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Production of *Spirulina platensis* Using Inexpensive Local Resources in Palestine

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Abstract

Spirulina platensis is a special and unique cyanobacteria that is produced worldwide with a varied cost of cultivation media. In this study, five main experiments with different treatments were performed to evaluate the possibility of using cheap aquaculture water for Spirulina production, to test if solutions made by plant ash (PAS) could be used for Spirulina production, to determine if brackish water (BW) and mining water have a good impact on Spirulina production, to create a medium composed of cheap chemicals and fertilizers to be used for Spirulina cultivation, and to test if a mixture made from local components could be used to produce Spirulina. All experiments were performed via growth and dry weight measurements, including determination of chemical and physical characteristics of the samples with a comparison with Zarrouk medium (ZM) as a reference for each experiment, and all experiments were performed for 21 days to determine the best media type that lasts longer for commercial purposes. In all experiments, pH values were between 8 and 11, and EC was between 9.8 and 30 ms/cm, while temperature was at 30˚C and 35˚C, and light was at 1500 and 5000 Lux for 16 h light and 8 h dark. Spirulina can grow in (FW). It can also grow in FW diluted with BW. Also a 3% PAS can be used as a source to cultivate Spirulina at a very low price compared to ZM. The chemical fertilizer formula was one of the best types among all treatments with a good price. A mixture of these local resources could be a very good cheap alternative source. The main result that was obtained from all experiments in this study is the ability of Spirulina to grow within a wide range of chemical parameters at a lower price.

Keywords

Spirulina platensis, Tilapia, Brackish Water, Zarrouk Medium

1. Introduction

Spirulina platensis is an old prokaryotic cyanobacteria that was rediscovered in the last few decades [1], and Spirulina is a unicellular organism that has the ability to grow under different environmental conditions [2]. Spirulina was first isolated in the 16^{th} century from Lake Texcoco in Mexico [3] [4]. National Aeronautics and Space Administration (NASA) studied the cultivation of *Spirulina platensis* as a food source [5], and the World Health Organization (WHO) reported that *Spirulina platensis* has no risk and is a good food supplement for health [6]. *Spirulina platensis* has a protein content range of 50% - 70% with 5% - 10% lipids and 10% - 20% carbohydrates, and all essential amino acids in complete balance; it also has 10 vitamins, especially vitamin B12, pro-vitamin A (β-carotene), and minerals such as iron [7] [8].

Spirulina platensis is naturally found in tropical regions with alkaline lakes (pH 11) with high concentrations of NaCl and bicarbonates [9] [10]. It can survive at pH 8.5 - 11 [11] and an electrical conductivity of approximately 20,000 μS/cm, while the best light illumination of 0.5 - 1.0 L of *Spirulina platensis* samples is approximately 67.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (5 Klux). It can also survive down to 1.5 Klux [12]. Spirulina, like other organisms, needs a proper temperature to reach their optimum growth, and the best temperature for Spirulina growth is 35˚C [13]. Mixing *Spirulina platensis* is a suitable way to support it with carbon dioxide; without mixing, the algae will ascend toward the surface, and in the case of any unsuitable conditions, it may sink and die.

Mass production worldwide is, however, constrained by the high cost of the growth medium, which mainly depends on Zarrouk medium [14]. However, there are several efforts from different studies that have been made to develop a convenient and cost-effective culture medium [15]. Michael *et al.*, that can produce spirulina biomass of comparable quality to Zarrouk medium. Murugan *et al.* [16] cultivated two species of Spirulina on cultivation medium formulated with seawater, and the results revealed that sea water medium forms a better alternative natural cheap resource to cultivate Spirulina. Regarding biomass yield, the *Spirulina platensis* (filamentous type) yield increased (2.72 g/l) when compared to the control (2.48 g/l) (Zarrouk) medium.

Kumari *et al.* [17] formulated a cost-effective fertilizer-based culture medium for the mass production of *Spirulina platensis*. The results showed that NPK fertilizer was superior to standard culture media, and 50.0% cost savings were achieved using a newly formulated NPK-10:26:26 fertilizer medium for Spirulina growth in comparison to Zarrouk medium. Raoof *et al.* [18] formulated a new medium for the mass production of Spirulina sp. By incorporating selected nutrients of standard Zarrouk medium and other cost-effective alternative chemicals, from a scale-up point of view, the revised medium was found to be highly economical because it is five times cheaper than the standard Zarrouk medium.

Introducing a new economical agricultural activity, such as the production of *Spirulina platensis,* to Palestine is vital to overcome malnutrition in Palestine and introduce a new economical industry that could be sold worldwide for the benefit of the Palestinian economy and local farmers. The main problem of Spirulina cultivation in Palestine is the high cost of the growth medium. Zarrouk medium cost approximately 2.5$/L if it is prepared in the lab and if it is used for commercial scale; it could have a lower price (1.6$/L) because the chemicals will be purchased in large quantities, but this price is still high. The main goal of this study was to conduct experiments on the cultivation and production of large amounts of *Spirulina platensis* using cheap, local, and environmentally friendly resources in a sustainable procedure.

2. Methodology

2.1. *Spirulina platensis* Cultivation at Al-Quds University

Spirulina platensis was cultivated in fish aquariums using Zarrouk medium [19] in the Aquatic Environmental Research Center at a temperature of 32˚C, pH of 10.5, sunlight (undershade) of ~25 K-Lux, and aeration using an aquarium air pump.

2.2. Experimental Design

This study included five separate experiments with different parameters. The experimental design for all tests is described in **Tables 1-5**. In all experiments, Zarrouk medium was used as a reference control, which in the first experiment was a comparison between Zarrouk medium (ZM) and fish rearing-waste water (FW); in the second experiment, a comparison was performed between ZM and FW, with the addition of plant ash solution (PSA) at a concentration of 3%, diluted brackish water, and mixed solution between all of the treatments except for ZM. In the first and second experiments, the temperature, light illumination, and volume of the sample were the same. The third experiment compared ZM, FW, PAS 1.5%, diluted BW, mining water (MW), IMJ, IMJ-1, and a mix of all types except for ZM. In the fourth experiment, ZM and IMJ-2 were compared, and in the fifth experiment, ZM and FW were compared with varied treatments composed of 90% FW and 10% of different types.

