in dairy effluent as a nutrient source for biodiesel production compared to the standard growth medium (Jitha and Madhu, 2016). *Chlorococcum* sp. was cultivated in dairy effluent for biofuel production (Ummalyma and Sukumaram, 2014). Recently, a dual approach for treating a dairy waste stream from a specific medium-scale industry and producing algal biomass for biofuel was proposed (Kothari *et al.*, 2012). The present study aims to use whey waste as inexpensive nutrient source, for generate microalgal biomass and producing clean biodiesel, simultaneously with reducing environmental pollutions by bio-treatment of waste.

MATERIAL AND METHODS:

Anabaena oryzae Fritsch (blue green algae) *Chlorella vulgaris* Beijerinck (green algae) were selected for this study, both were obtained from the Culture Collection of Botany and Microbiology Department, Faculty of Science, Cairo University, Egypt. Microalgal identification was confirmed phylogenetically using 16S rDNA *Anabaena* and 18S rDNA for *Chlorella* it was carried out at Animal Health Research Institute (Figs 1 & 2).

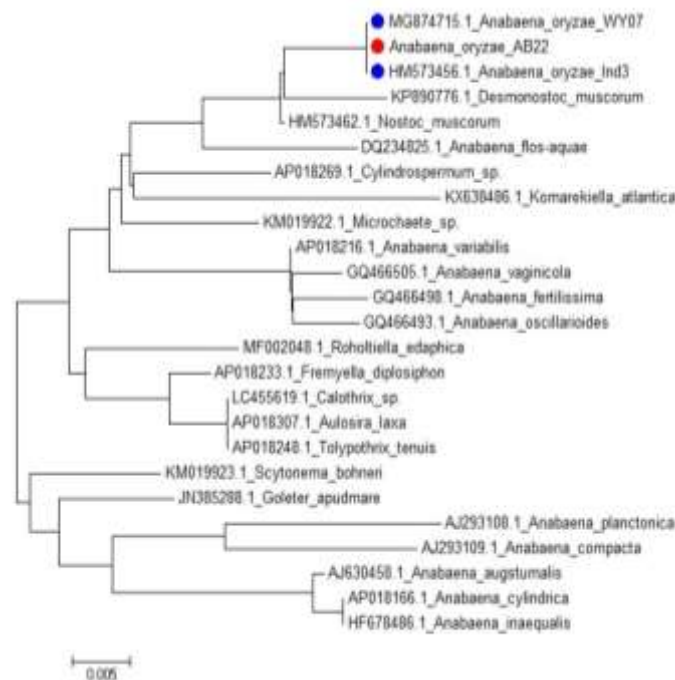


Fig. 1. Phylogenetic tree showing the relationships between the tested microalgal strain (*Anabaena oryzae* AB22) and the most similar sequences retrieved from NCBI nucleotide database.

