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Potential of Microalgae Cultivation in Dairy Wastewater as a Step in Low-Cost Biofuel Production

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ABSTRACT

The present study addresses adopting the organic and nutritious materials in dairy wastewater as media for cultivation of microalgae, which represent an important source of renewable energy. This study was carried out through cultivation of three types of microalgae; *Chlorella sp.*, *Synechococcus*, and *Anabaena*. The results shows the success the cultivation of the *Synechococcus* and *Chlorella* Sp, while the *Anabaena* microalgae were in low-growth level. The highest growth was in the *Synechococcus* farm, followed by *Chlorella* and *Anabaena*. However, the growth of *Synechococcus* required 10 days to achieve this increase that represents a negative indicator of the adoption of this type of microalgae in this media to meet the desired aims. While *Chlorella* needs less than two days to start growing. Moreover, the data obtained from the experiment show that removal of chemical oxygen demand in *Chlorella* cultures was (72%) more than that obtained from cultivation of other microalgae. Thus this microalgae is more efficient in wastewater treatment than other types.

Keywords: dairy wastewater, microalgae, *Chlorella*, nutrients.

امكانية زراعة الطحالب الدقيقة في المخلفات المائية للالبان كخطوة في انتاج وقود حيوي منخفض الكلفة

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الخلاصة

تناولت الدراسة الحالية اعتماد المواد العضوية والمغذية في مخلفات صناعة الألبان كمواد وسيطة رئيسية لزراعة الطحالب الدقيقة التي تمثل مصدرا هاما للطاقة المتجددة. وقد أجريت هذه الدراسة من خلال زراعة ثلاثة أنواع من الطحالب الدقيقة؛ *Chlorella sp.* ، *Synechococcus* ، و *Anabaena*. أثبتت النتائج نجاح زراعة *Synechococcus* وزراعة *Chlorella sp.* ، في حين مستوى نمو *Anabaena* كان منخفضا. أعلى نمو وجد في مزرعة *Synechococcus* ، تليها *Chlorella sp.* و *Anabaena*. مع ذلك، فان نمو *Synechococcus* قد تطلب 10 أيام لتحقيق هذه الزيادة, والتي تمثل مؤشرا سلبيا على اعتماد هذا النوع من الطحالب الدقيقة في هذا الوسط في تحقيق الأهداف المرجوة. في حين يحتاج *Chlorella sp.* أقل من يومين لبدء النمو. وعلاوة على ذلك، فإن النتائج تبين بأن إزالة محتوى طلب الأوكسجين الكيميائي

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في مزرعة *Chlorella sp.* كان (72٪) أكثر من تلك التي تم الحصول عليها من زراعة الطحالب الأخرى. وبالتالي هذا الطحالب ستكون اكفاء لمعالجة مخلفات المياه من الانواع الاخرى.
الكلمات الرئيسية : مخلفات الالبان , طحالب , كلوريلا , مغذيات

1. INTRODUCTION

Up today, the main economic crises and negative climate change are still identified as resulting from using the fossil fuels as a major source of energy. Despite the availability of alternative and environmentally friendly sources (for example energy produced from solar, wind, and hydroelectric processes). The amount of energy generated from these sources is far from meeting the global energy requirement. Indeed, it was reported that the high production cost is also one of the main reasons for the development of these sources **Schenk, et al., 2008** and **Dryzek, 2011**.

The energy generated from biomass may be a sustainable alternative and a reasonable competitor to fossil fuels **Chisti, 2008**, for instance, the fuel produced from crops. Portability, lower aromatic and sulfur content are important features of that biofuel than conventional fuel. In addition, the high temperature of fuel ignition makes the transporting process easier. Moreover, the biofuel machines have a higher default life than that operate with traditional fuel, since the biofuel produced from crops is more lubricating than those found in conventional fuels **Goldmints, 2009**.

However, the high viscosity, high emission of NOx, the problem of cold start, high price represent major obstacles to make this source at the forefront of important sources to confront the fossil fuels **Goldmints, 2009**. Technically, the biofuel is better, but it costs about three times the cost of traditional fuel. The raw material that is used in the production of this type fuel is already foodstuffs suitable for human consumption, for example, corn **Rosgaard, et al., 2012**. Therefore, it is difficult to determine the priority, whether conversion of this basic materials into bioenergy or supply to countries that already suffering from poverty **Eriksen, 2008**. These problems and others have prompted the researchers to find other alternatives that contribute to minimizing the damage from the use of these species of plant. One of the choices is the utilization of the organic materials that are found in the industrial food waste, and even the kitchen waste, and converting them all into biogas using certain types of anaerobic bacteria to disintegrate them biologically. However, the operational conditions of this biological unit with the associated units to purify the resulting gas, as well as the special environment (such as providing unit free from oxygen), which must be provided for these bacteria need the extra cost to complete them. Therefore, considering this source as a strong competitor to the fossil fuel source would be an illogical question, **Salmon,** and **Petazzoni, 2006**.

