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Original Article

Cultivation of *Spirulina platensis* in human urine medium or/and fish liver oil medium (home design)

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ABSTRACT

Spirulina platensis is one of the most significant algae that has attracted a lot of business interest as a novel natural product, which necessitates the development of a low-cost cultivation system to produce high yields. This study aims to evaluate the 30-day culture of *S.platensis* in a human urine mixture with or without fish liver oil medium (home design). The cultivation included eight conditions: Zarrouk's medium (control), human urine medium at 1:1 and 1:2 (v:v) concentrations, fish liver oil medium at 5/100 and 10ml/100ml concentrations, and finally mixture medium. The results show that *S.platensis* grew in all tested media with stable productivity. Nevertheless, Zarrouk's medium was significantly ($P<0.05$) higher than all tested media in all growth indicators. On the other hand, it was observed that *Spirulina* cultivation in the mixture medium was very satisfactory, yielding biomass (4.4g/L), a specific growth rate of 0.14 mg/L/day, a generation time of 2.7 days and total carbohydrate 53.0% (w/w), which was comparable to Zarrouk's medium, with biomass (5.2 g/L), a specific growth rate of 0.18 mg/L/day, a generation time of 3.2 days and total carbohydrate 38.0% (w/w). This study is a step in the right direction toward developing an affordable medium for *Spirulina platensis* cultivation.

1. Introduction

Spirulina spp. is one of the most commonly used natural ingredients and is becoming increasingly popular as a dietary supplement among humans. In the 16th century, it was considered one of the main meals of a Kanembu tribe that still lives in Chad and Niger [1], It is also used as a food supplement for animals, especially for poultry and fish, due to high protein content, vitamins, fatty acids, pigments, minerals and amino acids [2]. *Spirulina* has attracted many international companies and is manufactured as a healthy food product because, in addition to removing toxins and heavy metals, it also has special properties such as anti-inflammatory, antioxidants, diabetes and treating muscular cramps [3,4]. *Spirulina platensis* belongs to the phylum Cyanobacteria (blue-green algae), It is a microalga that is a filamentous, spiral multicellular, lives in soil, seawater, freshwater and alkaline environments [5]. Like any other blue-green algae, this alga needs important nutritional elements to continue growing, especially nitrogen and phosphorus [6]. The synthetic media for the cultivation and growth of algae are considered to have high costs [7]. human urine could be nutrient-rich, due to its content of 70% nitrogen stack and 30% phosphorus stack in wastewater [8], Focus of recent studies on microalgae cultivation using alternative media from various waste materials [9], Recently, an attempt has been conducted to tune the carbohydrate content of *Spirulina* using a bubble photobioreactor under concentrations of nitrogen and phosphorous [10]. In a similar vein, *spirulina* grown on Kosaric Medium and Papaya Skin Powder Medium produced good commercial results [11].

The purpose of this study was to assess the evaluation of the growth performance of *Spirulina platensis* in different concentrations of human urine medium and fish liver oil medium with home design.

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2. Material and Methods

2.1. Spirulina platensis Inoculum Preparation:

- *Spirulina platensis* was obtained from the Centre for VBX and was cultured in the standard culture medium described by Zarrouk (1966) [12], for the growth of cyanobacteria.
- 50 ml of S.platensis brood stock was inoculated into each 200 ml of media. Cultivation of S. platensis was carried out for one month in a glass tank. Prior to starting the studies, the standard curve was established, through spirulina platensis cell concentration was monitored by measuring the optical density of the culture at the wavelength 670nm using the spectrophotometer (biomass concentration curve), over 6 days under optimal growth inside the Zarrouk's medium.

