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Effect of different recycled fisheries and domestic waste compost on the production of *Spirulina platensis*

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Solid wastes of finfish and shellfish could be converted into bio-manure through composting process and it can be used for protein (*Spirulina*) production through aquaculture and bioremediation. The study is aimed for *Spirulina* production from different recycled fisheries and domestic wastes under homestead condition. The different wastes were collected from different sources and were converted into manure by composting process. The *Spirulina* is produced in five culture treatments fertilized with composts *viz*., trash fish compost (T-1), cephalopod compost (T-2), crustacean compost (T-3), mangrove compost (T-4) and kitchen waste compost (T-5) and the control without fertilizer compost (C) for sixty days. The population of *Spirulina platensis* was higher in cephalopod composted tanks (T-2) when compared to the other treatments. The compost made by cephalopod waste, and trash fish waste, may profitably be recycled for the production of protein-rich *Spirulina* under homestead condition.

[**Keywords**: Bio-manure, Bioremediation, Homestead, Population, Protein]

Introduction

Algae are used to treat wastewater and improve the water quality. These algae use solar energy, absorbs nutrients and other chemicals including heavy metals from the waste water and fix carbon substances producing plant biomass. Utilizing the available nutrients from sewage environment, algae oxidize the organic matter through photosynthetic activity[1]. Photosynthetic microorganisms are able to metabolize organic carbon, nitrogen and phosphorous, thereby treating waste water and simultaneously produce useful biomass[2]. With this concept, *Spirulina* is widely used to treat wastewater and for algal production.

Spirulina is a blue-green, multicellular, unbranched, trichomatous and helicoidal cyanobacterium. *Spirulina* belongs to the division Cyanophyta and family Oscillatoriaceae. The genus *Spirulina* consists of 15 species and the most commonly available species is *S. platensis* and *S. maxima*[3]. The protein content in *Spirulina* ranges from 55 to 70 % by dry weight[4]. It contains various vitamins including the B-complex range, minerals and growth factors like gamma-linolenic acid. It is one of the good protein sources with other essential nutrients which are highly nutritious and are produced in tons every day to meet the demands globally. *Spirulina*, which is a source of single-cell protein and mainly used in the nutraceuticals products is hailed as the "Food for the Future", and is also considered as an ideal food for astronauts by NASA[5]. The photoautotrophic *Spirulina* species are used to treat the wastes, human faeces[2] and urine[6]. In Nigeria, the effluents from a fertilizer company were used for the cultivation of *Chlorella* and *Spirulina* species in laboratory-scale[7]. The wastewater treatment by microalgae culture has a major advantage that is it does not generate additional pollution when the biomass is harvested and it allows efficient recycling of nutrients[8]. There are about 15 sea food processing plants operating in the Tuticorin district and these processing plants annually process 22,294 tonnes of raw materials into 15,996 tonnes of various products of sea food including frozen fish, cephalopod, shrimp, lobster, crab and fish meal. These sea food processing plants generate solid wastes of 6,298 tonnes per year[9]. The disposal of these wastes poses a major problem leading to environmental contamination. Such trash and unutilized fisheries waste along with the domestic wastes can be bio-converted into composts and recycled in the production process of protein rich microorganisms like *Spirulina* and in other aquaculture activities[9]. Disposing of the organic wastes is one of the severe issues and it leads to an environmental problem in fisheries avocation, especially in the seafood processing sector. Solid wastes can be used for protein production through aquaculture and bioremediation in an eco-friendly manner. Considering the above facts, this study has been aimed in *Spirulina* production from different recycled fisheries and domestic wastes under homestead condition.

Material and Methods

Collection of raw material

The trash fish wastes were collected from the Thoothukudi fishing harbour, mangrove litters from Thoothukudi coastal line near the Thermal Bridge and the kitchen waste from the Fisheries College and Research Institute Students' hostel. These waste materials were used as the principal fertilizer source for growing the microalgae though it contains carbon and nitrogen source. These wastes were converted to composts[9] to make them suitable for manuring the culture medium with the required C:N ratio.

Experimental design

The experiment was conducted for sixty days with three replications in 300 L water holding capacity cement tanks filled with bore well water. Five culture treatments fertilized with composts *viz*., Trash fish compost (T-1), Cephalopod compost (T-2), Crustacean compost (T-3), Mangrove compost (T-4) and Kitchen waste compost (T-5) and the Control without fertilizer compost (C) were tested in the study. *Spirulina platensis* requires sufficient light intensity for the photosynthetic process and relatively high pH to inhibit the growth of other algae. All the treatment and control tanks were added with bicarbonate (@ 2 g L^{-1}) to create an alkaline medium and the tanks were exposed to sufficient sunlight. Aeration was provided to all the tanks to avoid the stagnation as it turbulates the water. The respective treatment tanks were applied with 50 % concentration of each compost. The composts were applied fortnightly to ensure continuous nutrient supply, at the rate of the recommended cow dung fertilizer dose of 10,000 kg/ha (100 %).