Table 1. Experiment design for the first experiment.

Experiment		One
Parameters		**Treatment**
T (˚C)	30	ZM
Light (Lux)	1500	FW
Period (days)	21	
Rep. No.	5	
Volume/Replicate	100 mL	
Spirulina: medium	1:1	

Table 2. Experiment design for the second experiment.

Experiment		Two
Parameters		Treatment
T (˚C)	30	ZM
Light (Lux)	1500	FW
Period (days)	21	PAS 3%
Rep. No.	3	BW 1:2
Volume/Replicate	100 mL	Mix
Spirulina: medium	1:1	

Table 3. Experiment design for the third experiment.

Experiment		Three
Parameters		Treatment
T (˚C)	30	ZM
Light (Lux)	5000	FW
Period (days)	21	PAS 1.5%
Rep. No.	4	BW 1:2
Volume/Replicate	250 mL	MW
Spirulina: medium	1:1	IMJ
		IMJ-1
		MIX

Table 4. Experiment design for the fourth experiment.

Experiment		Four
Parameters		Treatment
T (˚C)	35	ZM
Light (Lux)	5000	IMJ-2
Period (days)	21	
Rep. No.	4	
Volume/Replicate	250 mL	
Spirulina: medium	1:1	

Table 5. Experiment design for the fifth experiment.

Experiment		Five
Parameters		Treatment
T (˚C)	35	ZM
Light (Lux)	5000	FW
Period (days)	21	FW-BW
Rep. No.	4	FW-MW
Volume/Replicate	250 mL	FW-PAS 3%
Spirulina: medium	1:1	FW-IMJ-1
	9:1	FW-MIX

2.3. Growth Media Preparation

In all experiments, ZM was prepared from the components shown in Table 6 and 1 mL of the mixture prepared from the components in Table 7. All components except $NaHCO_3$ were dissolved together in 500 mL of distilled water, while $NaHCO_3$ was dissolved separately in 500 mL of distilled water (to prevent salt precipitation), and both were mixed after autoclaving. FW was collected from the Tilapia fish farm in the Arab Development Society (ADS), where in all experiments, it was filtered three times to remove all large particles and autoclaved. PAS was prepared in all experiments after dissolving plant ash in clean distilled water, and the solution was filtered four times to remove any excess unwanted particles. BW was collected from the rejected RO unit in the PepsiCo factory in Jericho city, diluted with distilled water and treated the same way in all experiments. The mixed solution in all experiments was prepared according to each experiment alone, and all components in each mix had equal percentages. MW was received from mining stones in Hebron city, and it was autoclaved after filtration of any residuals. IMJ, IMJ-1, and IMJ-2 chemical fertilizer types were prepared as shown in Tables 8-10, respectively, with the addition of 1 mL of the micronutrient solution in Table 7 for each type. All prepared media in all experiments in this study were autoclaved at 121˚C and 105 KPa for 15 min.

Table 6. Zarrouk medium components.

Component	Mass (g/L)
$NaNO_3$	2.5
K_2HPO_4	0.5
$MgSO_4·7H_2O$	0.2
$CaCl_2$,	0.04
$FeSO_4·7H_2O$	0.01
Na-EDTA	0.08
$NaHCO_3$	16.8
NaCl	1.0
K_2SO_4	1.0

Table 7. Micronutrient solution.

Component	Mass (g/L)
HBO_3	0.62
$MnCl_2·4H_2O$	0.012
$ZnSO_4·4H_2O$	0.044
Na_2MoO_4	0.012
$CuSO_4·5H_2O$	0.02

Table 8. IMJ medium components.

Component	Mass (g/L)
KNO_3	3.0
K_2HPO_4	0.2
$MgSO_4 \cdot 7H_2O$	0.15
$CaCl_2$	0.04
$FeSO_4 \cdot 7H_2O$	0.06
Na-EDTA	0.1
$NaHCO_3$	5.2
NaCl	2.5
K_2SO_4	2.0

Table 9. IMJ-1 medium components.

Component	Mass (g/L)
$NaHCO_3$	16.8
NaCl	1.0
Urea	0.5
$CaCl_2 \cdot 2H_2O$	0.04
$FeSO_4 \cdot 7H_2O$	0.01
Na-EDTA	0.08
K_2SO_4 (50%)	3.0
$MgSO_4 \cdot 7H_2O$	0.2
K_2HPO_4	0.5

Table 10. IMJ-2 medium components.

Component	Mass (g/L)
$NaHCO_3$	10.0
NaCl	2.0
Urea	0.35
$CaCl_2 \cdot 2H_2O$	0.16
$FeSO_4 \cdot 7H_2O$	0.07
Na-EDTA	0.08
K_2SO_4 (50%)	3.0
$MgSO_4 \cdot 7H_2O$	0.4
K_2HPO_4	1.0

2.4. Growth Monitoring

Growth monitoring (change in color) via absorbance was performed using a UV/Vis single beam spectrophotometer (Jenway 7305). The optical density of all treatments was measured at 880 nm. Two milliliters of each replicate were filtered on Whatman filter paper (porosity 11 μm) during the experiment to calculate dry weight. Growth rate was taken depending on absorbance data according to the following formula:

$$\left(\frac{A_2 - A_1}{A_1}\right) \times 100\%$$

where A_1 is the initial absorption value and A_2 is the absorption value at a later time. The growth rate percentage was calculated from the increase in optical density from the previous day in each point.

3. Results

3.1. The First Experiment

The first experiment was performed to evaluate the possibility of using wastewater from Tilapia fish ponds in ADS to cultivate Spirulina compared to ZM. In this experiment, chemical and physical parameters were measured for all treatments before and after mixing with *Spirulina platensis* using pH and EC meters. The Zarrouk medium treatment had a whitish color and a slightly turbid appearance, while the fish wastewater treatment had a brownish color before filtration. The fish water trial was very turbid, but after filtration, the effluent was clear and had a clear–transparent appearance. All pH values were over 10 and less than 11, where the electrical conductivity was between 20 and 30 mS/cm, the temperature of the experiment was 30˚C, and the light was 1500 Lux for 16 h light and 8 h dark periods.