Changing this situation can be achieved through biological energy produced from microalgae source **Juneja, et al., 2013** and **Das, et al., 2016**. It can be a promising way to address energy economically and environmentally problems **Chisti, 2007, Li, et al., 2008** and **Hossain, et al., 2017**. In general, microalgae are organisms with micro-size (1.5-10 microns), growing by photosynthesis and resemble surface plants in terms of their work, but are more efficient in converting light into energy resulting of a simple structure in their cellular **Sheehan, et al., 1998**. As they are immersed in the biological solution, the availability of nutrients and aeration will be more than soil for plant cultivation. The oil produced from this source is 30 times higher than that produced by other biomass for the same area of land used for agriculture. Some species of algae have oil content more than its half dry weight. In addition, the operational conditions for growing microalgae are easier than growing crops with more biomass productivity. Moreover,

the microalgae are a good consumer of carbon dioxide, since the carbon element is about 50% of its content **Sheehan, et al., 1998**.

Cultivation of microalgae requires sunlight, water, nutrients and carbon dioxide as a regulator of pH and source of carbon.

Generation of glucose is carried out by photosynthesis process when the water and CO_2 are available according to flowing equation:

$$6\,CO_2 + 6\,H_2O \longrightarrow C_6H_{12}O_6 + 6\,O_2$$

This reaction is impossible to occur thermodynamically. But the stored energy ATP, **Curien, et al., 2016,** from the sunlight works as a catalyst to transform that reaction into a spontaneous reaction.

Sunlight, water, and carbon dioxide are key elements in the growth of algae. However, nutrients are an essential factor in the persistence of algae growth **Juneja, et al., 2013**. The most important elements are phosphate and nitrogen, while micronutrients are iron, magnesium, zinc, copper that required with trace amount. These nutrients are found in different forms; dissolved inorganic, dissolved organic and sediment. The dissolved inorganic matter is transferred from water to algae and plants, then to other living organisms, but it returns to water through soluble excretion from these organisms, decomposition of organic sediments and detritus, and finally through hydrolysis of dissolution organic nutrients **Bot** and **Benites, 2005.**

The most commonly known nutrients in the cultivation of algae are Chu's Medium No. 10 and BG11 Broth **Fayyad** and **Dwaish, 2016**. They are standard solutions containing phosphates and nitrogen as main compounds as well as other mineral salts. Through the complex structure of these solutions, they are a cost factor that increases the cost of producing microalgae. Despite the relentless attempts to find alternatives to those standard nutrients that are supposed to be available and less expensive, these proposed solutions need to be studied, developed and applied reasonably **Wang, et al., 2014**.

The current research addresses this issue seriously to find convincing alternatives to these nutrients. The wastewater produced from the food industries can be used to provide the basic elements of algae growth, trying to stay away from complexity or applications that are illogical or cost-bearing. This study suggested a dairy waste as a step in those attempts since the dairy wastewater is already enriched with organic materials. Although there are the chemical and biological processes for this purpose, these elements remain in the final product, even if they are small in quantity. However, they can cause the growth of surface algae and plants in the rivers or basins to which they are exposed. In the current study, the samples were taken as soon as the wastewater left the factories, and from the stream entering the wastewater treatment plant to take advantage of the basic elements before consuming by other microorganisms. The objective was to investigate the possibility of farming in these types of wastewaters.

2. MATERIALS AND METHODS

Current experiments have been preceded by many initial experiments using standard solutions and different type of wastewater. The samples from the dairy factories were taken at different times and from different regions. But the recorded in the current research was the dairy wastewater during its left the factories and before arriving the wastewater treatment plant. **Fig. 5** shows a simple schematic diagram of treatment units for wastewater that coming out of Abu

Ghraib plants. Three farms were established in the current study with a size of 500 ml for each farm in an incubator with a temperature controlled by 30 ° C as optimum temperature **Sri-Uam, et al., 2015.** In addition, **Ramaraj, et al., 2016**, used the same temperature for their experiment with the same type of chlorella. All the experiments were carried out with cool white fluorescent light. One milliliter of microalgae was placed in each flask with handshaking periodically. While the sampling was taken every two days to give enough time to reproduce.

2.1 Collection of microalgae strain

Three types of microalgae (*Chlorella Sp*., *Synechococcus*, and *Anabaena* microalgae) were taken from the Department of Biological Sciences at the University of Baghdad. The isolation of these microalgae was done using a continuous dilution method, **Pachiappan, et al., 2015**, to obtain pure isolates. These isolates were maintained using standard methods to keep this type of microalgae and prevent any contamination during the keeping process.

2.2 Collection of wastewater

The wastewater produced from the dairy factories was collected from the General Company for Food Industry in Abu Ghraib City. The dairy wastewater was analyzed to measure the total nitrogen, total potassium, total phosphate, and chemical oxygen demand (COD) as can be seen in **Table 1**.