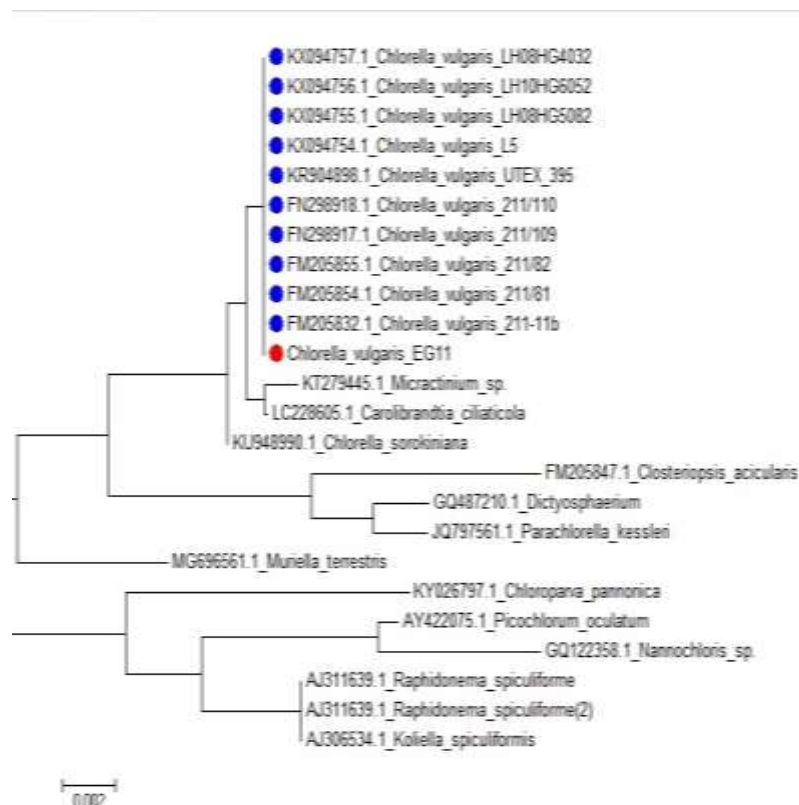


Fig. 2. Phylogenetic tree showing the relationships between the tested microalgal strain (*Chlorella vulgaris* EG11) and the most similar sequences retrieved from NCBI nucleotide database.

Methods of experimentation and growth conditions:

Raw dairy wastewater was obtained from dairy Plant of the Faculty of Agriculture, Cairo University. Serial dilutions were prepared by mixing tap water free of chlorine (left for 48 h. in jars before use) and sweet whey waste to prepare concentrations of 10 %, 25%, 50%, 75%, and 100% (v/v) which were selected from a preliminary test. pH was adjusted to 7.3. Erlenmeyer flasks (500 ml) used as bioreactors for batch cultures, BBM as control, three flasks for each dilution and three for control. All the flasks (18) were autoclaved, after cooling all were inoculated with 20 ml *Anabaena oryzae* stock culture. A second set of flasks were inoculated each with 20 ml *Chlorella vulgaris* stock culture. A third set of flasks were inoculated with 20 ml mixture of both *Anabaena oryzae* and *Chlorella vulgaris*.

Prior to each experiment initial chlorophyll (a) content, dry weight, lipid content and whey analysis were determined. All flasks were incubated in optimum growth conditions (40.5 μ E m⁻²S⁻¹ & 25 \pm 2°C) till the stationary phase. Microalgal cells were harvested, by centrifugation. At the end of incubation period (14 days) by centrifugation and chlorophyll (a) content, dry weight, and total lipid content were determined. Fatty acid analysis according to Pandey *et al.* (2014) was done using GC/MS. At the end of the experiment, whey analysis for the

concentration which gives the highest total lipid accumulation and growth (50% whey concentration) (TSS, TDS, COD, TN, NH₃, NO₃,

and P) were estimated according to APHA (2005). Biomass productivity for *Anabaena*, *Chlorella* and their mixture was calculated according to Abomohra *et al.* (2013). The lipid productivity was calculated according to Converti *et al.* (2009). Lipid extraction was carried out for microalgal tissues produced after 14 days growth at 50% concentration by Soxhlet Extractor using chloroform / methanol 2:1 v/v (Afify *et al.*, 2010). Transesterification for fatty acid analysis takes place by mixing the produced oil with a mixture of catalyst (0.25 g NaOH) and 24 ml methanol with stirring properly for 20 min, the mixture was kept in shaker at 300 rpm for 3 hrs. Predicted biodiesel properties based on fatty acid profile were estimated using computer software (biodiesel analyzer© ver.2.2.) available on "http://www.brteam.ir/biodiesel_analyser" This would eliminate the need for analysis steps and cost production (Talebi and Tabatabaei, 2016).

Statistical analysis was carried out using SPSS software. Results are presented as mean \pm standard deviation (SD). Multiple comparisons of means were made with Duncan's multiple range tests at 95% (Dytham, 1999).

RESULTS AND DISCUSSION:

One of the main objectives in this study is to find out alternative growth medium for microalgal growth and lipid accumulation for biodiesel production. Algal community can produce superior biodiesel quality (Marella *et al.*, 2019).

