IECAG 2021

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Proceedings

Bio-remediation of agro-based industry's wastewater and mass production of Spirulina (*Spirulina platensis* (Gomont) Geitler 1925)

Jerentulina Vijayarasa [1], Kandiah Pakeerathan [1,*], Nagarathnam Thiruchchelvan [1] and Gunasingam Mikunthan [1]

[1] Department of Agricultural Biology, Faculty of Agriculture, University of Jaffna, Ariviyal Nagar, 44000, Kilinochchi, Sri Lanka.

* Correspondence: pakeerathank@univ.jfn.ac.lk; Tel.: +94-077-696-9511

† Presented at the 1st International Electronic Conference on Agronomy, 3–17 May 2021; Available online: https://sciforum.net/conference/IECAG2021

Abstract: The Spirulina [*Spirulina platensis*] (Gomont) Geitler 1925, is a cyanobacteria and used as ecologically sound nutrient-rich dietary supplement. These microalgae have the capability to produce the least-cost protein per unit area than livestock, therefore, being investigated to address malnutrition and food security. Apart from the variety of components like animal feed, fertilizer, and cosmetics produced from Spirulina, phytoremediation of wastewater using Spirulina is an economically viable and environmentally sound tactic. A study carried out with the objectives of quick removal of waste from selected wastewater from agro-based industries, and identify the suitable organic wastes as the costless media for growing *S. platensis* for its powder production. Wastewater from the fish pond, poultry unit, grain soaked water, and parboiled rice liquid waste was selected as treatments and inoculated with the stock culture of S. platensis. Treatments were replicated three times along with the Zarrouk's medium as standard control and arranged in a randomized complete block design. The chemical parameters of wastes such as OD value, pH and EC(mS/cm), and the data on the growth of Spirulina using UH5300- Spectrophotometer with a wavelength of 560nm, were measured in 2 days' interval. The derived data were analyzed using SAS 9.4, and significance among treatments was determined according to the Duncan Multiple Range Test at P <0.05. The results show that the poultry wastewater was identified as a suitable medium for S. platensis growth with the harvestable density of 0.8 at a very low concentration (25 %) in 7 days compare to the standard Zarrouk's medium. The Maximum and significant OD value of 1.313 was observed on the 15th day in poultry wastewater, and non-significant among other treatments at P <0.05. In fish pond wastewater the maximum OD value of 0.567 was obtained on the 15th day. pH value of poultry wastewater declined from 9.28 to 7.5 after 15 days. The EC values among the treatment were not significant. Among the selected liquid medium poultry wastewater promotes better growth of *S. platensis* than other locally available wastewater tested. This experiment concludes that agro-based industry's wastewater can be bio-remediated by growing Spirulina, and nutrient-enriched wastewater can be used for mass culturing of Spirulina without nutrient supplements.

Keywords: Agricultural waste; Costless media; *Spirulina platensis*; Optical density

Citation: Vijayarasa, J.; Pakeerathan, K.; Thiruchchelvan, N.; Mikunthan, G. Bio-remediation of agro-based industry's wastewater and mass production of Spirulina (*Spirulina platensis* (Gomont) Geitler 1925). **2021**, *4*, x. https://doi.org/10.3390/xxxxx

Published: date

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1. Introduction

Spirulina (*Spirulina platensis*) is an important photo-autotrophic blue green microalgae [1]. It can be used for effective wastewater treatment [4]. Main sources of nutrients required for its growth are nitrate, urea, and ammonium salts. Treating the wastewater will provide effective waste recycling produced from fish farming. Waste materials which

are rich in a variety of concentrated nutrition, especially the waste generated from "*Pangasius*" fish farming produce more ammonia which causes major water body pollutant [3].

Spirulina can be grown well in swine wastewater that was a possible solution for treating the waste produced in swine farming [2]. Growing microalgae in wastewater creates more economic feasibility as a wastewater treatment [12]. Waste includes cheese whey, cow urine, and rain water has extensive potential for growing Spirulina [11]. Treating wastewater biologically will provide better benefits by reducing the environmental pollution by wastewater discharge and provide low cost media for the growth of microalgae.