2.3 Concentration of microalgae in culture media

The concentration of microalgae in the culture media was determined by examining and calculating the concentration of chlorophyll contained within the algae cell itself. Chemical digestion approach with 80% acetone was followed in the present study for extracting the chlorophyll concentration. The sampling was carried out every two days with 10 ml each time. The sample was centrifuged (CAPP, CR-656, Denmark) at 5000 rpm for 10 min. The supernatant was removed, while the sediment (pellet) was re-suspended with 4 ml of 80% acetone (99.7%, Romil-pure chemistry, UK). The product was heated up to 60 ^{o}C for 60 min in a water bath (Model: EH(V.2), Germany) in a dark area with shaking them each 5 min. After that, the sampling tube was centrifuged again with 5000 rpm for 10 min. The absorbance of supernatant was read via U.V. spectrometer (UV-160A spectrometer, Shimadzu) with blank (80% acetone). By application the following equation, the chlorophyll was determined:

$$Chl.a\ \left(\frac{\mu g}{ml}\right) = \frac{A_{663} \times Kf \times Volume\ of\ aceton}{volume\ of\ sample}$$

Where $Chl.a$ is concentration of chlorophyll, A_{663} is absorbance at 663 nm, and Kf is 12.63 according to **Najem, et al., 2016.**

2.4 COD Measurement

Chemical oxygen demined (COD) was used in the present study as a vital test to determine the number of organic matters that exist in wastewater before and after the treatment process. This test was carried out by reaction of sulphuric potassium solution and any substance in wastewater that has oxidation ability to create a green color. This color is represented as an indicator to the amount of these substances. The color intensity was determined by using the spectrometer. The cuvette was mixed manually and inverted to mix the sediment and suspension materials. Then, 2 ml of wastewater was pipetted to cuvette carefully. The cuvette was closed and inverted again and was heated up 150 ^{o}C for 148 minutes (RD 125, Germany). After the heating process, the hot

cuvette was removed outside to cool to 25 ^{o}C. Finally, the COD was measured via putting the cold cuvette in a spectrometer (Lovibond MD 200 COD Vario) as a standard method according to Lovibond procedure.

2.5 pH measurement

Value of pH is important in order to monitor biological processes since the microorganisms are strongly affected by this value, **Liu, et al., 2016** and **Khatikarn, et al., 2016**. The current study deals with more than one type of algae, therefore it was expected that the value of pH may play an important role in the process. Thus, this value was measured regularly using (pH-Basic 20, CRISON) to show the effect of microalgae growth rate on it.

2.6 Biogas measurement

Hydrogen and Hydrogen sulfide ratio was measured using a biogas analyzer (Biogas 5000 Geotech, UK) at a single atmospheric pressure and at a temperature of 20-23 °C.

2.7 Dissolved oxygen measurement

The measurement of dissolved oxygen ratio was necessary for the current research to determine the activity of microalgae in dairy wastewater water. The measurement was carried out by using a dissolved oxygen device (OXI 45+, CRISON, Spain).

3. RESULTS AND DISCUSSIONS

Fig. 2 shows *Chlorella Sp.* Cultivation in dairy wastewater after 10 days. It is generally greenish; however, the suspended microalgae in that media are much smaller than that deposited in the bottom of the flask, as shown in the figure. In fact, all the flasks were shaken several times during the day to ensure a homogenization of heat, acidity, light, and feedstock. But the sedimentation velocity is rather high, indicating the density of the biomass formed.

Nevertheless, the natural mixing, which otherwise can generate by the movement of oxygen fine bubbles during metabolic processes, may play an important role in generating a simple mixing that somewhat helps homogeneity all above parameters. Generally, the natural mixing is important in biological processes, especially if one of their products is a gas as well as the anaerobic bioprocesses such as an anaerobic system for bio-methane or bio-hydrogen production **Karim, et al., 2005**. However, bioprocesses that deal with heavy or viscous solutions unreasonably to depend on that property **Terashima, et al., 2009**. Fortunately, in the present study, the dairy wastewater is characterized by natural water properties, therefore the natural mixing will a positive effect on the efficiency.

The motion of fine bubbles in the biological medium was observed in the current study although it was not clear in **Fig.3** due to colure of culture media. Some of these bubbles are free to move. Others, however, cling to formed microalgae clusters to generate a higher buoyancy force than the weight of those clusters, which leads to raising them to the top of the flask. When this power is lost, as a result of the disconnection between oxygen bubbles leaving into the atmosphere and the microalgae clusters, these clusters begin to move down. This homogeneous action leads to moving the cultivation liquid from top to bottom and vice versa due to competition between both forces (drag force and upward force for media and gas phase respectively). Several studies have demonstrated an effect of fine bubbles in a sparged bioreactor on the circulation of the media around the draft tube, **Ying, et al., 2013**, **Rengel, et al., 2012, Šimčík, et al., 2011** and **Vesvikar**

and Al-Dahhan, 2005. However, in this study, it was observed that the movement of the media can also be obtained by movement of the microalgae cluster during rising and falling process. Therefore, it is possible to observe a simple movement in the media between different periods. Thus, this behavior will improve fluid movement to obtain homogeneous distribution in system variables.

Fig. 4 shows the growth of *Synechococcus* after 10 days. The problem of sedimentation is the same as in the cultivation of *Chlorella* microalgae. However, the suspended *Synechococcus* microalgae are more than *Chlorella*.Sp microalgae. The *Synechococcus* microalgae are small in size compared with other types of algae, is 0.9 um as average according to **Morel, et al., 1993**, that helps it move through the liquid media.