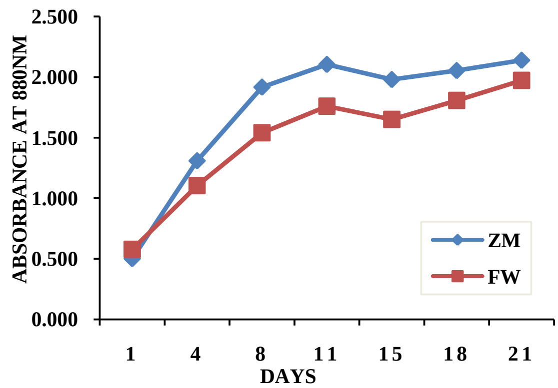
All samples were measured at 880 nm, which is the maximum wavelength florescence for chlorophyll [20]. Both the wastewater fishpond and the Zarrouk medium treatments correlated very well with each other. Both treatments began with low concentrations, and the initial absorbance was 0.5 at 880 nm. ZM tripled after 8 days, while FW doubled in the same period, and both treatments stayed at the same level until the end of the experiment (see **Figure 1(a)**). *Spirulina platensis* grew very well in fish wastewater; during the first 4 days, growth doubled, and it increased to reach 1.309 for the ZM treatment and 1.104 for the FW treatment. By Day 8, the reading increased again to reach 1.917 for the ZM treatment and 1.540 for the FW treatment, and it grew in a similar way to reach 2.104, 1.980, 2.054, and 2.139 for the ZM treatment and 1.759, 1.651, 1.806, and 1.972 for the FW treatment for Days 11, 15, 18, and 21, respectively.

Spirulina platensis absorption in the ZM treatment increased from 0.502 at 880 nm at the beginning of the experiment with a growth rate of 161% in the first 4 days (see **Figure 1(b)**) to reach 2.139 at 880 nm at the end of the experiment with a growth rate of 4% after 21 days. The sum of growth rate with all conditions (increase and decrease) was 219% from day one with a huge increase in the first week. *Spirulina platensis* cultivated in the FW treatment increased from 0.578 at 880 nm at the beginning of the experiment with a growth rate of 91% in the first 4 days to reach 1.972 at 880 nm at the end of the experiment with a growth rate of 9% after 21 days. The sum of growth rate with all conditions (increase and decrease) was 157% from day one with a huge increase in the first week. The decrease in the growth rate percentage for both treatments on Day 11 was based on the decrease on Day 15, while it increased again because the absorption reading increased in the next days. The growth rate curve in **Figure 1(b)** begins with very high growth values (161% for ZM, 91% for FW), and it decreased gradually in an opposite way to the absorbance curve in **Figure 1(a)** due to the increase in Spirulina cells number that reduced light to reach all Spirulina trichomes, where the increase in cells means an increase in wastes and a decrease in nutrients in the food source.

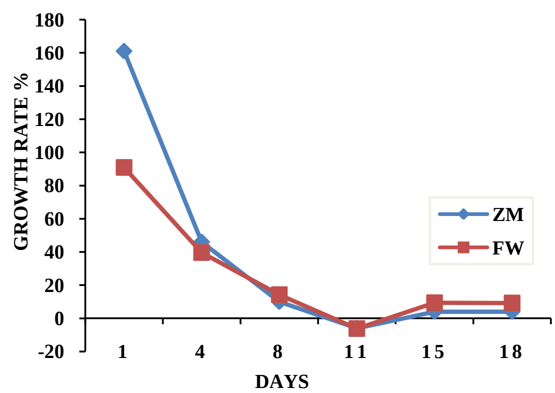
Spirulina platensis dry weight was very close and did not increase substantially in the first two weeks for unknown reasons despite a large increase in absorption reading at 880 nm, as shown in **Figure 1(c)**, while it increased sharply from 5.05 g/L to 13.68 g/L in the third week for ZM treatment and from 5.34 g/L to 8.34 g/L for FW treatment; in the fourth week, dry weight decreased to 9.34 g/L and 5.10 g/L for ZM and FW treatments, respectively, although the absorption values were more than in the third week. According to the experimental results, there is no clear relation between the measurement of absorption reading and dry weight.

3.2. The Second Experiment

The second experiment was performed by running treatments of fish wastewater, diluted brackish water, plant ash solution, and a mix of them compared with



(a)



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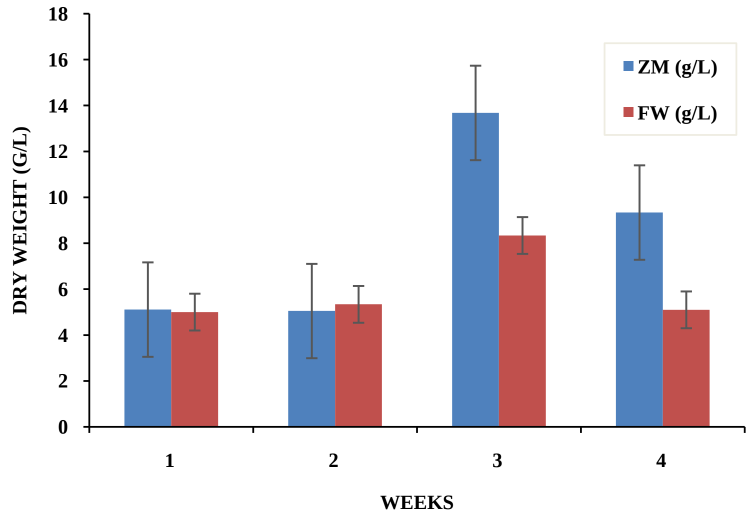


(c)

Figure 1. Experiment one results; (a) *Spirulina platensis* growth compared between Zarrouk medium (ZM) and Fish Water (FW) on an optical density of 880 nm, (b) Growth rate (%) depending on experiment optical density where increase of growth shows positive rise on the curve and vice versa, (c) Dry weight (g/L) is shown in each week alone. These measurements were the mean of five replicates.

