



Biological Co-existence of the Microalgae – Bacteria System in Dairy Wastewater using photo-bioreactor

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Abstract

Aeration system in the cultivation of *Chlorella Sp.* Microalgae using dairy wastewater as culture media was addressed in the current study. This research aimed to study the effect of aeration in the bubble column bioreactor on the biological synergy between microalgae and bacteria if they are present in the same place. The results show that the sterilization stage is not the dominant step in the success of microalgae cultivation in water-rich organic waste. There is a clear convergence between the growth rate of *Chlorella* microalgae in the sterilized and non-sterilized culture media, which gives realism if the proposal is applied industrially. Through the information obtained the aerobic bacteria in the non-sterilized media, with free of algae, are able to consume all dissolved oxygen within a very short period of time. The aeration factor is, therefore, important in that case. However, the experiments show that co-existence of bacteria and microalgae can occur even if there is no aeration system. Consequently, the microalgae in the dairy wastewater are capable of preserving the environment of cultivation. The gases produced due to metabolic processes in bacteria or microalgae remain in solution for a certain period and are not easily removed, especially if the solution is exposed to intermittent sparging. Thus, this will give enough time for both microorganisms to consume those gases. However, the results show that the sparging system for 15 minutes and three times a day improves biomass production by 60%. Therefore, the cultivation of microalgae in addition to its desired goal can play an important role in the dairy wastewater treatment units by maintaining the appropriate environment for aerobic bacteria even in the absence of an aeration system.

Keywords: Chlorella, Aeration, Photobioreactor, Microalgae

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1- Introduction

1.1. Background of The Problem

Identifying the biofuel as alternative sources of conventional fuels has become a scientific concept.

However, those sources, with their high costs or lower production, to be an ideal substitute for fossil fuels are still illogical topic [1], [2]. Crops source, as an example, is in a competitive position with global food demand [3]-[5]. In fact, biofuel produced from this source require large areas of arable land with water for cultivation purpose [6]. In addition, these crops can meet the needs of some countries to prevent famine [7].

Recently, the microalgae source was considered as best chose to deal with environmental problems and greater productivity of biofuel than other sources [1],[8],[9].

From an economic perspective, microalgae are an important source of many products. Energy, chemicals extracted, food, and healthcare constituents are the most important products that are available from those organisms [10].

Compared with biofuels produced from agricultural crops, the source of microalgae is less expensive and

more productive than other sources, and above all does not cause a problem in the global food crisis [11].

Based on some studies, half of their dry weight is oil content that can be extracted to be an environmentally friendly fuel and a good alternative to fossil fuels [1].

However, the growth requirements are still obstacles to achieving that goal [12]. The nutrients, an example of these requirements, are one of the main necessary for living the microorganism. The difference in nutrient quality depends on the type and nature of the organism that will consume it. In general, phosphorogenic and nitrogenous compounds are considered to be the most important elements for the growth of microorganisms, including microalgae. While, carbon source can be obtained from the carbon dioxide or from carbonic compounds that found in the media. However, there are other essential compounds that can improve the number of products such as micronutrients [13].

The most commonly known nutrients in the cultivation of microalgae are Chu's Medium No. 10 and BG11 Broth [14]. 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 [15].

It is known that the development of industries still produces a large quantity of wastewater. In addition to its contribution that is already facing energy problems, the resulting wastewater and its environmental pollutants have become an obstacle to these developments. These pollutants are related to human health. Thus, it has become necessary to use applied studies to address industrial problems and minimize energy requirements.

This is accomplished by identifying the most efficient means of exploiting bio-energy sources to provide the necessary energy. Nevertheless, that process, if accompanied by the improvement of the source itself, it will be increased the benefits to the maximum extent possible. In the food industry, as an example, the amount of wastewater produced from these processes has increased significantly recently. This increase requires numerous physiochemical, and biological treatment to control contaminants. Despite the evolution of these processes, high costs and inefficiency still remains the main obstacle to the development of these processes. On the other hand, there is a loss of organic matter exist in untreated wastewater. These materials, if utilized, will achieve significant economic benefits as well as contribute to reducing processing costs.

There are several studies that have included the cultivation of microalgae in dairy waste [16]-[18], but the operating conditions, microalgae species, and type of study case may differ from research to another. In order to cost-effective, the current study sought out to be close to the possibility of application industrially through moving away from the complexities or the addition of other industrial units.