In the present study, estimation of chlorophyll a content and dry weight revealed gradual increase as the whey concentration increased from 10% to 50%. At (50%) concentration chlorophyll a content and dry weight recorded maximum values (4.79, 6.85, and 6.00 mg/L) and (208.5, 405.23, and 378.80 mg/L) for *Anabaena*, *Chlorella* and their mixed culture respectively, with insignificant difference with control cultures (Table 1). Previously using of dairy industry wastes as alternative media for microalgal cultivation was discussed by number of researchers including (Freyssinet and Nigon, 1980; Riaño *et al.*, 2016). It is to be noted that a sharp drop in chlorophyll a content and dry weight occurred at 100% whey concentration. In compatible with the findings of Kothari *et al.* (2013) who reported that the growth of *Chlamydomonas* was stimulated by 75% concentration of dairy wastewater, when compared with that obtained at 100% concentration of wastewater. It is clearly observed from the study results, that lipid accumulation of *Anabaena*, *Chlorella* and their mixed culture were positively affected by (50%) whey concentration, where there was a significant increase in lipid content and

lipid percentage over control. The obtained results revealed that 50% concentration of sweet whey was the most suitable for growth (chlorophyll a content and dry weight) of *Anabaena*, *Chlorella* and mixed culture of both. Lipid percentage (13.64, 19.97, and 18.04%) was also higher than control cultures. In similar studies, Borges *et al.* (2016) reported that cheese whey and cheese whey permeate should be diluted to adjust lactose concentration for best microalgal growth. Kothari *et al.* (2013) reported that dairy wastewater can be utilized as rich nutrient source compared with BG-11 growth medium for biomass production of *Chlamydomonas*. Also, Espinosa-Gonzalez *et al.* (2014) indicated that whey is a promising carbon source for heterotrophic batch culture cultivation of *Chlorella protothecoides* and can be utilized as a feedstock for biomass and lipid production of microalgae. Results of the present study revealed that the highest biomass productivity of microalgal cultures treated with different concentrations of sweet whey was recorded at 50% concentration (14.89, 28.95, and 27.1 mg/L/day) for *A. oryzae*, *C. vulgaris* and their mixed culture, respectively (Fig. 3). In the same context the highest values of lipid productivity were recorded at 50% whey concentration recording 2.03, 5.78, and 4.88 mg/L/day for *A. oryzae*, *C. vulgaris* and their mixed culture, which was more than that of control cultures in this experiment (Fig. 4).

Table 1. Effect of different concentrations of sweet whey waste on growth and lipid production of microalgae after 14 days growth.

| Whey concentration % | <i>Anabaena oryzae</i> (parameters mg/L) | | | | <i>Chlorella vulgaris</i> (parameters mg/L) | | | | Mixture of <i>A. oryzae</i> and <i>C. vulgaris</i> (parameters mg/L) | | | |
|----------------------|--|-----------------------------|---------------------------|---------------------------|---|-----------------------------|---------------------------|---------------------------|--|-----------------------------|---------------------------|---------------------------|
| | Chl. a | Dry weight | Lipid content | Lipid % | Chl. a | Dry weight | Lipid content | Lipid % | Chl. a | Dry weight | Lipid content | Lipid % |
| (0.0) (Control) | 5.26 ± 0.41 ^c | 218.00 ± 07.00 ^e | 23.02 ± 2.77 ^c | 10.55 ± 1.20 ^a | 7.22 ± 0.39 ^c | 406.10 ± 18.75 ^e | 71.43 ± 2.76 ^c | 17.61 ± 0.76 ^a | 6.26 ± 0.32 ^d | 389.80 ± 9.95 ^e | 60.30 ± 2.62 ^e | 15.48 ± 0.81 ^b |
| 10 | 1.43 ± 0.13 ^a | 112.71 ± 11.84 ^c | 15.02 ± 1.37 ^b | 11.76 ± 0.36 ^a | 1.69 ± 0.25 ^a | 158.70 ± 4.10 ^b | 29.17 ± 1.72 ^a | 18.38 ± 0.94 ^a | 1.51 ± 0.12 ^a | 150.00 ± 4.37 ^b | 26.58 ± 1.84 ^b | 17.76 ± 1.72 ^b |
| 25 | 2.28 ± 0.35 ^b | 174.73 ± 05.23 ^d | 22.10 ± 1.45 ^c | 12.64 ± 0.47 ^a | 3.75 ± 0.23 ^b | 326.10 ± 7.19 ^d | 58.97 ± 2.93 ^b | 18.08 ± 0.60 ^a | 2.17 ± 0.11 ^b | 281.37 ± 10.43 ^d | 49.33 ± 3.49 ^d | 17.65 ± 2.54 ^b |
| 50 | 4.79 ± 0.32 ^c | 208.50 ± 09.17 ^e | 28.37 ± 1.40 ^d | 13.64 ± 1.18 ^a | 6.85 ± 0.14 ^c | 405.23 ± 7.12 ^e | 80.90 ± 1.83 ^d | 19.97 ± 0.69 ^a | 6.00 ± 0.24 ^d | 378.80 ± 9.76 ^e | 68.30 ± 1.91 ^f | 18.04 ± 0.79 ^b |
| 75 | 2.37 ± 0.27 ^b | 94.30 ± 03.17 ^b | 13.20 ± 0.30 ^b | 14.01 ± 0.62 ^a | 3.34 ± 0.28 ^b | 295.27 ± 7.10 ^c | 56.43 ± 4.71 ^b | 19.11 ± 1.58 ^a | 2.78 ± 0.12 ^c | 240.00 ± 6.95 ^c | 42.03 ± 3.05 ^c | 17.50 ± 0.77 ^b |
| 100 | 0.97 ± 0.14 ^a | 48.30 ± 04.33 ^a | 6.33 ± 0.61 ^a | 13.21 ± 2.07 ^a | 1.87 ± 0.08 ^a | 131.33 ± 13.72 ^a | 24.73 ± 1.72 ^a | 19.06 ± 3.26 ^a | 1.36 ± 0.13 ^a | 131.33 ± 3.21 ^a | 5.14 ± 0.48 ^a | 3.91 ± 0.29 ^a |