ZM. In this experiment, pH values were over 9 and below 10, while EC values were between 14 and19 mS/cm. The temperature was 30˚C, and the light was 1500 Lux for 16 h light and 8 h dark. ZM had a whitish color, and its appearance was slightly turbid. The FW before filtration was very turbid and had a brownish color. After filtration, it had a clear appearance. Additionally, BW was diluted with distilled water with a dilution factor of two, and it had a pure color with a slightly turbid appearance. Thirty grams of plant ash (PAS) was mixed with 1000 mL of distilled water to form a 3% solution. The solution had a black-turbid color, while after filtration, it had a clear, slightly turbid appearance. The mixed solution was created by mixing fish wastewater, plant ash solution (3%), and brackish water (1:2), and it had a clear appearance.

The second experiment was also run for 21 days, but the measurements were taken every 3 - 4 days. All treatments began with low concentrations, as in experiment one, with an absorbance of approximately 0.6 at 880 nm, and most of it tripled after 14 days. ZM was the lowest among all treatments; therefore, from this experiment, FW, diluted BW, PAS 3% or a mixture could be used to cultivate Spirulina at a very low price compared to ZM. Figure 2 illustrates a comparison between all treatments: Zarrouk medium (ZM), Tilapia wastewater (FW), diluted brackish water (BW 1:2), plant ash solution (PAS) (3%), and a mixed solution for the cultivation of *Spirulina platensis*. The growth in terms of optical density started with a similar concentration for all types, while ZM began with absorbance of 0.636 at 880 nm and increased to reach 1.561 after 21 days. FW started with an absorbance of 0.709 and increased to reach 2.035 at the end of the experiment, while BW 1:2, PAS 3% and the mix began with 0.563, 0.712, and 0.720, respectively, and after 21 days, it reached 1.740, 1.757, and 1.817, respectively.

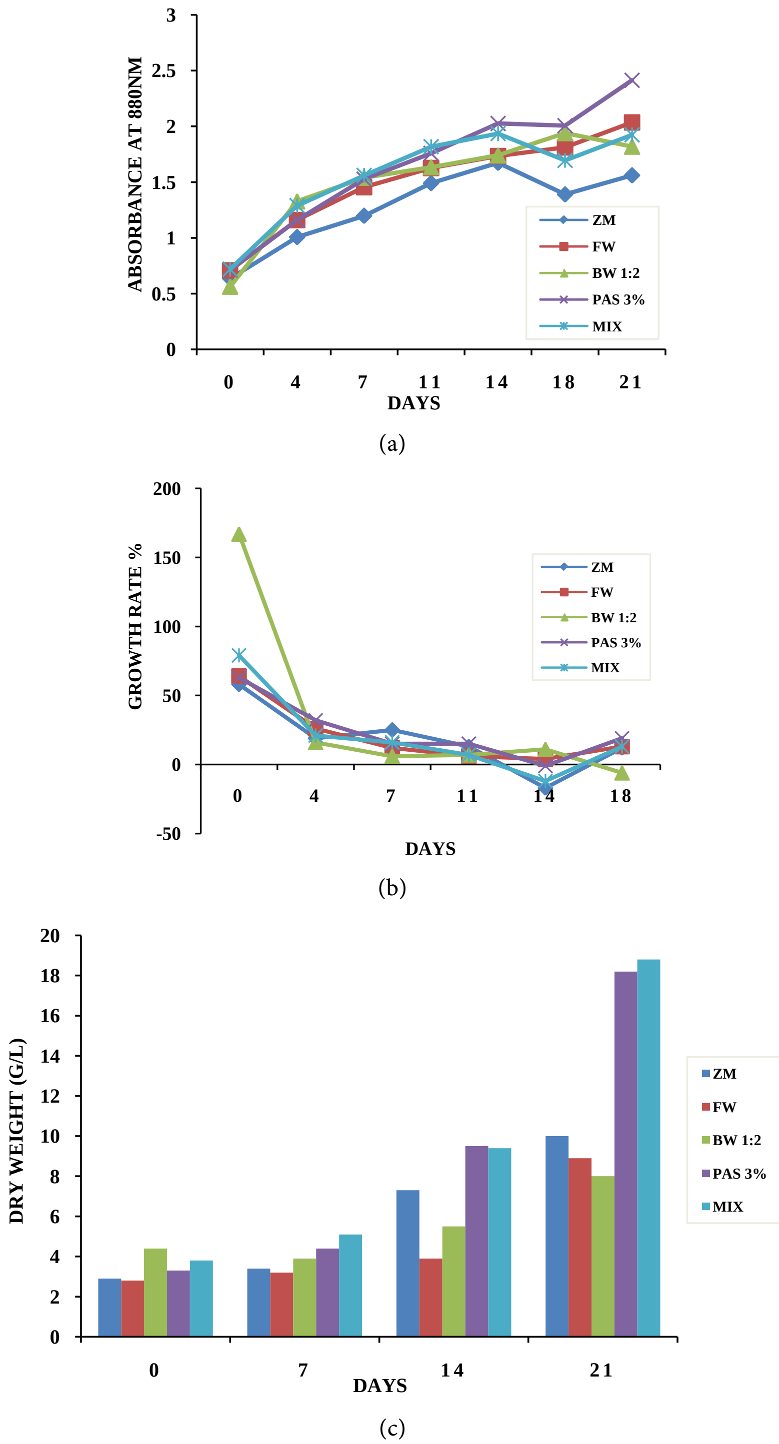


Figure 2. Experiment two results; (a) *Spirulina platensis* growth compared between Zarrouk medium (ZM), Fish water (FW), diluted Brackish water (BW), Plant Ash Solution (PAS) 3%, and mix solution on an optical density of 880 nm, (b) Growth rate (%) depending on experiment optical density where increase of growth shows positive rise on the curve and vice versa, (c) Dry weight (g/L) is shown based on 7 days interval.

According to the absorption readings for this experiment, PAS (3%) was the best medium to cultivate *Spirulina platensis* in the long term, while according to the growth rate, diluted brackish water was the best medium to cultivate *Spirulina platensis* rapidly because it increased 167% in the first 4 days with an average of 41.75% for each day. It decreased gradually to reach −6% at the end of the experiment and increased by 201% from its initial concentration. ZM treatment increased by 58% for the first 4 days, and it was increased by 110% from its initial concentration. FW increased by 64% for the first 4 days and increased by 125% from day one, while PAS 3% increased by 63% after 4 days and increased by 143% after cultivation from the first day. The mixed solution was increased by 79% in the first 4 days with an average of 19.75% for each day, and by the end of the experiment, it was increased by 124% from the first day.