With this size, it was difficult to coalesce. Thus the disadvantage of cultivation of this type of algae is to stay stuck for a long time in culture media. Therefore, the previous studies suggested different approaches that can be used in the harvest stage to treat the cultivation media using the physical, chemical or biological process **Barros, et al., 2015, Vandamme, et al., 2013, Vandamme, et al., 2012**, **Salim, et al., 2011** and **Grima, et al., 2003**. These methods seek to creating the microalgae clusters that have enough weight dominating over their buoyancy force and are oriented downward or as micro-flotation if micro-technology is used as treatment approach such as used by **Hanotu, et al., 2012**.

However, in this study and subsequent experiments, the focus was on a harvesting question for *Chlorella* algae. It has been found that at the stationary stage when the concentration of algae reaches a certain level, the harvesting occurs spontaneously and without external interference. **Fig. 5** shows the deposition of the *Chlorella* microalgae model in 10 minutes and with higher efficiency than expected, which is a good advantage if such algae are grown in such type of wastewater.

Considering cultivation of *Anabaena* microalgae as the third choice for cultivation in such aqueous residues, there was no clear green, only a little. This was the primary evidence of the failure of cultivation of this type of microalgae in this type of waste, as can be seen in **Fig. 4**.

In both cases, access to food and energy depended mainly on natural mixing as well as manual shaking. Nonetheless, and through the observation in the present study, the phenomenon of sediment occurs after 6 days from the beginning of the growth phase.

Fig. 6 shows the cultivation of the three types of microalgae (*Chlorella SP.*, *Synechococcus*, and *Anabaena*) using wastewater produced from dairy factories through 14 days. The assessment of the comparison was evaluated via chemical digestion for the cells using 80% acetone, while the chlorophyll was measured at OD663. From the response, it is noted that growth rate of *Synechococcus* was more than other species (*Chlorella SP*. and *Anabaena* microalgae) at the end of the experiment. However, the amount of chlorophyll response showed that this increase in growth rate required a period of adaptation more than 10 days to reach this level. In fact, initially, *Chlorella* was the dominant growth. While the other microalgae did not grow well. Indeed, the growth rate of *Chlorella Sp.*was started from the second day and did not require that duration with a faster growth value. The experimental data shows that its growth naturally passed in its four known phases (lag, exponential, stationary, and death phase).

pH value was almost under the required tolerance as shown as in **Fig. 7**, especially in the culture of the Anabeina, indicating the low-growth of the *Anabaena*, whereas the slight rise was evident in the other cultures. The significant increase in the value of pH is due to the consumption of carbon dioxide by the microalgae itself, being the important source of carbon in the metabolic

processes of the production of glucose. Concerning of production of the *Anabaena*, although clearly expressed via **Fig. 4** and chlorophyll figure, it is also possible to speculate through this response. pH stability is due to poor growth of this type of microalgae with this environment.

Other microorganisms may be a good competitor to microalgae, not only in terms of the abundance of food but of providing the appropriate environment for the growth of microorganisms. The growth of aerobic or anaerobic bacteria, for example, depends on the available environment **Al-Mashhadani, et al., 2016**. If the biological medium is not sterile, it will certainly contain different types of microorganisms. Thus the chance of growth of algae in this medium may depend on the strength and activity of that microalga and the predominance of microorganisms present with it. On the other hand, the weak growth of microalgae may contribute to a good environment for the growth of anaerobic bacteria such as sulphate-bacteria or hydrogen-bacteria, which required media free from oxygen **Radha** and **Murugesan, 2017, Speda, et al., 2017, Capson –Tojo, et al., 2017, Isa** and **Anderson, 2005**. This is done by not putting up enough dissolved oxygen by the microalgae, which was already consumed by the air bacteria initially and the subsequent recovery of anaerobic bacteria.

This hypothesis was investigated by measuring the gases in the headspace at the top of the flask using the biogas analyzer. It was found that there is a certain amount of hydrogen or H_2S. For instance, the amount of hydrogen was about 6 ppm in the *Anabaena* flask. These gases are typically generated in oxygen-free solutions. This is not limited to Anabaena microalgae, but with any low-growth rate, the biological medium is full of aerobic bacteria. If there is insufficient sparging, the potential for anaerobic bacteria growth will be high **Al-Mashhadani, et al., 2016**. Therefore, when the dissolved oxygen was measured in *Anabaena* flask, the result was 0.32 ppm, while other successful models ranged from 4.4 ppm to 8.5 ppm. Hence, it is possible to conclude the importance of aeration system in the cultivation of microalgae in food industrial wastewater.

The effect of microalgae culture on the quality of wastewater was also investigated. It was found that the amount of COD removed from wastewater with *Chlorella* cultivation was greater than in *Synechococcus* or *Anabaena* microalgae. The percentage of COD removal was about 72% when the *Chlorella* was cultivated in wastewater, while it was 64% and 61% with *Synechococcus* and *Anabaena* respectively.

As a result, a *Chlorella* microalga is more suitable for cultivation in dairy wastewater than other microalgae species. The ease of harvesting, growth with lower lag phase, removal of larger organic matter, greater integration of aerobic bacteria and microalgae, and the prevention of the growth of anaerobic bacteria in solutions are the main reasons achieved from this research. Moreover, the economic product of *Chlorella* cultivation is relatively better than the *Synechococcus*, since the first contains higher oil content than the second, **Becker, 1992**.