1.2. Sterilization Challenge

The sterilization stage enables the cultivation of a certain type of microalgae in isolation from other organisms, along with food and their suitable operational conditions, especially if the microalgae were cultivated in standard media.

This media usually contains precise compounds and nutrients that suitable for certain species of organisms. But, using alternative culture media, may contribute to the wideness of living organisms that can grow as soon as they are exposed to the atmosphere if the appropriate operating environment is available. Therefore, a need to sterilization stage represents an important step [19].

Sterilization is done using high temperatures (121 ° C) and pressure of about 2 bar for 15 minutes using autoclave device. Thus, application of cultivation of microalgae industrially with huge amounts of biological media may be impractical and adds considerable economic cost. Previous studies have addressed the subject as with the Ding [20], but its use of the principle of dilution models needs to lose large amounts of water.

In dairy factories, the pasteurization, chilling, and homogenization are processes used in manufacturing the butter, cheese, and milk [21].

These processes produce wastewater with a high level of chemical and biological oxygen demand, thus it gives a clear idea of the amount of food enriched in those wastes (i.e Eutrophication).

During the manufacture of these food products, large quantities of water are used to complete the manufacturing process. As a result, the used water contains large amounts of fat, proteins, and sugars, which are nutrients for the growth of a huge group of microorganisms, including aerobic bacteria. Discharge the dairy wastewater into the river without biological treatment would inevitably cause a major economic problem and hazardous to the environment. In fact, its negative impact will not only be on humans but on fish and marine resources as a result of the consumption of oxygen from the bacteria that will feed on those wastes.

The current research addressed this problem seriously by verifying the cultivation of microalgae in dairy wastewater in the case of sterilization using the autoclaves and comparing the results with that obtained from the cultivation of the same algae in non-sterilized wastewater. Then, the biological synergy between microalgae and bacteria was investigated in dairy wastewater using bubble column bioreactor.

2- Experiments and Methodology

2.1. Experimental Setup for Sterilization Study

Three farms were established in the current study with a size of 500 ml flask. Incubator with a temperature controlled by 30 ° C as the optimum temperature was used for this culture. All the experiments were carried out with cool white fluorescent light.

One millilitre of microalgae inoculums was placed in each flask with handshaking periodically. While the sampling was taken every two days to give enough time to reproduce.

2.2. Experimental Setup for Biological Synergy Study

Two cylindrical photo-bioreactors with a diameter of 120 mm and 260 mm height were constructed for the current study as shown in Fig. 1 and Fig. 2. One of the bioreactors was provided by a ceramic diffuser (diameter 80mm) for sparging system purpose.

This experimental set-up was used to grow microalgae in non-sterilized dairy wastewater. One of these bioreactors was aerated with a 500 ml/liter, while the second reactor was left without ventilation for comparison purposes.

The aeration system was carried out for 15 minutes with three times a day separated by one stopwatch without aeration.

According to initial experiments, this sparging period is sufficient to dissolve the oxygen level of equilibrium.

Temperatures in both reactors were controlled at 30 °C, while dissolved oxygen and pH were monitored daily.

The lighting process was carried out via cool-white fluorescent (T5 Led, China).

The light intensity was $110.6 \mu\text{E m}^{-2}\text{s}^{-1}$, which was measured using light intensity meter (Milwaukee SM700, Italy).

Microalgae growth rate was confirmed by the optical density in the first part of the study and by dry biomass weight in the second part. The density of the biomass was calculated using the wavelength of 680 [22].

The biomass weight was obtained using standard methods by separating the microalgae from the solution by centrifugation (5000 rpm) and then drying it for 24 hours, then drying it for one hour in the oven at 60°C [23].

The standard media used in the current study was BG11(HIMEDIA M1958). Table 1 shows the ingredients of this media with their amount.

Table 1. BG11 media composition

Ingredients	Amount g/l
Sodium nitrate (NaNO ₃)	1.5
Dipotassium hydrogen phosphate (K ₂ HPO ₄)	0.04
Magnesium sulphate, heptahydrate (MgSO ₄)	0.075
Calcium chloride dihydrate	0.036
Citric acid	0.006
Ferric ammonium citrate	0.006
EDTA, disodium salt	0.001
Sodium carbonate	0.02
Trace metal	
Boric acid (H ₃ BO ₃)	2.86
Manganese chloride, tetrahydrate	1.81
Zinc sulphate, heptahydrate	0.222
Sodium molybdate, dihydrate	0.39
Copper sulphate, pentahydrate	0.079
Cobalt nitrate, hexahydrate	0.0494