Spirulina platensis cultivated in 3% PAS and mixed solution had the highest dry weight per liter. PAS (3%) began with a dry weight of 3.3 g/L, and after 21 days, it increased to 18.2 g/L with a positive difference of 14.9 g/L, while the mixed solution began with a dry weight of 3.8 g/L and reached 18.8 g/L with a positive difference of 15 g/L after 21 days. ZM, BW at 1:2, and FW started with 2.9 g/L, 4.4 g/L, and 2.8 g/L, respectively, and increased to 10.0 g/L, 8.9 g/L, and 8.0 g/L, respectively, while the lowest difference in dry weight was 3.6 g/L for DW at 1:2, and the highest difference was 15.0 g/L for the mixed solution.

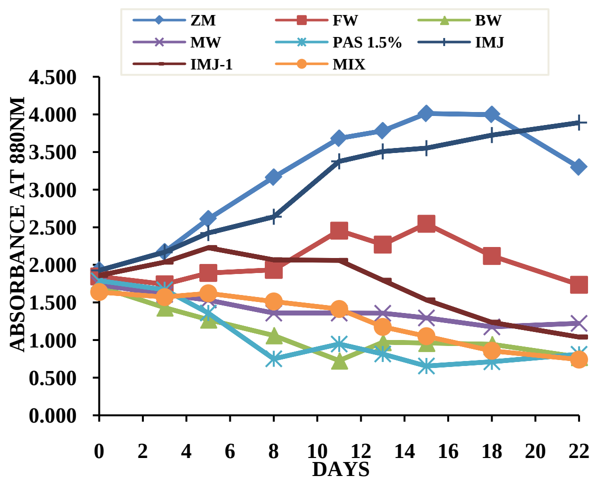
3.3. The Third Experiment

The third experiment was conducted to evaluate the same components as in experiment two with some modification and addition of new treatments, such as mining water and creating new chemical mediums as IMJ and IMJ-1. In this experiment, pH values were over 9 and below 10, while EC values were between 12 and 21 mS/cm. The temperature was 30˚C, and the light was 5000 Lux for 16 h day and 8 h night. PAS (1.5%) has the lowest electrical conductivity and total dissolved solids from the natural types, but it has the highest pH value. This makes it the best solution to raise the pH level, while FW, BW, and MW have lower pH values and higher electrical conductivity and total dissolved solids. The pH of the undiluted brackish water was 8.3, EC was 21,300 µS/cm, TDS was 10,700 ppm, and salinity was 12.2 ppt. At the same time, the ZM and IMJ treatments were very close in pH value and differed in EC, TDS, and salinity. The mixed solution was a combination of MW and PAS (1.5%). All medium types were whitish and slightly turbid, while FW and PAS were turbid before filtration and slightly turbid after filtration after mixing growth medium types with *Spirulina platensis*.

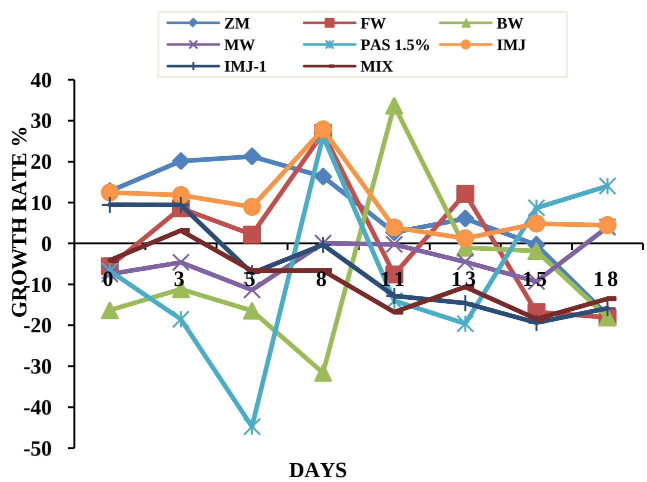
All types began at a similar concentration (**Figure 3(a)**), and the absorbance of all samples was between 1.6 and 1.9. *Spirulina platensis* that grew in ZM increased from 1.927 at Day 0, increased gradually to reach its maximum on Day 15 and began to decrease gradually, while Spirulina grown in FW decreased in the first 3 days and then increased on Day 5 and continued going to Day 15. Then, it decreased again, and that grown in BW, MW, and the mixed solution failed to grow and that in BW failed quicker than the one grown in lime mining water; in the same time period, *Spirulina* grown in IMJ and IMJ-1 treatments grew well and IMJ competed with ZM, but the fertilizers failed after Day 8, while the one grown in PAS (1.5%) started to grow on Day 11 but decreased again.

The growth rate results were different from treatment to treatment; ZM and IMJ were the best types, with growth rates of 13% and 12% for ZM and IMJ, respectively. In the first 3 days, both media had increased and decreased growth percentages, while the other types unfortunately grew negatively except for some types, such as FW and PAS (1.5%).

Although some growth medium types were not satisfactory for the growth of *Spirulina platensis,* they gave a good indicator for the dry weight, ZM as usual gave a higher dry weight than others, and FW was competing with it and even surpassed it in the third week, as presented in Figure 3(c). The other types showed a good increase in dry matter that reached a minimum of 13 g/L in the mixed solution and a maximum of 35 g/L in FW from the first and initial week to the third week.