Spirulina microalgae promote better recycling by the process of bioremediation which involves with the utilization of living organisms. Effluent collected from dairy discharge point from the dairy farming provide better yield of Spirulina growth than the control (NCIM growth medium) and also it effectively removes COD/Phosphate/EC from that effluents [9]

2. Experiments

2.1. Collection of Mother Culture

Spirulina platensis mother culture was collected from OFFER Ceylon NGO, Jaffna, Sri Lanka.

2.2. Media preparation and inoculation

Zarrouk's medium was prepared and 250ml of purified mother culture was added into the freshly prepared Zarrouk's medium for growth [8]. Optical density (OD) value was measured at 560nm wavelength by using UH-5300 spectrophotometer at alternative day's intervals for 15days [11].

2.3. Sample Preparation

Various substrates of fish pond wastewater, poultry waste water, grain soaked water and parboiled rice liquid waste were collected and autoclaved at 121℃ for 15 minutes. Treatment ratios were arranged with various concentrations with water as T1 – T4; to the total volume of 1000ml (Table 1).

2.4. Culturing of Spirulina in different waste water

Pure Spirulina culture of 250ml of was inoculated in twelve treatment flasks containing 1000 ml culture media. Temperature of the medium was maintained at 28-32℃. All liquid wastes were aerated at the rate of seven liters/min using an air pump Lp-60 (Resun) for 24h continuously. The setup was placed in well indirect sunlight receiving point at 30±2℃. pH was adjusted daily to the range between 8.5-10 pH. Culture was agitated by manual shaking [14].

2.5. Statistical Analysis

Data collected in the whole study was analyzed by Microsoft Excel 2013 and SAS software (9.4 version). Duncan's Multiple Ranges Test (DMRT) was used to determine the least significant differences among the treatments at $P < 0.05$.

Table 1. Different sets of treatments.

Type of waste	Treatment	Volume of waste	Volume of water
Fish pond wastewater	T1	250ml	750ml
	T2	500ml	500ml
	T3	750ml	250ml
	T4	1000ml	-
Parboiled rice water	T1	250ml	750ml
	T2	500ml	500ml
	T3	750ml	250ml
	T4	1000ml	-
Poultry waste water	T1	250ml	750ml
	T2	500ml	500ml
	T3	750ml	250ml
	T4	1000ml	-
Grain soaked water	T1	250ml	750ml
	T2	500ml	500ml
	T3	750ml	250ml
	T4	1000ml	-

3. Results and Discussion

3.1. Chemical parameters of various selected liquid waste

The parameters like pH and EC were measured at the beginning and the end of the culturing period (Table 2). The pH of the parboiled rice waste water reduced from day one to the final day from the value of 5.21 to 6.01, and pH increase was non-significant at P <0.05. But in poultry, fish pond grain soaked wastewater, pH value was lowered significantly, and highly significant in poultry wastewater. Similarly, EC value was not significantly declined in parboiled rice wastewater, where as in other all wastes EC values were increased significantly at P <0.05.

Table 2. Chemical parameter of various selected liquid waste.

Substrate	Chemical characters at the beginning of culturing		Chemical characters at the end of culturing	
	pH	EC(mS/cm)	pH	EC(mS/cm)
Fish pond wastewater	7.89[a]	1.87[b]	6.9[b]	9.98[a]
Parboiled rice liquid	5.21[a]	1.79[a]	6.01[a]	1.01[a]
Poultry unit	9.28[a]	1.501[a]	7.5[b]	9.11[a]
Grain soaked water	8.21[a]	1.99[a]	7.1[b]	8.34[a]

Values with the same alphabets are not significantly different according to the DMRT at 95% confidence interval.

3.2. *Determination of Spirulina platensis growth in different wastewater*

Growth of *Spirulina* in Zarrouk's medium was steady and growth indicator OD value was increased from 0.129 at the day of inoculation to 1.112 after 15days of inoculation (Figure 1).