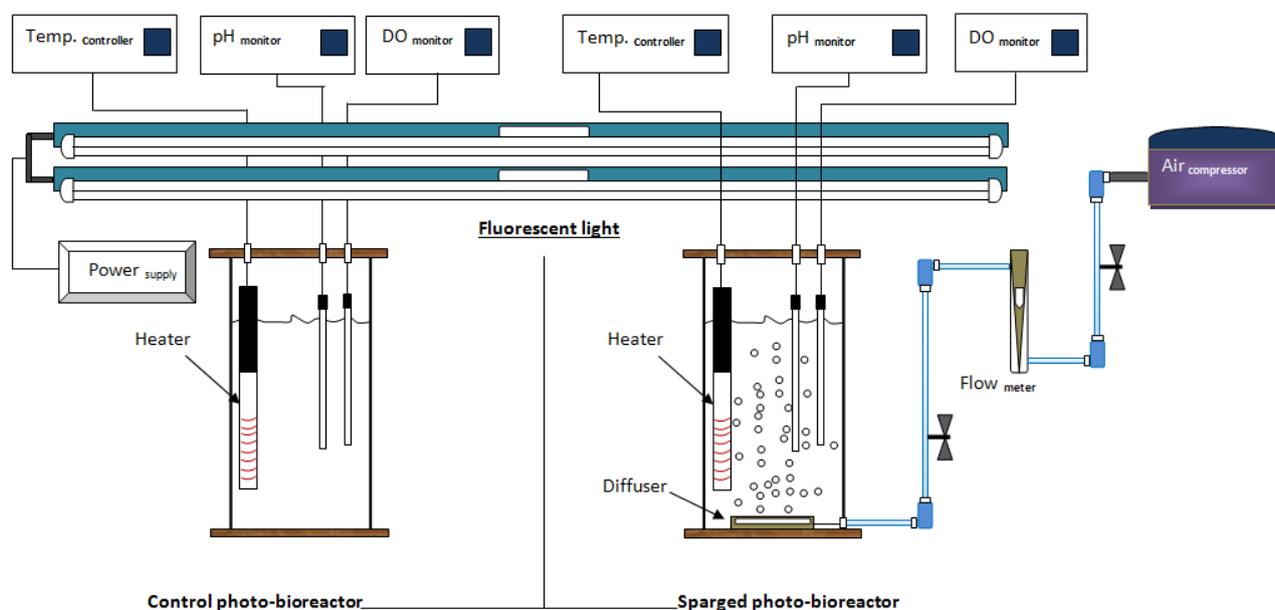


Fig. 1. Schematic diagram of the control photo-bioreactor and sparged photo-bioreactor



Fig. 2. Screenshot of the experimental set-up used in the present study

2.3. Characteristics of the Dairy Wastewater

The current experiments have been preceded by many initial experiments using standard solutions and dairy wastewater. The samples were taken from the General Company for Food Industry in Abu Ghraib City. The dairy wastewater was analyzed to measure the total nitrogen (TN), total potassium (TK), and total phosphate (TP) as requirements nutrients for the cultivation of the microalgae as well as chemical oxygen demand (COD). It was found that the total nitrogen, total potassium, total phosphate, and chemical oxygen demand are 1000, 18000, 6000, and 2083 mg/l respectively. The first three parameters were measured in were measured in the Department of Water and Environment- Science and Technology Laboratories.

2.4. Collection of Microalgae Strain

Chlorella 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 [24], to obtain pure isolates.

These strains were maintained using standard methods for keeping and prevent any contamination during the keeping process.

The specific growth rate of microalgae was calculated in exponential phase and according to the following equation [25]:

$$\frac{dX}{dt} = \mu X \quad (1)$$

Where X is dry weight biomass (mg), μ is specific growth rate (day^{-1}), and t is generation time.

Integrate:

$$\int_{X=X_0}^{X=X} \left(\frac{dX}{X}\right) = \mu \int_{t=0}^{t=t} dt \quad (2)$$

While the doubling time was estimated via the following equation:

$$T_d = \frac{\ln 2}{\mu} \quad (3)$$

Where T_d is doubling time (day)

2.5. 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). Before using this device, it was calibrated and activated according to the procedure of provided company.

2.6. Turbidity Meter

The relative clarity of the media after the agriculture process was determined by the turbidity meter. This device was used to measure the scattering of micro-algae particles in the seed solution. Therefore, the turbidity meter that has used in current investigation is Turbidity meter (TurbDirect, LOVIBOND).