Means marked with the same superscript letters are not-significant ($P > 0.05$), whereas others with different superscript letters are significant ($P < 0.05$). Data are average of three replicates; each value represents the mean ± SD. (Control): cultivated on BBM.

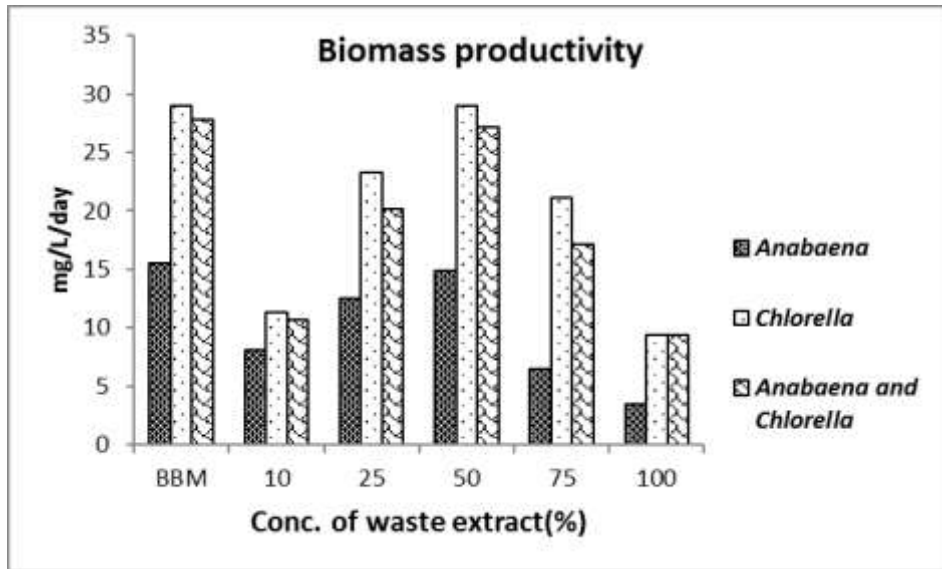


Fig. 3. Effect of different concentrations of sweet whey waste on biomass productivity of *Anabaena oryzae*, *Chlorella vulgaris* and mixed culture of them after 14 days growth.