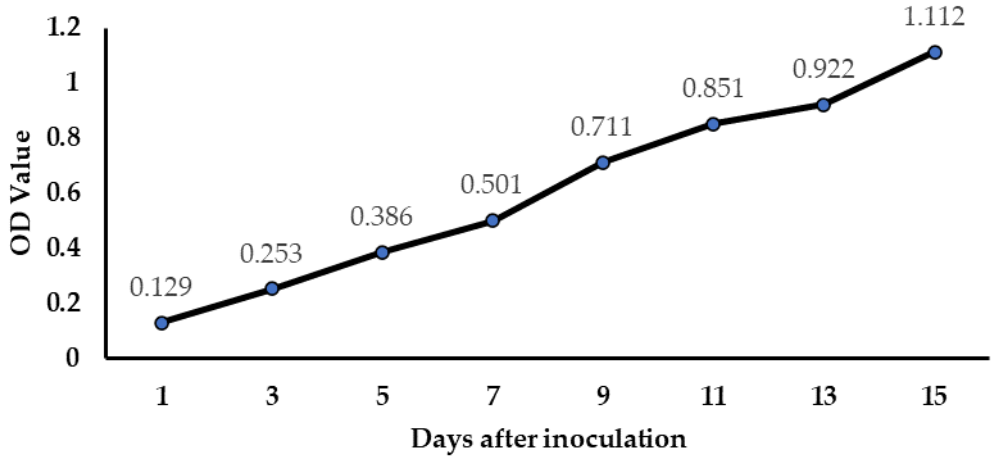

Figure 1. Growth of *Spirulina platensis* in ideal Zarrouk's medium.

Growth of Spirulina in terms of OD in the fish pond wastewater was shown in figure 2(A). In all concentrations, the growth of Spirulina was observed, but the growth in fish pond wastewater increased as the ratio of 750ml of wastewater to the 250ml of fish tank wastewater (T3) have the high potential for Spirulina growth than other treatment concentrations. This shows increasing OD value from 0.047 to 0.567 in fifteen days of growth. On the day7 to day 9 the OD values decreased in all concentrations, and then a exponential increase was observed from day 9.

In parboiled rice waste water, the growth of Spirulina in terms of optical density (OD), was decreasing trend of OD values was observed (Figure 2(B)). When considering the treatment T1 (wastewater to distilled water volume is 250ml: 750ml) has a steady and slow increasing trends of OD values of 0.091 to 0.470 with days. The best performance was observed at lower concentration of parboiled rice effluent. Overall the growth of Spirulina in parboiled rice water was very lower than the fish pond wastewater.

In poultry wastewater, all treatments had an effect on the growth of Spirulina over the period (Figure 2(C)). Among the four concentrations, ratio of waste to the water 250ml: 750ml (T1) shows a better environment for the growth of Spirulina, and in T1 the growth increased with the OD range of 0.269 to 1.452 than all other treatments.

The overall performance of growth of Spirulina in grain soaked water was not satisfied with its OD values. The maximum OD value obtained was 0.298 with the minimum of 0.25 in all concentration. In comparison to standard Zarrouk's medium, growth of Spirulina was better in poultry wastewater at low concentration of 1:3. Fish pond wastewater and the poultry waste water are rich in nitrogen, carbonate and bicarbonate which are essential nutrient for Spirulina growth [15]. This grain soaked water and parboiled rice wastewater do not contain the source of nitrogen and bicarbonate [6, 13].