Collection of *Spirulina platensis* inoculum

The *S. platensis* stock solution was collected from the ornamental fish farm at Madurai. The pure culture was inoculated into the culture tanks at the rate of 300 ml per tank having the capacity of 300 L. The concentrated stock solution was maintained to replenish the tanks with *S. platensis*, periodically, at weekly intervals.

Estimation of hydro-biological parameters

Sampling was done on every day to estimate the water quality parameters such as temperature, pH, dissolved oxygen, water hardness and total alkalinity. The temperature was measured using a standard thermometer with an accuracy of 0.1 °C and pH was analysed by digital pH meter (ELICO). The dissolved oxygen content of the algal culture water and control water was estimated by modified Winkler's titration method[10]. Similarly, water hardness and total alkalinity were determined following the procedure of APHA[10]. Nutrient contents of the water were analysed fortnightly with optical density measured at different wavelength levels. The nutrient values such as ammonia-nitrogen, nitrite-nitrogen, nitrate-nitrogen and phosphate-phosphorous were determined fortnightly following the procedure of APHA[10].

Production of *Spirulina platensis*

Chlorophyll-*a* content of water was analysed following Boyd & Pillai[11]. The production of *Spirulina* was calculated through the algal count, once in three days, in the exponential growth phase, taking into account the lag, growth and decline phases, as described by Santillan[12]. This was correlated with the chlorophyll-*a* content.

Estimation of proximate composition

The estimation of moisture content, protein, fat and ash content in *Spirulina* cultured using different organic composts was done by following the procedures laid down in AOAC[13].

Statistical analysis

All statistical analyses were performed using SPSS 16. Water quality parameters were compared by two-way repeated-measures ANOVA with treatment as the main factor and sampling date as repeated measures factor. Oneway analysis of variance (ANOVA) was performed to examine the difference in the proximate value of *Spirulina* among the treatments and control. The analyses were run at 5 % significance level.

Results

Water quality parameters

The water temperature recorded in the treatment tanks and control are given in Table 1. The treatment T-3 and T-4 recorded significantly higher average water temperature than other treatments and control. Treatment T-2 showed higher pH whereas the treatment T-4 showed a lower pH than the control. The

Table 1 — Water quality parameters of different compost experimental groups

Parameter	Control	Treatment				
	C	T-1	T-2	T-3	T-4	T-5
Temperature (°C)	32.83[a]±0.09	31.71[b]±0.09	32.68[a]±0.04	32.33[ab]±0.41	32.22[ab]±0.28	31.67[b]±0.30
pH (no unit)	7.87[c]±0.12	8.53[b]±0.16	9.23[a]±0.02	8.01[c]±0.12	7.98[c]±0.01	8.41[b]±0.06
DO (mg L^{-1})	4.13[c]±0.09	4.23[bc]±0.05	4.40[ab]±0.06	4.09[c]±0.02	4.59[a]±0.07	3.39[d]±0.10
Alkalinity (mg $CaCO_3$ L^{-1})	52.00[e]±8.72	98.95[c]±3.23	179.72[b]±1.73	67.43[a]±1.02	136.70[b]±3.36	84.52[d]±2.55
Hardness (mg $CaCO_3$ L-1)	277.67[c]±9.33	346.28[b]±7.79	388.09[a]±1.91	369.67[a]±9.12	288.57[c]±2.94	237.57[d]±8.92
Ammonia (µg L^{-1})	80.76[a]±0.93	84.94[a]±7.11	72.69[ab]±1.73	57.39[b]±5.82	76.49[ab]±13.15	67.34[ab]±2.86
Nitrate (µg L^{-1})	8.33[d]±0.88	76.23[b]±3.58	105.56[a]±19.05	24.56[cd]±7.64	39.47[c]±1.29	24.91[cd]±1.09
Phosphate (µg L^{-1})	120.00[b]±15.28	173.03[b]±14.59	504.81[a]±119.87	81.30[b]±5.40	18.08[b]±0.37	23.50[b]±0.24
Chlorophyll (mg L^{-1})	4.54[d]±0.01	36.48[b]±0.20	71.86[a]±0.50	28.69[c]±3.34	27.46[c]±1.27	28.44[c]±0.23