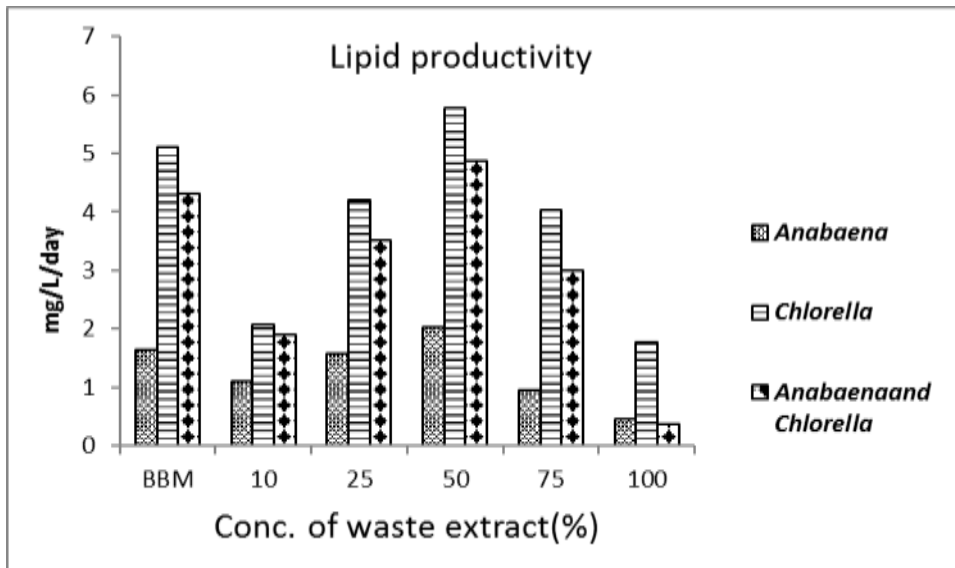


Fig. 4. Effect of different concentrations of sweet whey waste on lipid productivity of *Anabaena oryzae*, *Chlorella vulgaris* and mixed culture of them after 14 days growth.

Sweet whey treatment and utilization is of major concern, its high contents of organic matter, with the high produced volumes and limited treatment methods make whey a big environmental problem. High COD, N, P and ammonia reductions after microalgal cultivation indicated that they should be considered as an energy resource rather than a pollutant (Chatzipaschali and Stamatis, 2012). Many studies showed that microalgal cultivation is an important tool for treatment of different types of wastewater, efficiently removed nitrogen, phosphorus and rapidly consumed nutrients for photosynthetic activity (Samori *et al.*, 2013; Bohutskyyi *et al.*, 2015; El-Sheekh *et al.*, 2016; Eladel *et al.*, 2019). In the present study high lipid productivity were obtained with 50% whey concentration in *Anabaena*, *Chlorella* and mixed culture exceeding control. Biomass productivity of the tested microalgae in the same whey

concentration was similar to that obtained in control cultures in agreement with Mondal *et al.* (2016) who reported that whey permeate was the best among different carbon sources for total lipid production from microalgae and superior in enhancing biomass production. Algal-based bioremediation would be more attractive, if the resulting biomass could be applied as feedstock for the manufacture of bio-products (Shurin *et al.*, 2013).

The role of *Anabaena*, *Chlorella* and mixed culture in bioremediation and purification of 50% sweet whey concentration was prominent, where there was a reduction in all tested parameters (TSS, TDS, COD, T N, NH₃, NO₃, and P) after microalgal cultivation (Table 2). The maximum reduction percentage recorded with NH₃ and NO₃ followed by P and TN in all cultures. The most effective organism was *Chlorella* culture (NH₃, 81.45, NO₃ 92.64, P 69.56, and TN 55.35%). The same conclusion

was reported previously by Pizarro *et al.* (2002) who indicated that *C. pyrenoidosa* was found to remove 80 – 85% phosphorus and 60 – 80% of nitrogen from the dairy wastewater. Similarly,

using microalgae species for treating dairy wastewater also have the ability to sustain growth of microalgae that produce lipids which can be used for biodiesel production.