3- Results and Discussion

3.1. Sterilization Effect

Three types of biological solutions were used for cultivating the Chlorella Sp.; sterilized, non-sterilized dairy wastewater and standard media BG11. The cultivation period was carried out by two stages. The first one was cultivation without adjusting the pH during 16 days operating. While in the second stage; the pH was adjusted every two days as can be seen in Fig. 3.

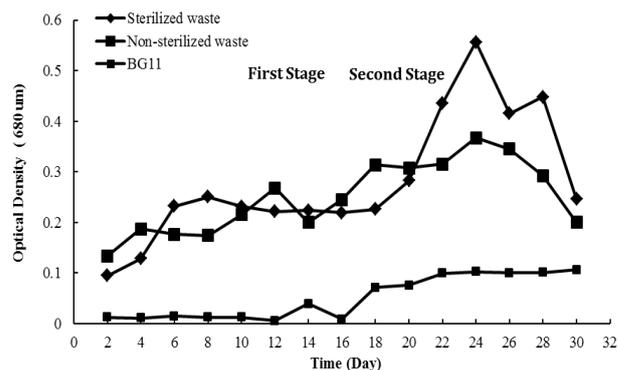


Fig. 3. Cultivation of microalgae in sterilized wastewater, non-sterilized wastewater, BG11 through two stages

According to this figure, it is noted that the growth rate of Chlorella microalgae in sterilized and non-sterilized wastewater is almost close.

However, the growth rate in the sterilized solution is more stable than that in the non-sterilized solution. While the growth rate of the Chlorella microalgae in the standard solution was somewhat slow.

The main reason is that adding 1 ml of inoculums of Chlorella microalgae was not enough to sense the growth rate and thus may need more time to grow. In previous studies [25], 15 ml was added to 350 ml media culture.

But in the present study, it was noted that adding the current amount (i.e 1:500 ml) of inoculums in dairy wastewater gives a significant growth rate of microalgae.

Fig. 4 displays the pH values in the three cultivate solutions during the two phases. The figure shows clearly that the pH value of all the models goes up during the microalgae growth period.

This increase was due to the reduction in dissolved carbon dioxide in these culture media. However, aerobic bacteria that can be originally grown in these wastes (whether sterilized or non-sterilized), may contribute significantly to the provision of carbon dioxide to the medium. This is because of the availability of materials for growth with the appropriate environment. Nevertheless, the pH values of the sterilized media are greater than that of non-sterilized media depending on the amount and activity of the bacteria present in the media.

In addition, the values of pH in standard solution are the highest compared with other cultures mediums.

The reason is the presence of aerobic bacteria in the wastewater and reducing them in the standard solution, if not already exist.

Thus, the consumption of carbon dioxide will not have opportunities to be compensated by another source. In case of poor bacterial performance, the pH value begins to rise.

Therefore, there is a close relationship between the activity of bacteria and pH value if there is an opportunity for the growth of microalgae active in the same media, since the microalgae, in general, is a good consumer of carbon dioxide.

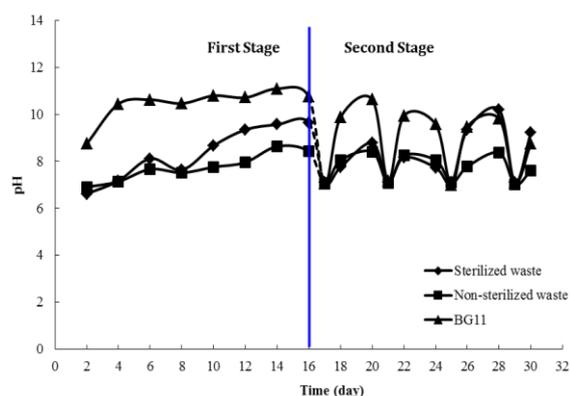


Fig. 4. pH values in sterilized wastewater, non-sterilized wastewater, BG11 through two stages

Measuring dissolved oxygen in samples is necessary to give a clearer idea of the effectiveness of microalgae. It is evident from Fig. 5 that the dissolved oxygen in the non-sterilized media during the first 16 days is the lowest compared to its measurement in the other culture media.

This is due to the presence of aerobic bacteria in the biological medium, which initiates metabolic activity very quickly if the appropriate operating conditions exist.

In the second stage and during the same experiment, the high pH value was adjusted in all models periodically (every two days) to give sufficient time for microorganism and know the effect of the pH on the growth of *Chlorella* microalgae.