Trash fish compost (T-1), Cephalopod compost (T-2), Crustacean compost (T-3), Mangrove compost (T-4) and Kitchen waste compost (T-5) and the Control without fertilizer compost (C). The values are means (±SE, n = 18) of three replications in six sampling date for the treatment and control. Mean values in the same row with different superscript differ significantly (P < 0.05)

Table 2 — Proximate composition of *Spirulina platensis* (mean ± SE) produced in different experimental compost groups (dry weight basis)

Parameter	Control	T-1	T-2	T-3	T-4	T-5
Protein	63.52[b]±0.02	64.77[c]±0.012	64.38[c]±0.006	63.59[b]±0.005	62.83[a]±0.005	62.63[a]±0.21
Lipid	7.10[c]±.0055	6.83[b]±0.012	6.8[a]±0.006	7.22[d]±0.0057	7.14[c]±0.0057	7.61[e]±0.0057
Ash	8.96[a]±.006	8.95[a]±0.0057	8.88[a]±0.0057	9.35[bc]±0.003	9.02[ab]±0.00	9.53[c]±0.302
Carbohydrate	20.42[c]±.004	19.45[a]±0.01	19.94[b]±0.0057	19.84[b]±0.003	21.01[d]±0.005	20.23[c]±0.078

Trash fish compost (T-1), Cephalopod compost (T-2), Crustacean compost (T-3), Mangrove compost (T-4) and Kitchen waste compost (T-5) and the Control without fertilizer compost (C). The values are means (±SE, n = 18) of three replications in six sampling date for the treatment and control. Mean values in the same row with different superscript differ significantly (P < 0.05)

treatment, T-5 (3.39 mg L^{-1}) had significantly lower DO than that of control and other treatments. Similarly, significant lower alkalinity was recorded in T-3 than that of control and other treatments. Further, higher water hardness is recorded in T-2 (388.09 mg $CaCO_3$ L^{-1}) and T-3 (369.67 mg $CaCO_3$ L^{-1}) than that of control and other treatments. Significant difference was observed in ammonia level among the treatments and control whereas higher NH_3-N is recorded in T-1 (84.94 µg L^{-1}). The treatment T-2 showed significantly higher nitrate (105.56 µg L^{-1}), phosphate (54.81 µg L^{-1}) and chlorophyll (7.86 mg L^{-1}) level than that of control and other treatments.

Enumeration of algae

The population of *Spirulina platensis* was higher in cephalopod composted tanks (T-2) *i.e.* 5023 numbers per ml compared to the other treatment (Fig. 1) followed by control and trash fish waste (T-1).

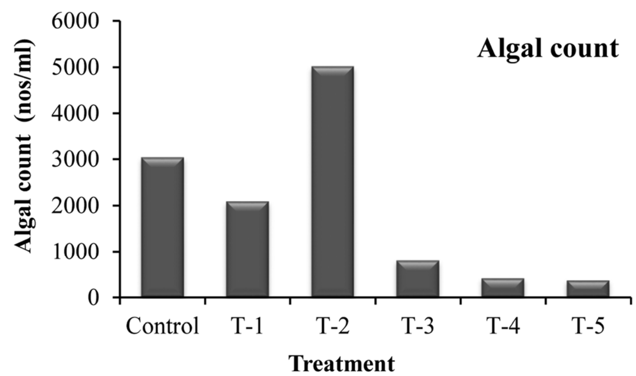

Fig. 1 — Population of *Spirulina platensis* in different treatments

Proximate composition

The proximate composition of *Spirulina* produced in this study is given in Table 2. The protein content was comparatively at higher range from 62.63 to 64.77 %. Whereas, the fat content observed was in low range *i.e.* from 6.8 to 7.61 %. The *Spirulina* contained 8.88 to 9.53 % of ash. The remaining portion in the sample constituted by carbohydrate (19.45 to 21.01 %). On an average, the moisture content in cultured *Spirulina* was found to be around seven per cent.

Statistical analysis

The "*F*" values (ANOVA) derived for drawing the relationship between the quality of the different organic wastes and *S. platensis* production rates are shown in Table 3. A significant difference was observed between the types of organic wastes used for the culture of *S. platensis* at 1 % level. Also, there existed a significant difference between the organic waste concentrations and *S. platensis* production at 1 % level.