Finally, according to above experiments and the analysis of the results, the current research proposed a modified scheme by adding the microalgae cultivation unit with the appropriate location for cultivation as can be seen in **Fig. 8**. The figure shows the utilization of organic and nutrients matters in dairy wastewater to convert them into bio-fuels without significant change in the existing units on the ground. Consequently, research has moved somewhat away from any complexity, radically changing, or economic cost.

4. CONCLUSIONS

The current research reported the possibility of cultivating microalgae in the wastewater produced by dairy factories. The paper studied the cultivation of three types of microalgae (*Chlorella Sp.*, *Synechococcus,* and *Anabaena* microalgae). The results obtained from the several experiments showed that *Synechococcus* was the most growing at the end of the test, nevertheless, it took a long time to reach that level. Cultivation of *Chlorella sp*. needed a shorter time to begin the known growth stages. In addition, the present research and through the observations studied the phenomenon of natural mixing that occurs during the cultivation. In fact, this factor has a positive impact on cultivation process due to the characteristics that are almost similar to tap water. In the bio-treatment field, the research showed that removal of organic matter from waste was better if chlorination was used as a stage of biological treatment in the wastewater treatment plant.

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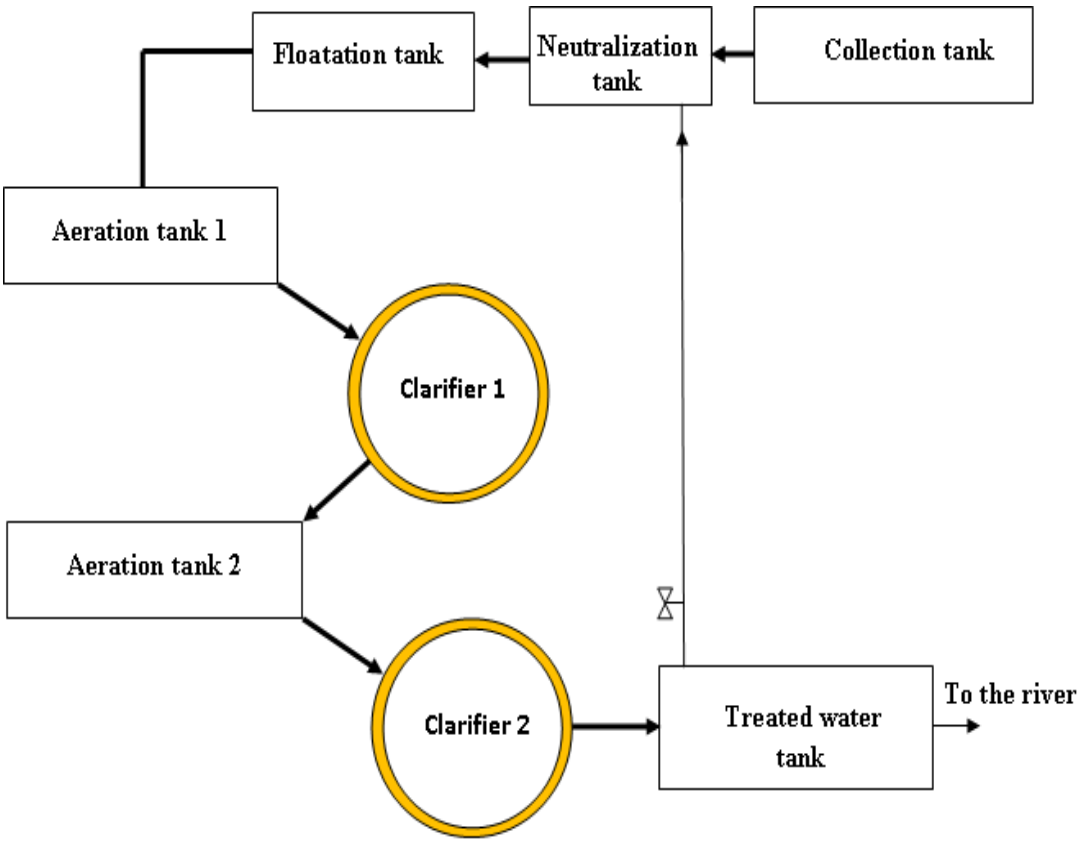

Figure 1. Schematic diagram of wastewater treatment plant in Abo-Ghraib dairy factories.

Table 1. Characteristics of the dairy wastewater used in the current study.