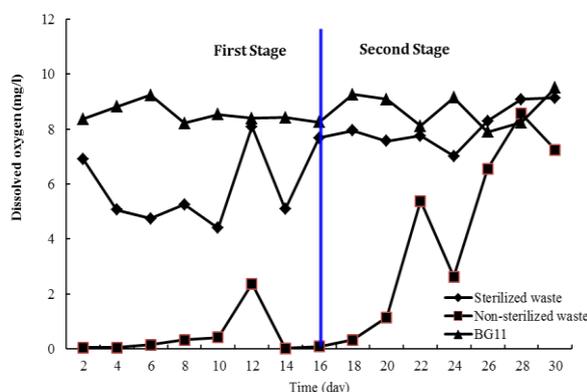


Fig. 5. Dissolved oxygen in the sterilized wastewater, non-sterilized wastewater, and standard solution BG11

The adjusting was carried out by adding 1 M of hydraulic acid to reduce the pH to 7 as shown in Figure 4. It can be seen in the same figure that the pH in all culture media and after 24 hours is returned to its original value. But its return to the standard solution is higher than that in other media. Fig. 3 shows also a significant increase in microalgae growth rate in all culture solution after adjusting the process. This reflects the impact of pH on growth of microalgae. Thus this indicates that enhancement in growth rate can also occur if the pH is maintained around 7.

This improvement was due to the convenient environment not only for microalgae but also for aerobic bacteria [26]. Nevertheless, reducing the pH reduces carbon dioxide solubility from the atmosphere. But, the presence of the main biological source (carbon dioxide of the bacteria) as well as of the carbonic compounds [27], [28] is already present in the solution is sufficient to compensate for that decrease.

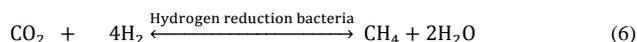
However, adjusting the pH by adding hydraulic acid may be a cost factor, and illogical for the application.

Therefore the use of carbon dioxide in the process of adjustment is better economically and environmentally as well as an adequate amount of carbon for metabolic processes.

Nevertheless, in the presence of microalgae and bacterium together in the same biological solution, the sparging of carbon dioxide may have a negative impact on aerobic bacteria with the encouragement of non-aerobic reactions to occur in acidogenesis anaerobic bacteria by converting the propionate and butyrate to acetate as the following [29].



Or via methanogenesis anaerobic bacteria by converting carbon dioxide and hydrogen to bio-methane [30], [31].



Effect of pH on microalgae growth rate has been verified in previous studies. Among these investigations, are those conducted by Khalil [32] and Çelekli [33].

They confirmed that the selection of the best-operating conditions of microalgae depends mainly on the type of microalgae that requires growing. Some achieve their highest growth rate at pH 9 and others from pH 7.

In both cases, with achieving this preference, the biological composition of algae itself can be also changed; such as protein and carbohydrates.

Moreover, the biological and chemical composition of the solution used in agriculture can play an important role in determining these preferential values. For example, Çelekli and Dönmez [33] have shown that salinity influences the determination of the best acidity value that produces the best growth.

Notwithstanding, they display that the pH 7 is possible to achieve that advantage.

Nevertheless, these researchers were conducted when the growth of one or two types of microalgae in the standard bio-solution. While in the current study the food-rich waste was used as a culture media, which is radically different.

The risk of this eutrophication greatly helps the growth of other microorganisms, which can directly or indirectly affect the microalgae growth.

A source of biological carbon produced from bacteria plays an important role in determining the optimal operating conditions for microalgae.

If microalgae lost that important source, they will have to change their pathway depending on the carbon sources found in the carbon as part of the heterotrophic process [34].

According to this study, the biomass produced by the hydrothermal process is much less than that produced by the photosynthesis process. In addition, the dependence on carbon dioxide in the atmosphere without aeration is impractical, since the quantity itself is few, as well as the limits of the mass transfer.

It is therefore noted that pH-adjusting has achieved growth rate for microalgae in both culture media (sterilized and non-sterilized). The other evidence is an increase in the concentration of dissolved oxygen in the non-sterilized solution in the second stage, which indicates the rapid growth of microalgae.

In fact, and through a series of experiments, it was observed that microalgae in the non-sterilized media undergo an anaerobic phase that may produce hydrogen sulphate or hydrogen. However, after the pH adjustment and activation of microalgae, the produced oxygen completely cut the pathway to continue the anaerobic bacteria.