(a)



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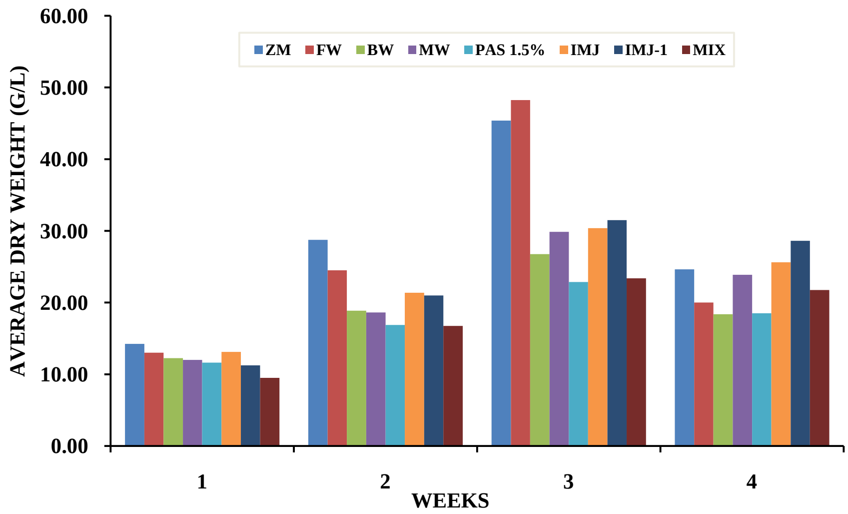


(c)

Figure 3. Experiment three results; (a) *Spirulina platensis* growth compared between Zarrouk medium (ZM), Fish water (FW), diluted Brackish water (BW), Plant ash solution (PAS) 1.5%, IMJ, IMJ-1, and mix solution on an optical density of 880 nm, (b) Growth rate (%) depending on experiment optical density where increase of growth shows positive rise on the curve and vice versa, (c) Dry weight (g/L) is shown based on weekly interval.

3.4. The Fourth Experiment

The fourth experiment was run at the same time as experiment five. It was a comparison between ZM and IMJ-2. In this experiment, pH values were between 9 and 10, while EC values were approximately 21 mS/cm, where temperature was at 35˚C, and light was 5000 Lux for 16 h day and 8 h night. The Zarrouk and IMJ-2 treatments started with similar concentrations at a temperature of 35˚C. Both treatments began with a moderate concentration with an absorbance reading of 1.25 at 880 nm. ZM doubled after nearly 14 days, while IMJ-2 increased by 1.76 times during the same period. Both treatments increased with an average absorbance reading of 1.25 in a similar way until Day 10 of the experiment. On Day 11, the growth of *Spirulina platensis* behaved differently, as shown in **Figure 4(a)**. On Day 14, both reached the highest growth level; ZM increased to reach an absorbance of approximately 3.052, while IMJ-2 reached an absorbance of approximately 2.295. After that, both decreased.

Growth rate performance was different for both medium types. ZM began with a low growth rate in the first 3 days, then increased gradually and then decreased after Day 10, while IMJ-2 began with a nearly constant rate and then declined gradually after Day 10; then, both increased again after that. The ZM growth rate was 13% in the first 3 days, then it increased to 22%, 30%, and 40%, respectively, then dropped down by −18%, and finally increased again by 3%. The IMJ-2 growth curve began with 21% in the first 3 days, then increased by 16%, 18%, and 6%, respectively, then dropped by −33%, and finally increased by 18%.

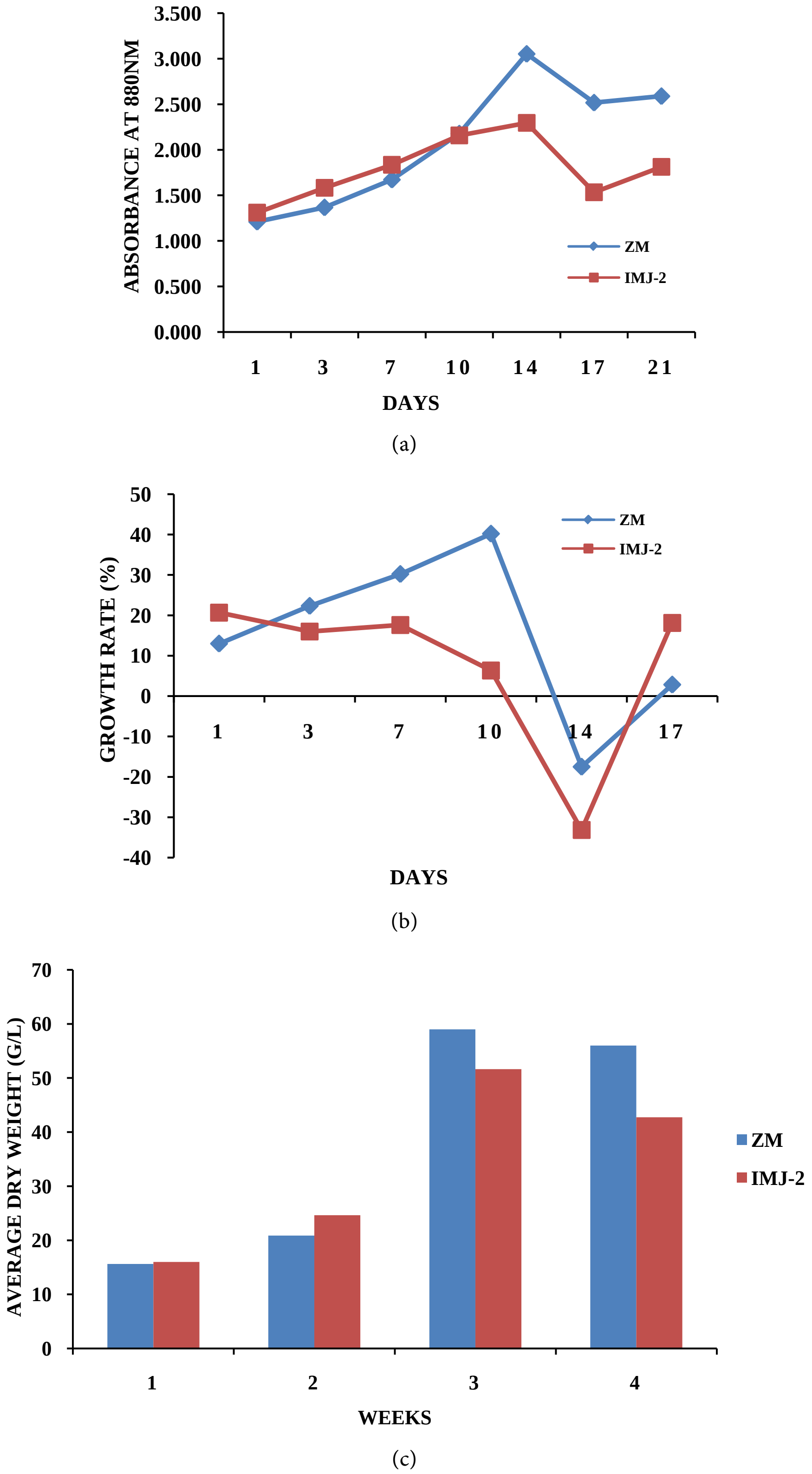
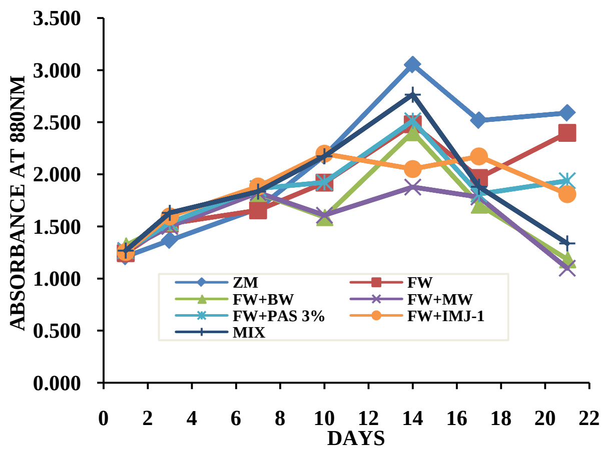