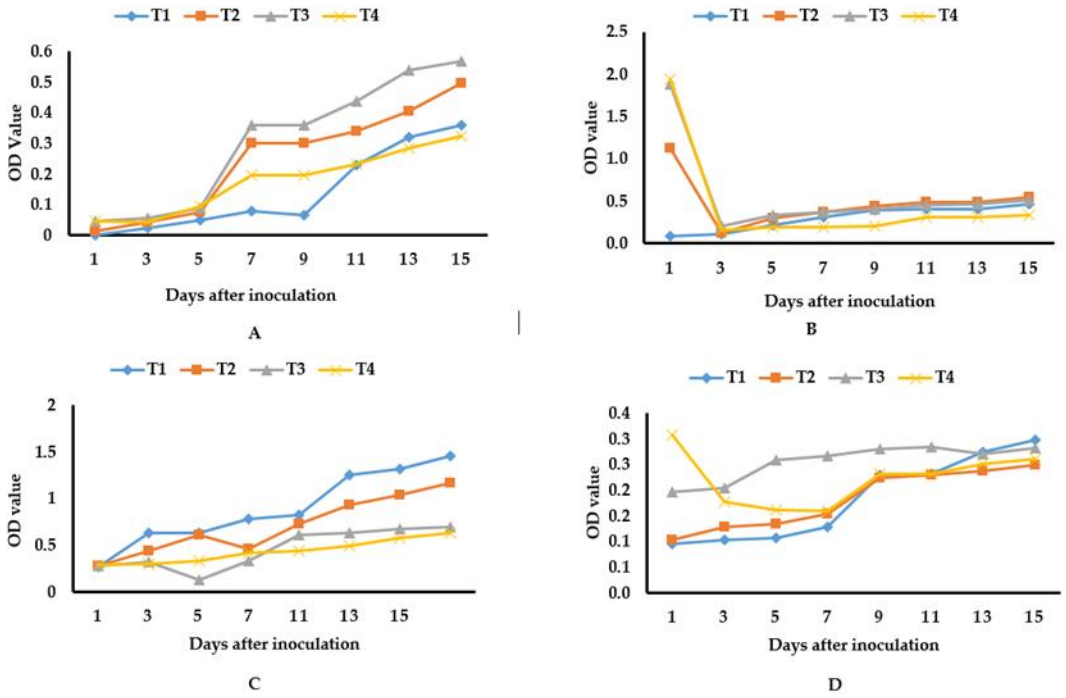

Figure 2. Growth of *Spirulina platensis* in (A); fish pond wastewater (B); parboiled rice water (C) poultry industry wastewater and (D) grain soaked water.

Initially after the inoculation OD value was increased due to the cultural adaptation period for the new environment where the growth is referred to as in the lag phase. At day 7- day 9 culture showing the start of exponential growth rate compared to other days which is due to the well adaptation to the environment [10, 16]. Soni et. al (2019) reported that the maximum OD value at 560nm was of 0.5 in open pond system. But in the current experiment, the maximum value of 1.112 was obtained in poultry wastewater at laboratory conditions, and the growth observed in this concentration shows a tremendous and well response with time. These variations were mainly due to the continuous agitation and O_2 supply to the cells in laboratory conditions and proper care of cultures [7-8,17-18]. Ability of *S. platensis* to absorb ammonia present in fish pond and poultry wastewater promotes desirable factor for microalgae growth [3]. Madkour et al. (2012) reported that *S. platensis* could utilize ammonium nitrate most efficiently and that growth was enhanced with increasing the concentrations of ammonium nitrate giving maximum biomass at 0.353 g/L. Further increasing the concentration limited growth. The growth parameters in urea showed a significant decrease associated with increasing urea concentrations [10,5]. These findings tally with the current investigation.

5. Conclusion

Among the selected liquid medium poultry wastewater promotes better growth of *S. platensis* than other locally available wastewater tested. This experiment concludes that agro-based industry's wastewater can be bio-remediated by growing Spirulina, and nutrient-enriched wastewater can be used for mass culturing of Spirulina without nutrient supplements.

Acknowledgments: This research was carried out in JICA granted Laboratory at the Department of Agricultural Biology, Faculty of Agriculture, University of Jaffna. The authors thank to Japanese government to donate research and laboratory complex through JICA grant. The authors wish to thank Mr. K. Uthayakumar, and Mr. S. Arunsan Department of Agricultural biology, Faculty of Agriculture, University of Jaffna, of their technical assistance throughout this research.

Author Contributions: All authors contributed equally. GM, NT and KP conceived the research idea; JV conducted experiments; JV and KP wrote the manuscript; and GM and NT edited the manuscript.

Conflicts of Interest: Declare conflicts of interest or state "The authors declare no conflict of interest

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