Therefore the present study suggested using the sparging system with air instead carbon dioxide to adjust pH and activation of aerobic bacteria and prevent anaerobic bacteria to grow.

3.2. Aeration System in Microalgae Culture

Effect of aeration system on microalgae growth rate with standard solutions was investigated by Al-mashhadani and Khudhair [25]. These solutions (standard solution) contain certain macronutrients and micronutrients suitable for the microalgae growth. Thus, this effect may be a positive factor to improve the growth of microalgae due to stripping the dissolved oxygen produced by metabolic processes of microalgae and adjusting the pH value.

In the current research, the scenario is different, since the culture media used as a biological solution is dairy wastewater, which is characterized by a food-enriching media. It is, therefore, susceptible to the growth of many microorganisms if their environmental conditions are met.

Thus, these organisms coexist with the growth of microalgae, which may force them into food competition or sometimes change their environment. The present research gave preference to the non-sterilized solution on sterilized media (as was concluded in the previous section) depending on the economic and applied requirements of the water treatment plant. In addition, it gave an advantage to the growth of microalgae and beneficial aerobic bacteria on other microorganisms as key targets.

This can be achieved by making the environment favorable to the desired organisms and controlling the growth of other undesirable microorganisms. Thus, the presence of a third factor in controlling the desired path became necessary at this stage.

Through our investigation with mass transfer of oxygen gas in distilled water and wastewater, it was found that the physical properties of solution play an important role in the dissolution process. Fig. 6 shows the dissolution of oxygen in the distilled water and dairy wastewater when the bioreactor was sparged by air.

This response was recorded when the solutions were free of microalgae. It can be seen that the dissolution of oxygen in distilled water is higher concentration and quicker than that with dairy wastewater. Approximately 2 minutes was required to get 7 mg/l oxygen concentration in distilled water, while 15 min gave about 5.7 mg/l oxygen concentration in wastewater.

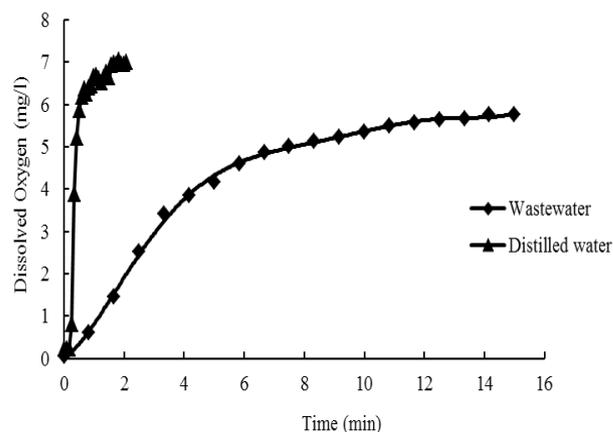


Fig. 6. Dissolved oxygen in the distilled water and dairy wastewater in the sparged bioreactor

The removal of gases from the solution required more time than from dissolution process. According to our previous experiments, the results showed that the volumetric mass transfer coefficient ($K_L a$) of the dissolution of oxygen gas in distilled water was about 3.18 min^{-1} , while in the removal process using the nitrogen the $K_L a$ was about 1.67 min^{-1} . The same thing was observed with the dissolution of oxygen and carbon dioxide in water. However, the chemical reactions of carbon dioxide with water for producing of carbonic acid and ions the dissolution and removal processes took longer time than that with oxygen. In fact, this behavior gave a sufficient time advantage to bacteria and the microalgae for the consumption of biogas resulting from the metabolic processes (i.e mutually beneficial).

Therefore, the aeration system using air to achieve the required path is the factor proposed in this study.

Two reactors were used in this section, one with a sparging system, while the second one was adopted as a control for comparison. Both reactors were operated with a non-sterilized solution with control and monitoring system of temperature, while pH and dissolved oxygen were monitored regularly. Fig. 7 shows the dry biomass weight during the growth stages of the two reactors.

The figure shows the accelerated growth rate in the sparged reactor compared to its growth in the control bioreactor.

While Fig. 8 shows snapshots of *Chlorella* Sp. microalgae in the sparged photo-bioreactor and unsparged photo-bioreactor.