Figure 4. Experiment four results; (a) *Spirulina platensis* growth compared between Zarrouk medium (ZM) and IMJ-2 solution on an optical density of 880 nm, (b) Growth rate (%) depending on experiment optical density where increase of growth shows positive rise on the curve and vice versa, (c) Dry weight (g/L) is shown based on weekly period.

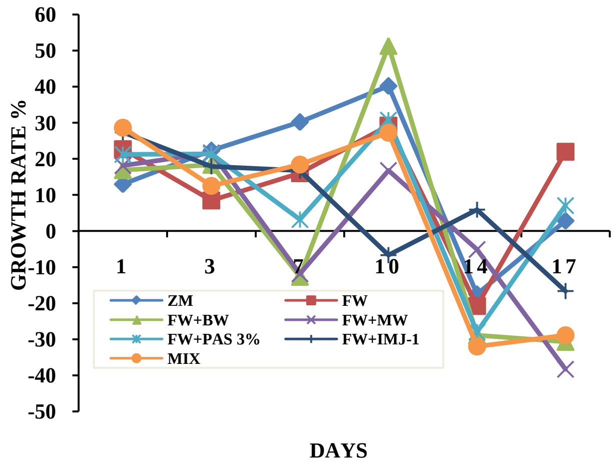
Dry weight in this experiment began with 16 g/L for ZM and increased after one week to reach 21 g/L, after which it increased substantially to 59 g/L and then decreased to 56 g/L. The IMJ-2 treatment also began at 16 g/L and increased to reach 25 g/L after one week, which was better than the Zarrouk treatment for this period. Then, it increased to be very close to the ZM treatment and reached 52 g/L, after which it dropped to 43 g/L.

3.5. The Fifth Experiment

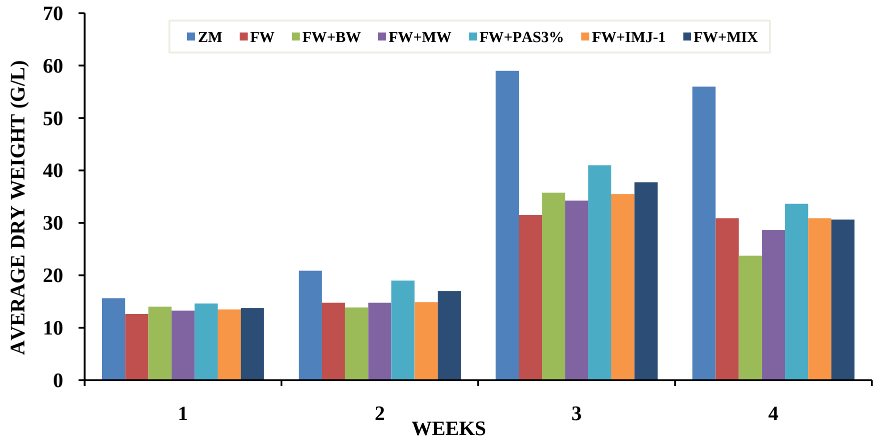
The fifth experiment was a comparison between ZM, FW, and the following types: FW:BW (9:1), FW:MW (9:1), FW:PAS-3% (9:1), FW:IMJ-1 (9:1), and a mixture composed of FW:(BW + MW + PAS-3% + IMJ-1) (9:1). In this experiment, pH values were between 8 and 9.30, while EC values varied between 9.8 and 21 mS/cm. All treatments began with moderate concentrations with an absorbance of approximately 1.25 at 880 nm. ZM and the mixture were the best types, while ZM, the mixture, and FW:IMJ-1 exhibited similar behavior until Day 10. All treatments started with similar concentrations (**Figure 5(a)**) and increased during the first week with similar behavior. At the beginning of the second week, all changed as follows: *Spirulina platensis* grown in Zarrouk medium, fish wastewater (including IMJ-1 medium), and mixed solution increased the most and reached approximately 2.177, 2.198, and 2.173, respectively, while most of the growth medium types increased by the end of week two and then decreased gradually. Growth rate measurements were different according to the medium used (**Figure 5(b)**). The best growth rate during the first 3 days was recorded for the mixed solution at 29%, while the lowest growth rate was for the Zarrouk medium at 13%, but this changed by the end of the first week and until the end of the experiment. The overall and best growth rate for 21 days was for Zarrouk medium with a growth rate of 90%, and next was fish wastewater with 77%, while fish wastewater (with PAS 3%), fish wastewater (with IMJ-1), fish wastewater (with Mix), fish wastewater (with BW), and fish wastewater (with MW) were at 55%, 44%, 26%, 13%, and 2%, respectively.