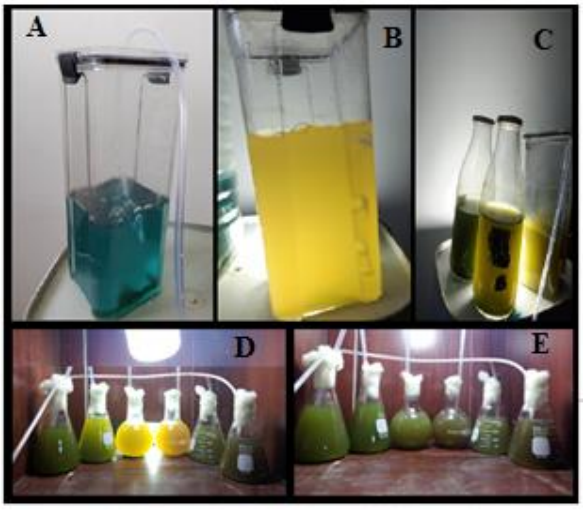

Fig. (1): Culture *Spirulina platensis* in a cultivation room, (a) First day, (b and c) Prior to starting the studies, (d) 15th day and, (e) 30th day.

2.2 Culture Conditions:

- Zarrouk's medium (control).
- The urine medium: was prepared by mixing human urine with distilled water of (1:1 and 1:2) (v/v) in an Erlenmeyer flask, after a routine urine test in the analytical laboratory to ensure that the urine was free of microbes. Calcium bicarbonate 2g/l was added to the medium, It was put in the light culture chamber at 30.0℃ under fluorescent light 3000lux with dark/light of 12:12h, were installed Air pumps as a source of oxygen with manual stirring, pH was adjusted to 9.5 with NaOH.
- The fish liver oil medium: was prepared by mixing fish liver oil with distilled water of (5/100 and 10ml/100ml) (v/v). Other cultivation parameters were kept constant from light and air, and no other nutrient substances were supplemented into this medium to avoid the introduction of new substances.
- The mixture medium: was prepared by adding 5ml of fish liver oil to the urine medium at a concentration (1:1). all the experiments were performed in triplicate.

2.3 Analysis physiological:

During the cultivation period, 10 ml of spirulina culture was harvested for all the test media at intervals (5, 10, 15, 20, 25 and 30 days). it was filtered using filter paper Whatman NO.1, then dried and powdered. The powder was added to 10 ml of methanol. Then, it underwent constant agitation in a vibratory shaker at 35 °C before being filtered and shaken in a centrifuge at 3500 rpm for 15 minutes. the obtained filtrate was freed from the solvent by evaporation under reduced pressure. Spirulina growth rates were followed to perform some metabolic measurements.

- Biomass productivity was calculated gravimetrically in the logarithmic phase using the following equation [13]:

1. $$Biomass-productivity(mg/L/d)=\frac{Biomass-yield(mg/L)}{Number-of-days}$$

The optical density was used as a parameter for Specific growth rate and production time of Spirulina platensis through absorption measurements at 750 nm [14].

2. $$\mu\,(d-1) = (\ln N1 - \ln N0)/(t1 - t0)$$

Where: N1 = Optical density at time t1, N0 = Optical density at time t0, t1 - t0= The time elapsed in days between two determinations of optical density.

- The generation time (G) or doubling of optical density) can be calculated as follows [15]:

3. $$G=\frac{\ln 2}{\mu}d$$

- Determination of Photosynthetic Pigments:

10 ml of spirulina platensis suspension was centrifuged and the growth media were decanted, The pigments were extracted in hot methanol (70°C) for 10 minutes, this was made daily during the period of the experiment (Marker, 1972). Cell debris was removed by centrifugation and the clear supernatant, which contains the pigments, was diluted to a definite volume. The absorbance was measured against a methanol blank at the wavelengths of 470, 652.4, and 665.2 nm using a spectrophotometer. Pigment fraction (µg/ml) was calculated using the following equation[10]:

4. Chlorophyll-a(µg/mL)=16.72×A665.2−9.16×A652.2.
5. Chlorophyll-b(µg/mL)=34.09×A665.4−15.28×A665.2.
6. Total carotenoids (µg/mL) = (1000 × A470 - 1.63 × Chl a - 104.9 × Chl b) / 221.

where A665.2, A652.4, A470 are the absorbance at 665.2, 652.4, and 470 nm.

- Determination of total carbohydrates.