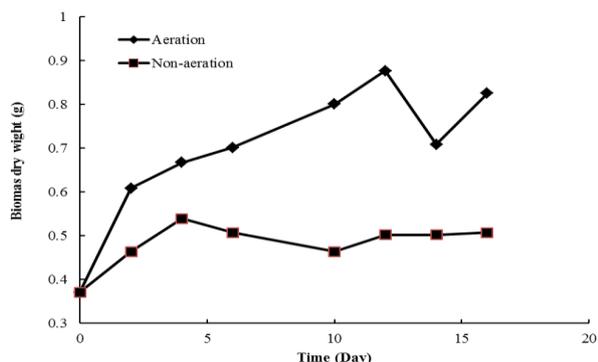


Fig. 7. Biomass dry weight of *Chlorella* microalgae in the sparged and unsparged photobioreactor

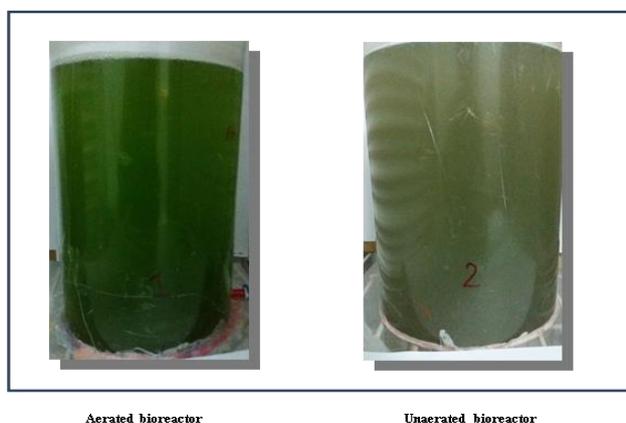


Fig. 8. Snapshot of *Chlorella* Sp. microalgae in the sparged photo-bioreactor and unsparged photo-bioreactor

The specific growth rate of *Chlorella* in the sparged bioreactor was 1.2 per day, while its value in control bioreactor was 0.2 per day. Moreover, the doubling time was 0.5776 day in a sparged bioreactor, while it was 3.465 day in control bioreactor. These values gave a clear idea of the importance of ventilation for microalgae if they were grown with aerobic bacteria in dairy wastes. In fact, the bubbling of air in the dairy wastewater used as a culture media in the current study contributes to reducing the pH value of the solution as shown in Fig. 9.

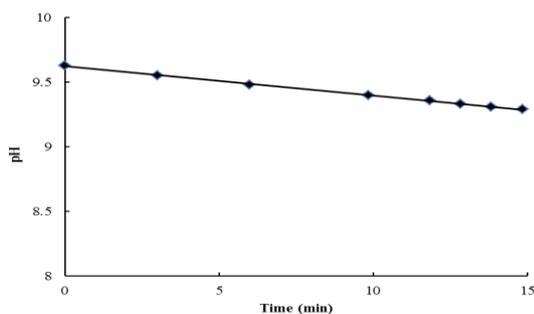


Fig. 9. pH value recorded during the sparging the microalgae cultivation

The figure also shows the pH readings during the *Chlorella* sp. microalgae cultivation process in this solution over a 15 minute pumping period. Increasing the pump period does not necessarily mean a constant decrease in pH.

This reduction was the result of carbon dioxide in the air that reacts with water producing two atoms of hydrogen ions. Because of its insignificant proportion (i.e CO₂) in the bubble air, it is unable to increase its concentration more than equilibrium concentration. In fact, the increasing in the carbon dioxide concentration in air increases that equilibrium concentration.

This can also occur if the concentration of oxygen in the culture media is higher than its equilibrium concentration.

This increase is possibly achieved if the growth of microalgae has increased over the growth of bacteria in the same solution. Therefore, the air sparging in the bioreactor may create an opportunity to decrease the concentration of oxygen in the solution as a result of moving of oxygen from the high concentration in the solution to the low concentration in the bubble air until to equilibrium concentration of oxygen based on the fundamentals of the mass transfer.

Fig. 10 shows the air sparging in the seeding solution and during the cultivation of microalgae has caused a sharp drop in the concentration of oxygen in the solution to settle to 7.63 after 4 seconds. In simple calculations, the equilibrium concentration of oxygen in the distilled water is supposed to be 7.54 at 30 ° C, while these experiments gave slightly higher value because of the different properties of the dairy wastewater to give a small percentage difference.