Parameter	Value	Units
TN	1000	ppm
TK	18000	ppm
TP	6000	PPm
COD	2083	ppm

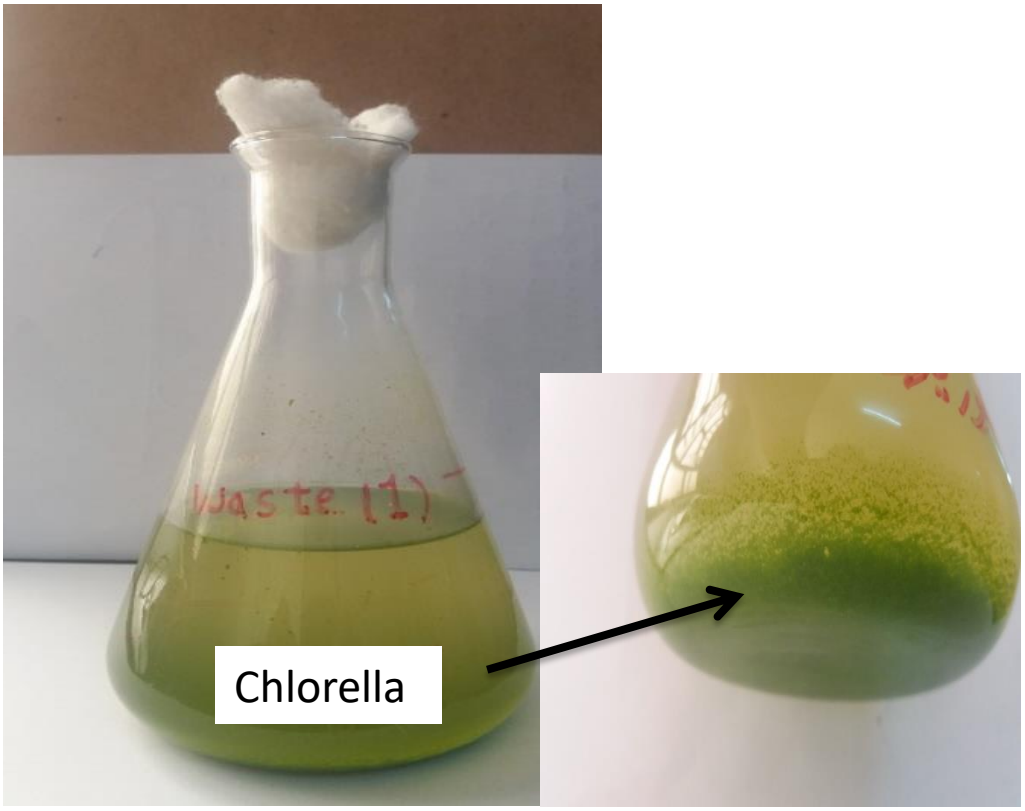

Figure 2. Cultivation of *Chlorella Sp*. in dairy wastewater after 10 days.

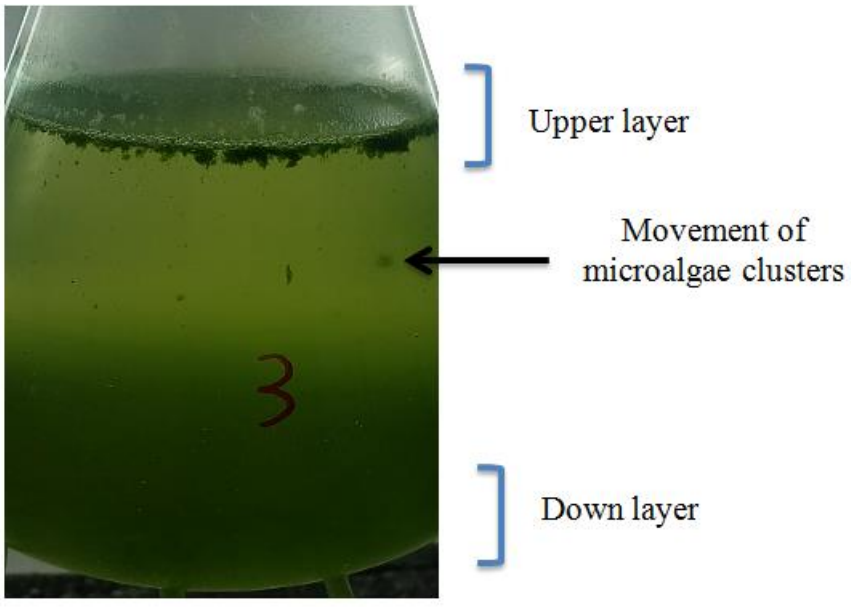

Figure 3. The layer formed at the top and formed at the bottom of the flask, and the movement of microalgae clusters between these two regions.