Table 2. Analysis of sweet whey waste (50%) before and after microalgal cultivation for 14 days.

| Parameter (mg/L) | Initial (Before inoculation) mg/L | <i>Anabaena oryzae</i> | | <i>Chlorella vulgaris</i> | | Mixed culture | |
|------------------|-----------------------------------|------------------------|----------------|---------------------------|----------------|-----------------|----------------|
| | | Final mg/L | % of reduction | Final mg/L | % of reduction | Final mg/L | % of reduction |
| TSS | 325.00 ± 4.58 | 190.33 ± 2.52 | 41.43 ± 1.24 | 183.67 ± 1.15 | 43.48 ± 1.15 | 161.67 ± 2.08 | 50.25 ± 0.32 |
| TDS | 700.00 ± 8.89 | 381.33 ± 3.21 | 45.52 ± 0.58 | 344.33 ± 4.04 | 50.81 ± 0.10 | 356.67 ± 3.51 | 49.04 ± 1.13 |
| COD | 5100.00 ± 30.00 | 2383.33 ± 76.38 | 53.26 ± 1.69 | 2053.33 ± 55.08 | 59.73 ± 1.23 | 1896.67 ± 15.28 | 62.81 ± 0.23 |
| TN | 68.76 ± 5.13 | 37.00 ± 2.00 | 46.05 ± 1.42 | 30.67 ± 2.52 | 55.35 ± 0.49 | 30.33 ± 1.53 | 55.72 ± 2.76 |
| NH ₃ | 23.33 ± 1.04 | 5.57 ± 0.15 | 76.12 ± 0.88 | 4.31 ± 0.21 | 81.54 ± 0.24 | 4.03 ± 0.05 | 82.69 ± 0.82 |
| NO ₃ | 21.23 ± 0.90 | 2.92 ± 0.15 | 86.23 ± 0.94 | 1.56 ± 0.16 | 92.64 ± 0.93 | 0.83 ± 0.02 | 96.09 ± 0.13 |
| P | 1.82 ± 0.08 | 0.73 ± 0.06 | 59.83 ± 2.08 | 0.55 ± 0.05 | 69.56 ± 2.69 | 0.63 ± 0.05 | 65.42 ± 3.56 |

Data are average of three replicates; each value represents the mean ±SD.

Reduction percentage of TSS was higher in mixed culture (50.25%). Similar results were obtained on dairy wastewater treatment by using the microalga *Botryococcus* represents a good example of phycoremediation by consuming the nitrogen and phosphorus components of the dairy wastewater (Shen *et al.*, 2008).

The present result was confirmed the finding of Kothari *et al.* (2013) who reported that microalgae grown on dairy wastewater reduced the load of nitrate, nitrite, phosphate, fluoride and ammonia after ten days of its cultivation. According to Mondal *et al.* (2016) mixotrophic cultivation of *Chlorella* and *Chlamydomonas* in cheese whey removed organic carbon and whey can be used as very cheap medium for microalgal cultivation. Chorus (2012) reported that microalgae require large amounts of costly phosphate and nitrogen fertilization for optimal biomass productivity, so culturing microalgae on dairy wastewater provides dual benefits of producing the biomass for oil production and wastewater treatment reducing pollution load of industrial wastewater (Pizarro *et al.*, 2002).

Results in table 3 of fatty acids analysis after esterification of microalgal extracted lipids showed that, nine fatty acids were recorded for *Anabaena* grown on 50% sweet whey for 14 days contained C14: 0, C 16: 0, C16: 1, C 16: 2, C 18: 0, C 18: 1, C 18: 2, and C 18: 3 as their major components in addition to C 20: 4 which is in agreement with the previous study performed by Li and Watanabe (2001). While number of fatty acids of *Chlorella* was higher (13 fatty acids) constituted mainly from C14: 0, C16: 0, C18: 0, C18: 1, and C18: 2. The present study was compatible with the findings of Yusof (2011). Fatty acids of mixed culture (*Anabaena* and *Chlorella*) was more or less similar to that represented in *Anabaena* and *Chlorella* individually, included 14 different fatty acids. Hu *et al.* (2008) reported that the main fatty acids synthesized in microalgal cells contained

fatty acids with chain lengths from C16: 0 to C18: 0. Maximum total identified fatty acids was recorded in mixed culture followed by *Chlorella* and *Anabaena*. While *Chlorella* contained maximum saturated fatty acids percentage (61.3%), due to the presence of Lauric fatty acid (5%), Myristic fatty acid (20.3%) and Palmitic fatty acid (17%), *Anabaena* contained maximum unsaturated fatty acid percentage (52.7%), due to the presence of Linolenic fatty acid (22.5%).