To estimate the total carbohydrates, a certain amount of algae, after being dried and weighed was hydrolyzed by (4N) HCl for two hours in a boiling water bath. After cooling, the hydrolysate was filtered and the filtrate was completed to a definite volume. One ml filtrate (containing carbohydrate solution) was introduced into a clean Pyrex test tube and mixed with 9ml anthrone reagent. The mixture was then heated in a boiling water bath for exactly 7 minutes, after which it was directly cooled under tap water. The developed blue-green color was read at the wavelength of 620 nm against a blank containing only water and anthrone reagent [16].

3. Statistical Analysis:

All statistical analyses were performed using SPSS version 24 (Statistical Package for the Social Sciences)—differences in culture conditions and biomass composition between all Media with Tukey's test. Statistical significance was set at $p < 0.05$ for all the analyses.

4. Results:

Figures 2, 3 and 4 show the indicators of *spirulina* cultivation in urine and fish liver oil media. The results show that *spirulina* grew in all the media tested, and took a short period of time to adapt to these media. Biomasses were estimated throughout the cultivation period every (5, 10,15, 20, 25 and 30 days). The maximum growth was observed for *Spirulina* for most media on days (15, 20 and 25), which decreased by day 30 for all tested media, as found that produced biomass increased with the passage of days, especially in the mixed medium, which recorded maximum biomass of 4.4 g/L at the experiment end. Furthermore, this medium achieved the highest specific growth rate (0.14 mg/L/day) with a generation time (2.7 days) compared to other media. Additionally, fish liver oil medium (10/100) produced 3 g/L biomass with a specific growth rate (0.11 mg/L/day) and generation time (2.2 days). The two urine media 1:1 and 1:2 produced 2.5 g/L biomass each, with a small specific growth rate (0.09 mg/L/day) and a short production time. The lowest biomass productivity throughout the experimental period was recorded with fish liver oil (5/100), with the minimum specific growth rate (0.06 mg/L/day) and generation time (1.0 days). compared to 5.2 g/L in the control (Zarrouk medium), with a specific growth rate (0.18 mg/L/day), and a generation time (3.2 days).

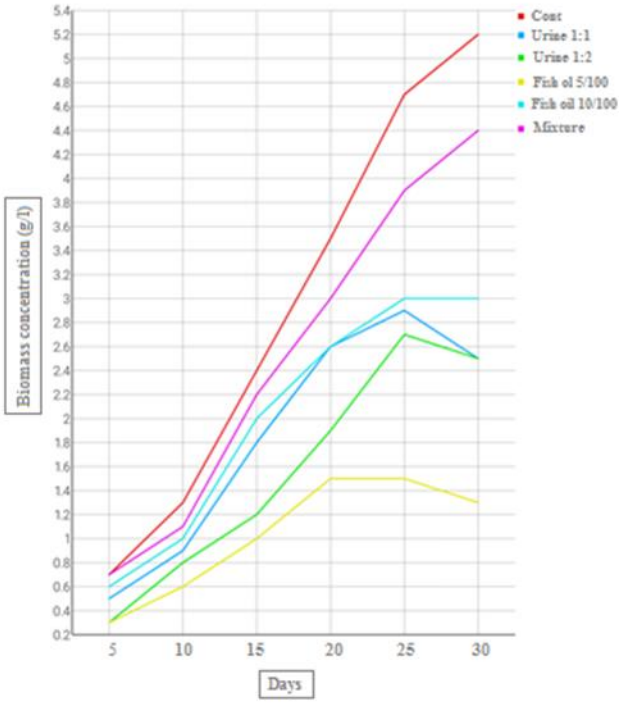

Fig. (2): biomass concentration curves to the culture of *Spirulina platensis* at 30days.