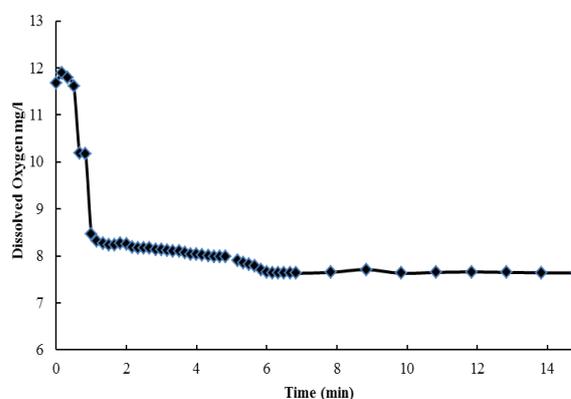


Fig. 10. The oxygen concentration in dairy wastewater during the sparging the microalgae

4- Conclusions

Biological synergy for *Chlorella* microalgae and aerobic bacteria in the dairy wastewater was investigated in the present study. The convergence of results between the sterilized and non-sterilized circles gave clear support for the growth of algae even in non-sterile environments. And therefore its applicability in water treatment plants would be economically acceptable. The results showed that the characteristics of the dairy wastewater play an important role in the removal and dissolved of biogases.

Nevertheless, this behavior may give sufficient time advantage to bacteria and the microalgae for the consumption of biogas resulting from the metabolic processes via mutually beneficial principle. The operational conditions of microalgae make bacteria able to grow and recover during the early hours of experimentation, even if sterilization was done. The results showed that pH control was an important step in improving the productivity of microalgae. The highest productivity was obtained at pH value 7, but its rise again will not take long.

Thus, the current study and through a series of experiments showed that ventilation may be the best way to control acidity. Not only does it contain carbon dioxide, it also drives the process toward increasing the amount of carbon dioxide produced by bacteria that need air.

Acknowledgment

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Nomenclatures

Parameter	Meaning	Unit
T _d	Doubling time	Day
X	Biomass dry weight	mg. L ⁻¹
X ₀	Initial biomass dry weight	mg. L ⁻¹
X _f	Final biomass dry weight in exponential growth phase	mg. L ⁻¹
μ	Specific growth rate	Day ⁻¹
K _L a	Volumetric mass transfer coefficient	min ⁻¹

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التعایش البيولوجي لنظام الطحالب الدقيقة والبكتريا في مخلفات الالبان مستخدما مفاعل حيوي ضوئي

الخلاصة

تناولت الدراسة الحالية نظام التهوية في زراعة الطحالب الدقيقة باستخدام مخلفات الالبان كوسط زرع. هذا البحث هدف الى دراسة تأثير التهوية في مفاعل الفقاعة العمودي الحيوي على التآزر الحيوي بين الطحالب والبكتريا اذا تم تواجدهما في نفس المكان. النتائج تبين بان مرحلة التعقيم ليست الخطوة المهمة في نجاح زراعة الطحالب في مياه غنية بالمخلفات العضوية. هناك تقارب واضح بين معدل نمو *Chlorella* في الاوساط الزرعية المعقمة وغير المعقمة، والتي تعطي واقعية إذا تم تطبيق الاقتراح صناعيا. من خلال المعلومات التي تم الحصول عليها ، يمكن للبكتيريا الهوائية في الاوساط غير المعقمة ، الخالية من الطحالب ، أن تستهلك كل الأوكسجين المذاب في غضون فترة زمنية قصيرة. لذلك ، فإن عامل التهوية مهم في هذه الحالة. مع ذلك ، تظهر التجارب أن التعایش بين البكتيريا والطحالب الدقيقة يمكن أن يحدث حتى لو لم يكن هناك نظام تهوية. وبالتالي ، فإن الطحالب المجهرية في المخلفات المائية للالبان قادرة على الحفاظ على بيئة الزراعة مناسبة. الغازات الناتجة بسبب عمليات التمثيل الغذائي في البكتيريا أو الطحالب الدقيقة تبقى في المحلول لفترة معينة وليس من السهولة ازلتها خاصة اذا كانت فترة ضخ الهواء متقطعة. وبالتالي ، فإن هذا سيعطي وقتا كافيا لكلا الكائنات الحية الدقيقة لتستهلك تلك الغازات. ومع ذلك ، فإن النتائج تظهر أن نظام انتعاش لمدة 15 دقيقة وثلاث مرات في اليوم يحسن إنتاج الكتلة الحيوية بنسبة 60 ٪. ولذلك ، فإن زراعة الطحالب المجهرية بالإضافة إلى هدفها المنشود يمكن أن تلعب دوراً هاماً في وحدات معالجة مياه الصرف الصحي من خلال الحفاظ على البيئة المناسبة للبكتيريا الهوائية حتى في حالة عدم وجود نظام تهوية.