Table 3. Fatty acids profiles of *Anabaena*, *Chlorella* and their mixed culture cultivated on 50 % sweet whey waste concentration for 14 days, data expressed as relative percentage.

| Fatty acids | <i>Anabaena oryzae</i> | <i>Chlorella vulgaris</i> | Mixed culture |
|-------------------------------------|------------------------|---------------------------|---------------|
| Capric (C10: 0) | nd | 1.8 | 2.0 |
| Lauric (C12: 0) | nd | 5.0 | 3.0 |
| Myristic (C14: 0) | 16.1 | 20.3 | 14.7 |
| Palmitic (C16: 0) | 17.3 | 17.0 | 18.3 |
| Palmitoleic (C16: 1) | 6.2 | 2.8 | 2.8 |
| Hexadecadienoic C16: 2 | 11.8 | nd | 3.5 |
| Margaric (C17: 0) | nd | 1.5 | 5.2 |
| Stearic (C18: 0) | 11.6 | 12.0 | 12.1 |
| Oleic (C18: 1) | 5.4 | 15.6 | 12.0 |
| Linoleic (C18: 2) | 6.1 | 11.0 | 8.0 |
| Linolenic (C18: 3) | 22.5 | 3.9 | 16.0 |
| Arachidonic (C20: 4) | 0.7 | nd | nd |
| Behenic (C22: 0) | nd | 1.7 | 0.4 |
| Lignoceric (C24: 0) | nd | 2.0 | 1.4 |
| Others | 2.3 | 5.4 | 0.6 |
| Total identified fatty acids% | 97.7 | 94.6 | 99.4 |
| Saturated fatty acids% (SFA) | 45.0 | 61.3 | 57.1 |
| Monounsaturated Fatty acids% (MUFA) | 11.6 | 18.4 | 14.8 |
| Polyunsaturated Fatty acids% (PUFA) | 41.1 | 14.9 | 27.5 |

Percentage of FAME was calculated based on peak area of individual peaks in the (GC-MS) spectrum.

nd: not detected.

Biodiesel characterization:

Since the experimental determination of the quality parameters of a biodiesel sample requires more time, money, effort and large quantity sample of a biodiesel from feed stock oil. Depending on the fatty acid composition of the feed stock oil used, biodiesel properties can vary greatly. An efficient software for estimation of biodiesel properties, especially when there is only small sample of oil, was applied. Physico-chemical properties of biodiesel in this study were estimated depending on fatty acid profile recorded in table 4 using computer software (Biodiesel Analyzer program, ver. 2.2). Different microalgae cultures showed different iodine values which was 86.25, 47.42, and 71.87 (g I/100 g), respectively were within the EN standards requirements. These variable contents were due to variable contents of

saturated fatty acids in their different cultures. More or less similar SV of the predicted properties of biodiesel were obtained (183.3 - 206.38) mg NaOH /g). Good ignition property due to high predicted values of CN was in all cultures (56.67, 62.1 and 56.58) where it matched with ASTM, EN and EPS standards. The most suitable (CFPP) for EN standards was the predicted value of *Anabaena* biodiesel. The major advantages of biodiesel from microalgae included higher oxidation stability than oil crop feedstocks (Rajvanshi and Sharma, 2012), where the predicted oxidation stability (6.7, 10.51 and 7.5 h) was higher the world standards and it is an important character of a good stable biodiesel. Generally, the predicted properties of the biodiesel produced from *Anabaena*, *Chlorella* and mixed culture were meet with the world standards (Nascimento *et al.*, 2013).

Table 4. Predicted physico-chemical properties of biodiesel based on fatty acid profile of *A. oryzae*, *C. vulgaris* and mixed culture of both cultivated on 50 % sweet whey waste concentration for 14 days using computer software (Biodiesel Analyzer ver. 2.2).