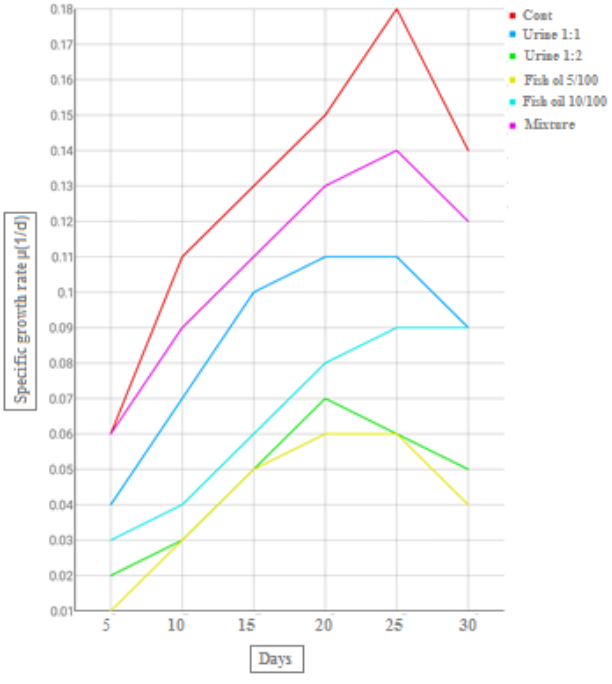

Fig. (3): Curves of Specific growth rate of *Spirulina platensis* in 30days.

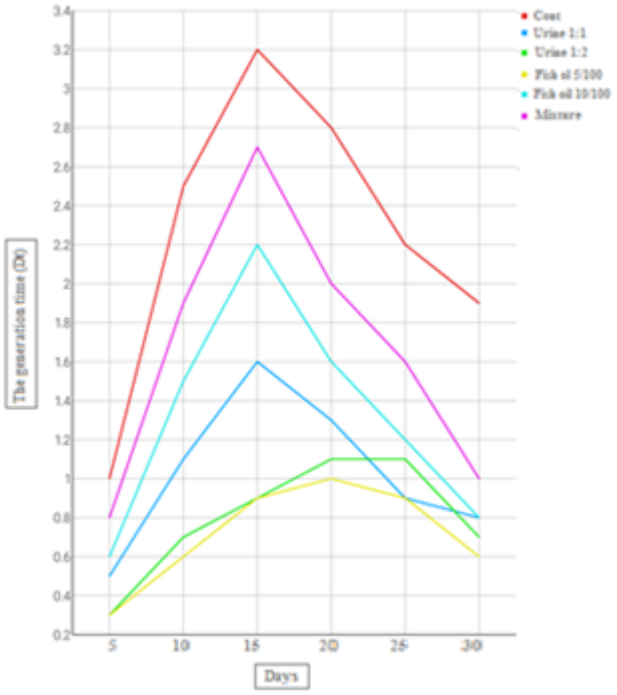

Fig. (4): Curves of The generation time of *Spirulina platensis* in 30 days.

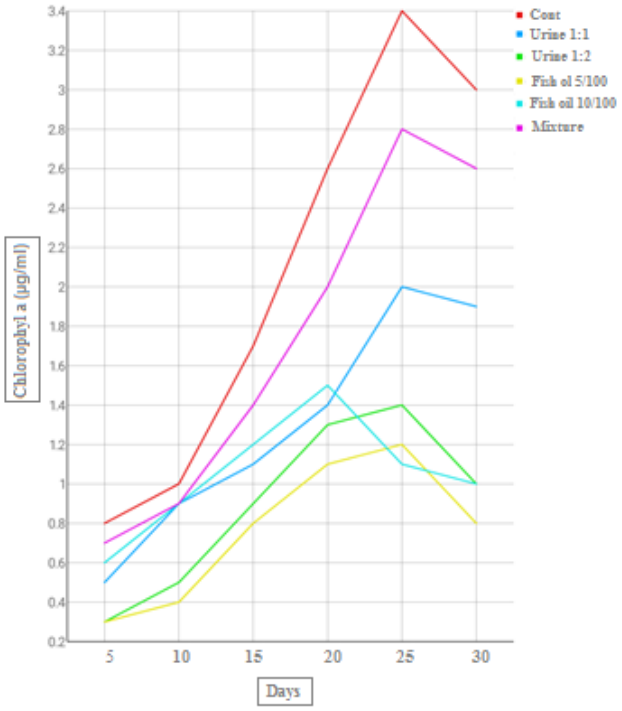

Fig. (5): chlorophyll-a curves to the culture of *Spirulina platensis* at 30 days.