(a)



(b)



(c)

Figure 5. Experiment five results; (a) *Spirulina platensis* growth compared between Zarrouk medium (ZM), Fish water (FW), and the following types FW:BW (9:1), FW:MW (9:1), FW:PAS-3% (9:1), FW:IMJ-1 (9:1), and a mix composed of FW:(BW + MW + PAS-3% + IMJ-1) (9:1). On an optical density of 880 nm, (b) Growth rate (%) depending on experiment optical density where increase of growth shows positive rise on the curve and vice versa, (c) Dry weight (g/L) is shown based on 7 days interval.

Spirulina platensis had a similar dry weight range from 13 to 16 g/L on day one in all growth medium types, while after one week and according to the behavior of Spirulina in each type, its dry weight changed and increased in some types. In Zarrouk medium, for example, the dry weight increased from 16 to 21 g/L, while in fish wastewater, it increased from 13 to 15 g/L, and in fish wastewater and brackish water, the dry weight of *Spirulina platensis* did not change. It was approximately 14 g/L. The rest of the growth medium types increased at a lower rate than the Zarrouk medium. All of that changed after week two, and most of them doubled their dry weight. The lowest one was fish wastewater, with 17 g/L increasing from week one to week two, while most of them increased by approximately 20 g/L during the same period. Zarrouk medium still had higher biomass, with an increase of 38 g/L for the same period.

4. Discussion

The result that was obtained from all the experiments in this study is that Spirulina was able to grow within a wide range of chemical parameters. The pH of the medium that supports the growth of *Spirulina platensis* can range between 7.08 and 10.72, while the electrical conductivity can be between 6600 and 21,500 µS/cm. The total dissolved solids ranged between 3300 and 10,200 ppm, and the salinity ranged between 3.30 and 11.6 ppt. The optimum conditions were always achieved when the parameters moved toward higher values. The best chemical parameters after mixing *Spirulina platensis* with the growth medium ranged between 8.6 and 10.65 pH values, and the best electrical conductivity ranged between 10,000 and 29,700 µS/cm. The best total dissolved solids ranged between 5500 and 19,500 ppm, and the best salinity ranged between 5.36 and 17.40 ppt. The best results were obtained with higher alkaline and salty conditions.

Spirulina platensis grows rapidly during the first three to four days with a huge increase in growth rate because in the first three days, low concentrations of Spirulina cells will have enough nutrients and enough light saturation. Light will pass easily and reach all Spirulina trichomes, while after three days, the number of cells will increase to be more concentrated, and nutrient concentrations will decrease; thus, light cannot pass the water easily due to high numbers, and the growth rate will decrease due to low nutrients, especially nitrogen, and less light [21]. Excessive light intensity may lead to photooxidation and inhibition, whereas low light levels will become growth limiting [22].

From our practical experiences, Spirulina growth can extend up to seven days depending mainly on light and nitrogen levels in addition to other minor parameters. *Spirulina platensis* should be treated carefully and supported with nitrogen every four days if urea is used as a nitrogen source or every two weeks if sodium or potassium nitrate is used as a nitrogen source. When Spirulina is grown in bottles or in a transparent container, the inner wall of the container or the bottle should be cleaned at least once per week to allow light passage and reach more Spirulina cells because Spirulina movement in the container will make it stick to the wall surface, especially if it is in a straight shape; and, day by day, it will create a coat on the surface.

Dry weight in most treatments used in this study showed a small increase during the first week and a large increase after the second week. Compared to the absorbance, we found that dry weight is not always a good indicator and does not represent the absorbance. We then tried another method and found that if dry weight measurements were based on the change in growth rate and absorbance after taking the weight on the first day, it would give related and corrected data.

Zarrouk medium was the best growth type, but it is not the cheapest. Fish wastewater with a price of 0.42 $/m^3 was a very good source to cultivate *Spirulina platensis* and needs to be controlled and tested for its elemental content, especially nitrogen. From our practical experience, fish wastewater, even with filtration and autoclaving, forms a type of coating on the inner walls of transparent bottles that prevents light passage and leads to Spirulina death. Plant ash comes from natural sources, such as weeds and grasses, that cause many problems for farmers, and it is considered a very good source to cultivate *Spirulina platensis* with a price of ~ 0.42 $/m^3$. Its quality varies depending on the concentration used. Brackish and mining water could be used in addition to other elements, such as nitrogen, phosphorous, and potassium, and it could be bought at approximately 0.42 $/m^3$. IMJ, IMJ-1, and IMJ-2 are very good for cultivating *Spirulina platensis* for short periods at a lower price than Zarrouk medium; also, all the types that are composed of chemicals could be cheaper if purchased in large quantities.

Common notes could be shared between this study and the previous studies mentioned in chapter two. In comparison with Danesi *et al.* [23], they found that the best results were achieved at 30˚C, and we tested Spirulina at 30˚C and 35˚C and obtained very good results. They also found that the best light intensity was 60 μmol photons $m^{-2}.s^{-1}$, which is close to the intensity we used (approximately 5000 Lux). They also obtained more cell productivity after using urea as the nitrogen source, which was clear in the fourth experiment when dry weight was compared between IMJ-2 and ZM. In this study, we looked for urea as a promising alternative as a cheap nitrogen source, and Soni *et al.* [24] observed the same thing after their experiments. They also concluded that growth media based on organic nutrients produce more biomass, and we noticed that when we compared FW with ZM in most of our experiments. After performing the experiments in this study and from our experience, we noticed that media used urea as a nitrogen source sustained for 10 days at maximum, and Sukumaran *et al.* [25] also noticed and recommended adding urea as pulse-fed throughout the cultivation period.

5. Conclusion

It is possible to introduce *Spirulina platensis* as a new economical agricultural product and for Palestine to overcome malnutrition and introduce this new economical industry to sell it worldwide for the benefit of the Palestinian economy and local people. *Spirulina platensis* could be used as a healthy food for many people in Palestine. The production price of *Spirulina platensis* varies between countries around the world. This study proved that cultivation in Palestine is possible at a low cost. This is the first study in Palestine that investigates the possibility of Spirulina cultivation and production in the Jordan valley environment with lower cost than other international growth medium types and with competitive quality. The field is new in Palestine and needs further research, including testing the composition of different cultivation sources, such as fish wastewater.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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