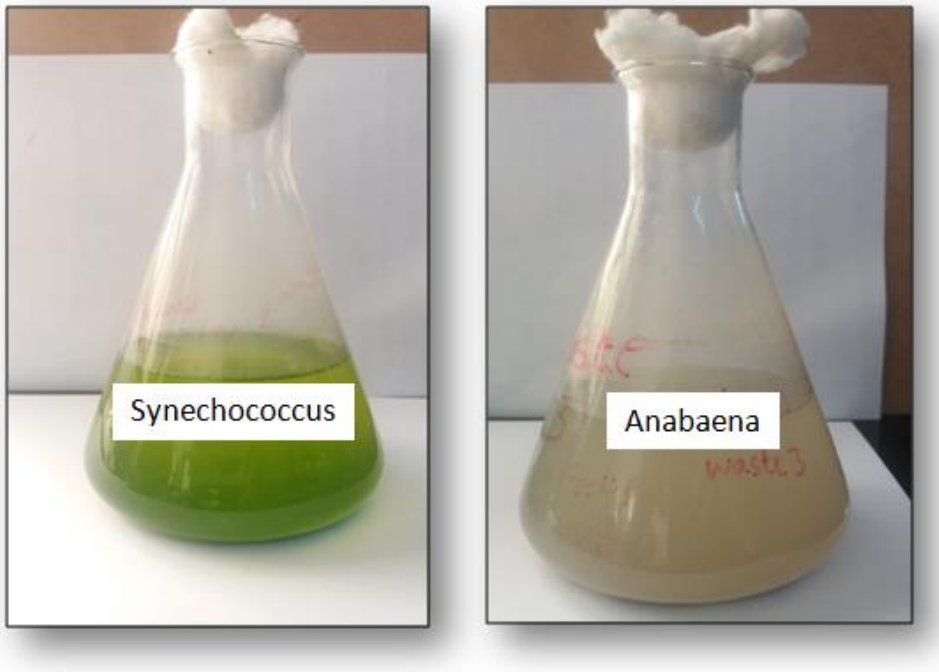

Figure 4. Snapshot of the two microalgae (*Synechococcus*, and *Anabaena* microlage) in dairy wastewater.

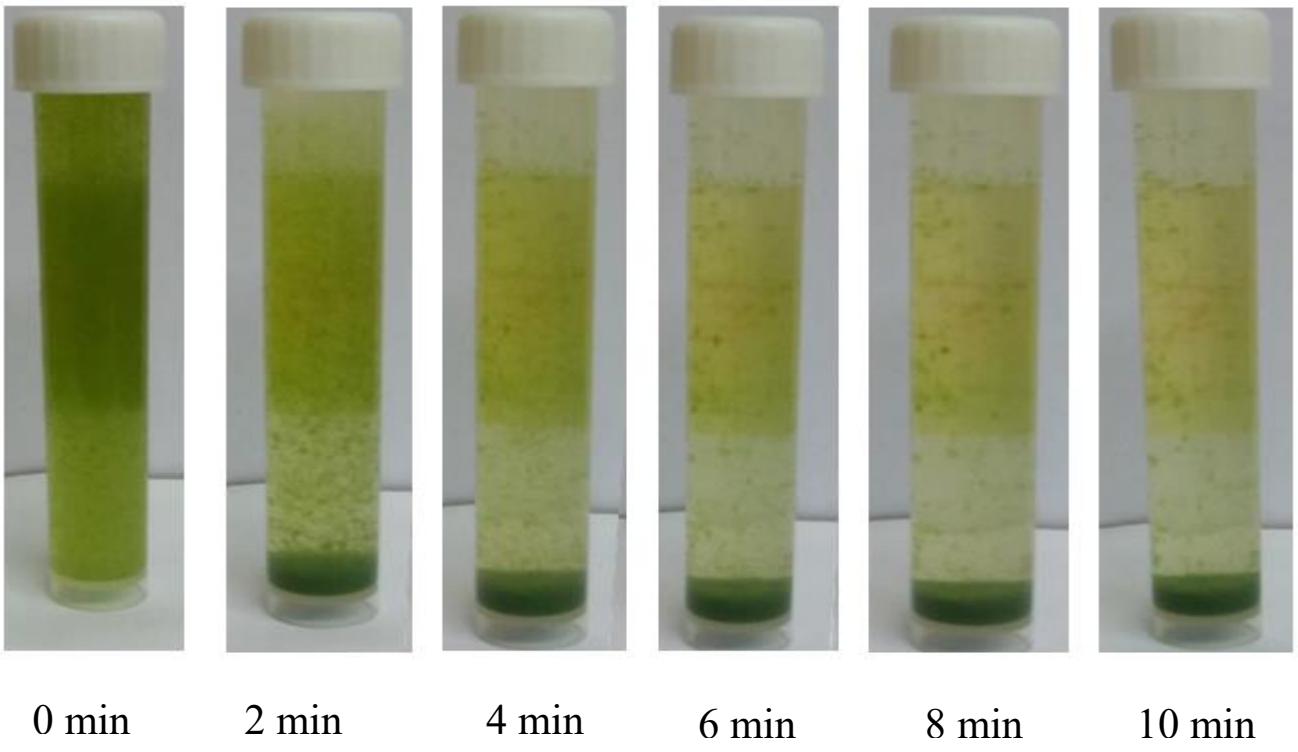

Figure 5. Natural harvesting of *Chlorella* microalgae with time.

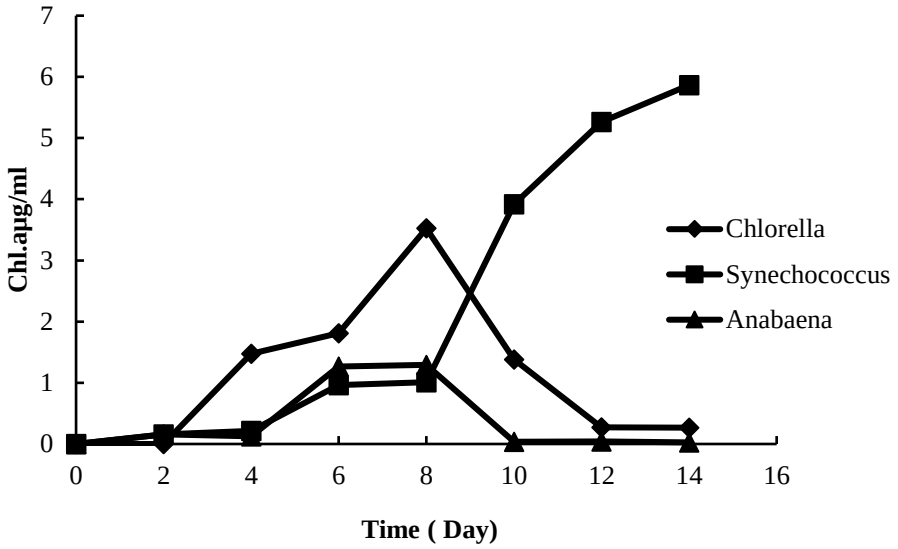

Figure 6. Chlorophyll content during the growth of *Chlorella*, *Synechococcus*, and *Anabaena* microlagae in dairy wastewater.