Estimation of pigments (chlorophyll a and chlorophyll b, and total carotenoids) was performed on days 5, 10, 15, 20, 25, and 30. The results revealed in Figures 5,6 and 7 that the Maximum chlorophyll content found in the mixed medium was higher significantly than all other media, by levels: Chlorophyll-a (2.80± 0.05 µg/ml), Chlorophyll-b (1.30 ± 0.01 µg/ml), carotenoids (0.10 ± 0.02 µg/ml). while the fish oil medium (10/100) contained the minimum level of pigments, Chlorophyll-a (1.20± 0.05 µg/mL), Chlorophyll-b (0.45 ± 0.01 µg/mL), carotenoids (0.02± 0.00 µg/mL), during the 25th day. Similar to these results, the carbohydrate content of the mixed medium increased by 38%. In contrast, a decrease in carbohydrate content was also recorded in all other media compared to the control Fig.(8).

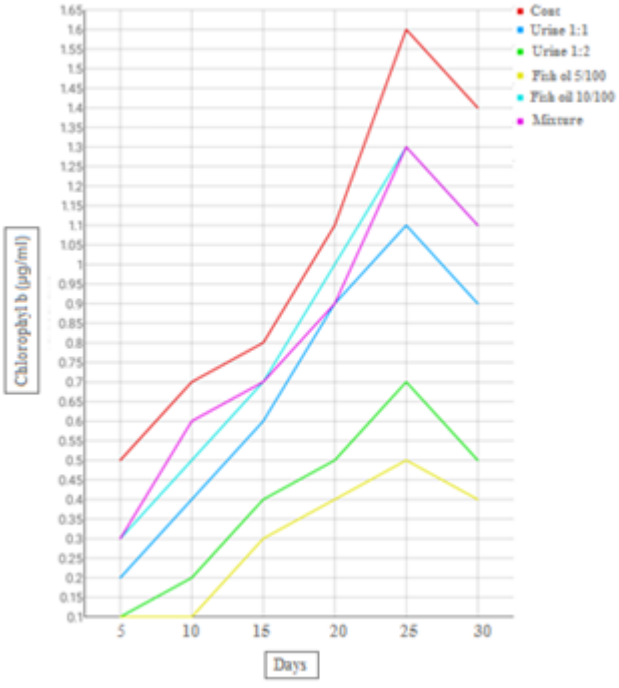

Fig. (6): chlorophyll-b curves to the culture of *Spirulina platensis* at 30 days.

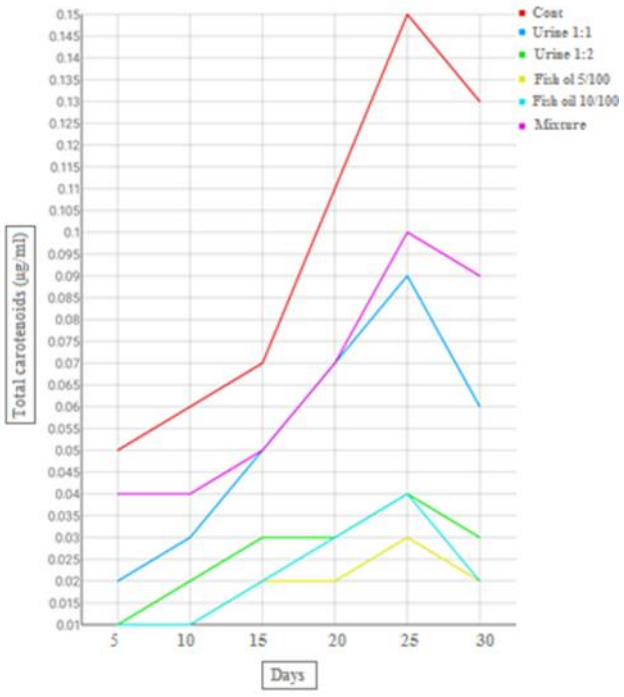

Fig. (7): Total carotenoids curves to the culture of *Spirulina platensis* at 30 days.