| Predicted biodiesel properties | Whey (50%) | | | Biodiesel Standards | | |
|--|-----------------|------------------|---------------|---------------------|------------|-------------|
| | <i>Anabaena</i> | <i>Chlorella</i> | Mixed culture | ASTM D6751 | EN 14214 | EPS |
| Degree of unsaturation (Du) | 70.2 | 48.2 | 62.8 | | | |
| Saponification value (SV) mg/g | 183.3 | 206.2 | 206.38 | | | |
| Iodine value gI/100 g (IV) | 86.25 | 47.42 | 71.87 | | ≤ 120 | |
| Cetane number (CN) | 56.67 | 62.1 | 56.58 | > 47 | ≥ 51 | > 55 |
| Long chain saturation factor (LCSF) | 7.5 | 14.25 | 11.28 | | | |
| Cold filter plugging point (CFPP) | 7.18 | 28.29 | 18.96 | -5 to -13 | 5 -20 | |
| Cloud point °C (CP) | 4.11 | 3.95 | 4.63 | | | |
| Pour point °C (PP) | - 2.36 | -2.54 | -1.79 | -15 to 10 | | 4.5 |
| Allylic position equivalent (APE) | 64.0 | 54.4 | 60 | | | |
| Bis Allalic position equivalent (BAPE) | 51.1 | 18.8 | 40 | | | |
| Oxidation stability/h (OS) | 6.7 | 10.51 | 7.5 | 3 | ≥ 6 | |
| Higher heating value (HHV) | 33.61 | 36.98 | 37.52 | >35 | > 32.9 | > 44.3 |
| Kinematic viscosity mm ² /s (ν) | 2.83 | 3.33 | 3.32 | 1.9 - 6 | 3.5 - 5 | 1.6 - 7 |
| Density g/cm ³ (ρ) | 0.75 | 0.825 | 0.84 | 0.878 | 0.86 – 0.9 | 0.82 – 0.87 |

CONCLUSION:

Some microalgal organisms could be successfully cultivated in sweet whey waste as a good alternative media which was rich in high valuable nutrients for growth of microalgae. Whey waste was stimulating for

enhancing algal lipid accumulation more than synthetic medium. Microalgae were an important factor in treating waste, reducing organic pollution and used for producing sustainable, clean valuable biodiesel and other valuable by-products.

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جدوى استزراع الطحالب الدقيقة في مخلفات الألبان لإنتاج الديزل الحيوي

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نسبة الدهون حققت ارتفاعاً ملحوظاً حيث بلغت الزيادة في إنتاجها 29.3 % في الأنابينا، 13.4% في الكلوريللا و16.5% في مزرعة الأنابينا والكلوريللا معاً. بلغت الإنتاجية القصوى للدهون 2.03، 5.78 و 4.88 (ملجم/لتر/يوم) في الأنابينا، الكلوريللا ومزرعة الأنابينا والكلوريللا معاً على التوالي. أظهرت النتائج دوراً فعالاً للطحالب الدقيقة في المعالجة البيولوجية للشرش، حيث سجلت النتائج نسبة اختزال عالية لكل من المواد الصلبة الذائبة، والمواد الصلبة العالقة، طلب الأكسجين الكيمائي المستهلك النيتروجين الكلي، الأمونيا، النترات، والفسفور. تحليل الأحماض الدهنية المنتجة من الطحالب بعد عملية الأسترة أظهر أن الديزل الحيوي المنتج ذو مواصفات جيدة تلي المواصفات الدولية والمصرية للديزل الحيوي.

حاز الديزل الحيوي في الآونة الأخيرة على الكثير من الاهتمام من قبل عدد من الباحثين، نظراً لكونه مصدر من مصادر الطاقة المتجددة، ويصلح ان يكون بديلاً للوقود الأحفوري الذي أصبح وشيكاً ولفوائده البيئية. التكلفة العالية لإنتاج وقود الديزل الحيوي هي العائق الرئيسي. لذلك كانت أهداف هذه الدراسة هي استزراع اقتصادي قليل التكلفة للطحالب الدقيقة على شرش الألبان (مصل اللبن الحلو) للحصول على كتلة حيوية كبيرة من أجل الحصول على كمية من الدهون. أظهرت نتائج البحث أن تركيز 50% من الشرش يعتبر وسط غذائي واعد لنمو الأنابينا والكلوريللا كلا على حده بالإضافة لنموهما معاً. كانت معايير النمو من الكلوروفيل والوزن الجاف متقاربة جداً مع نظيرتها في الوسط الغذائي العادي (الكنترول) ولكن