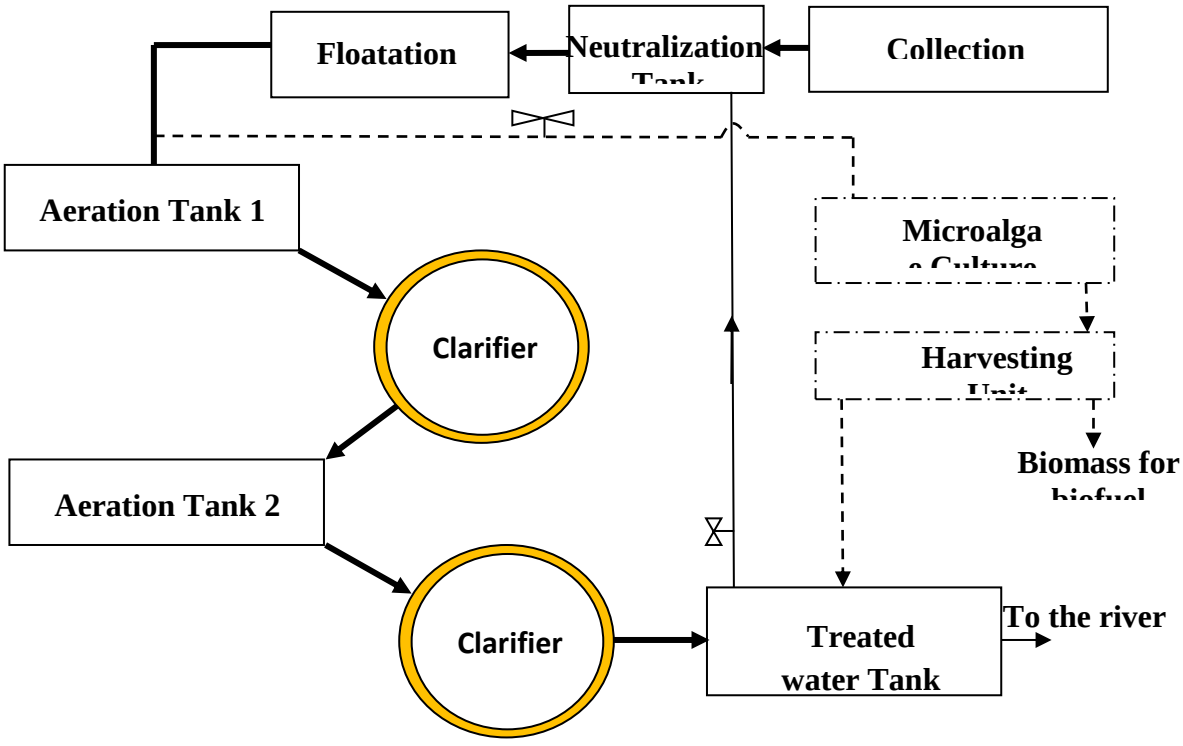

Figure 7. Values of recorded pH during the cultivation the three microalgae (*Chlorella, Synechococcus,* and *Anabaena* microalgae) in dairy wastewater.

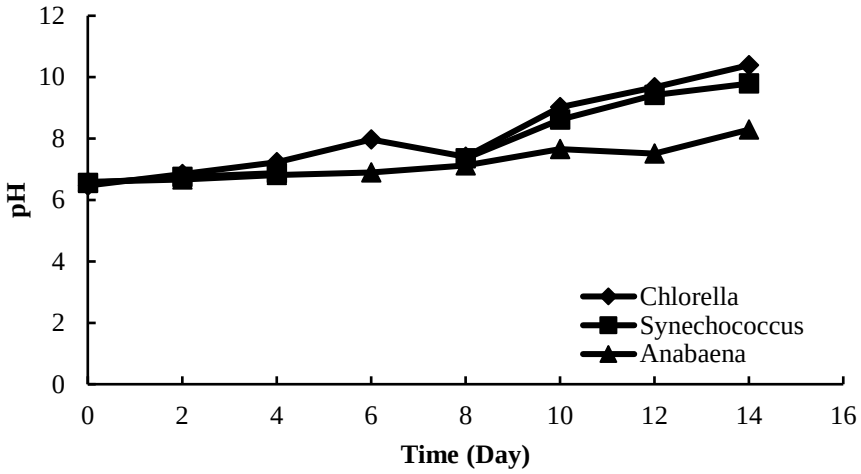

Figure 8. Suggested schematic diagram of wastewater treatment plant in Abo-Ghraib dairy factories.