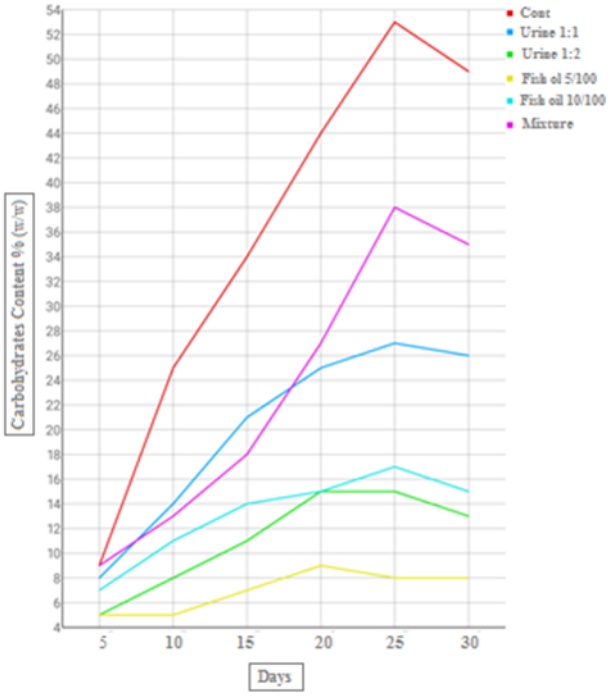

Fig. (8): Carbohydrate content curves to the culture of *Spirulina platensis* at 30 days.

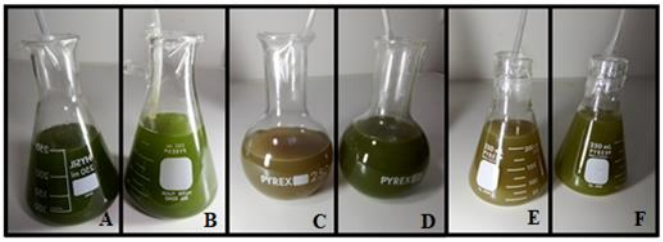

Fig. (9): Final productivity of *spirulina* in all media. A: Control, B:the mixture, C: Fish liver oil 10/100mL, D: Fish liver oil 5/100mL, E: Urine 1:1, F: Urine 1:2.

5. Discussions:

Numerous businesses and academics have made an effort to discover low-cost techniques for growing *spirulina* inside of media that resemble the Zarrouk medium and have the simplest capabilities possible. However, the majority of the outcomes of these efforts have not shown much benefit thus far [17,18]. This study showed that *Spirulina platensis* can be cultivated in a basic medium made of fish liver oil and human urine while still meeting other cultivation requirements including adequate illumination and oxygen, This could be because, in addition to the high concentrations of phosphorus and nitrogen found in human urine, fish liver oil also contains high concentrations of micronutrients required for the growth of microalgae, this is similar to the results of a study successfully grew *Spirulina maxima* in an outdoor urea-enriched medium [19]. Additionally, no discernible variations were found between the mixed medium and the Zarouk medium, the control medium, in a number of growth metrics, including biomass, productivity, and generation time. However, it was discovered that the urine and fish liver oil media individually were insufficient to produce high *spirulina* yields. However, according to this study, *S.platensis* can be cultivated with high fish liver oil, which is a great source of calcium, phosphorus, vitamin D, and selenium, among other vitamins and minerals [20]. Conversely, *Spirulina* was able to grow at low concentrations in human urine medium. This outcome for the treatment of human waste is almost satisfactory. This is comparable to a recent study [21] that established the viability of cultivating *Spirulina* for the treatment of wastewater in piggery farms. The 20th and 25th days in the majority of the tested media showed the highest rates of *S.platensis* cell division as well as the highest levels of carotenoids, total carbohydrates, and chlorophyll a and b. Nevertheless, these metrics declined dramatically in comparison to the control. It was underlined that following this time, the indicators decreased. This is consistent with many studies that have demonstrated a direct correlation

between the rate of cell division and the rise in pigments and carbohydrates [22,23].

6. Conclusion

Considering that this study was conducted at home, the results generally point to the success of culturing spirulina in a medium made of human urine and fish liver oil. This is a simple and affordable method that could replace the Zarrouk medium used to grow the blue-green medium used to grow blue-green algae. In addition to high concentrations of fish liver oil medium, the study found that spirulina can also grow at low concentrations in human urine medium.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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