Table 3 — ANOVA test for *Spirulina* production with different waste composts

Source of variation	Sum of squares	Degrees of freedom	Mean sum of squares	*F*-ratio	*F*-table	
					5 %	1 %
Between wastes	46351977	4	11587994	79.05916 **	3.837853	7.006077
Between wastes at different doses and *Spirulina* production	121174.9	2	6058747	9.413358 **	4.45897	8.649111
Error	1172590	8	1465737			
Total	47645742	14				

*Significant at 5 % level; **Significant at 1 % level

Discussion

This study was aimed to assess the production potential of different organic wastes. Several physicochemical and biological water quality parameters were monitored continuously throughout the experiment. In the present study, the values of water temperature recorded in the *Spirulina platensis* culture tanks manured with five different composts were found in between 28.5 and 34.3 °C on the culture days from 1st day to 60th day. Vonshak *et al.*[14] indicated that the effect of decrease in population density in a light-limited system is more pronounced at higher temperatures and much less at low temperatures. Confirming to the above, the alga was found to thrive well at an average temperature around 32 °C in this experiment. Danesi *et al.*[15] and Vonshak[16] reported the optimal growth temperature for *S. platensis* is between 30 and 35 °C and it's being deleterious at 40 °C. In terms of biomass increase, the best responses were obtained in this study at around 30 °C, which agrees with the findings of Danesi *et al.*[15] and Richmond *et al.*[17]. Danesi *et al.*[15] and Richmond *et al.*[17] emphasised the importance of pH as a growth limiting parameter in an algal culture that must be strictly monitored. The highest pH values reported in their experiment on *Spirulina* culture were 9.8, 9.9, and 11.2 in the pond, bag, and jar, respectively. Although *S. platensis* prefers alkaline media, values above 10.3 were found to be harmful to its culture. But in the current study, the average pH range was between 7.1 and 9, from the 1st day to 60th day. In this study, the optimum pH level (7.1 – 9.0) was in cephalopod compost trial which was found to be elevated when compared to the other compost trials. Addition of CO_2 would be beneficial in keeping the pH at optimum range particularly in small culture systems where high biomass concentrations are reached[17]. The dissolved oxygen level of the different compost media showed almost a declining trend, indicating high biological oxygen demand. The recorded values of average dissolved oxygen levels were around 4.2 – 5.0 mg L^{-1}, on the experimental days in different composts. Large scale production of the cyanobacterial biomass is essentially a complex process involving a large number of variables, and for their successful growth and to meet many of the essential requirements of the organism, the environmental needs are to be conditioned, particularly the oxygen, as reported[18]. Sudhir *et al.*[19] investigated that the incubation of *S. platensis* cells with 0.8 M NaCl resulted in decreased oxygen evolution. Their mean DO concentration was 8.3 mg L^{-1}, with a stable range of 8.1–8.7 mg L^{-1}. A high DO concentration over 30 mg L^{-1} in the culture water retards the photosynthesis rate, as the excessive O_2 evolved from the culture organism competes with CO_2 for photosynthesis[20]. The saturated O_2 concentration can be maintained in large ponds through small water circulation and turbulence in large ponds[21]. Even in the summer period, a stable DO concentration can be maintained by running the air bubblation[22]. The lipid saturation changes with temperature and at lower temperature more dissolved oxygen will be available in the culture medium since the process of denaturation of an enzyme is oxygen dependant[23]. In the present study, the cephalopod compost treated water showed good growth of *Spirulina* at 5 mg L^{-1} level of dissolved oxygen, which was sufficient enough to support the production of *S. platensis* population.

The water used for the production of *S. platensis* showed an average hardness range between 300 to 315 mg L^{-1} on the 1st day and the values fluctuated between the days of experiment up to the 60th day, from 298 to 398 mg L^{-1}. In this study, the alkalinity values of different compost media recorded during the culture period from 1st day to 60th day showed much fluctuation. In the cephalopod compost treated culture tank, the alkalinity level was found to be increased and stable. But the alkalinity level was low in the range between 52 and 93 mg L^{-1}, on the 60th day, in crustacean compost media of *Spirulina* production. Similarly, it showed low values and high fluctuation in the other compost trial tanks manured with mangrove compost, trash fish and kitchen waste composts. Zarrouk[24] investigated the presence of high amounts of sodium bicarbonate in the culture medium to sustain the alkalinity level and avoid fluctuations. Zarrouk medium which is rich in bicarbonate has used as a common culture medium in *Spirulina* cultures for years. *S. platensis* has a high bicarbonate requirement, which acts not only as a carbon source but also helps to maintain alkaline conditions which are favourable for its growth[16]. These facts are also endorsed by the relevant values recorded in the present study.

Chlorophyll-*a* content was found to be more in cephalopod compost tanks when compared to the other waste media indicating a high production level, and the average observed value was in the range of 68 to 79 mg m^{-3}. The other compost media tanks like trash fish compost, crustacean compost, mangrove compost and kitchen waste compost produced fewer concentrations of chlorophyll with the values ranging from 21 – 32 mg m^{-3} from 10^{th} day to 60^{th} day. Cornet *et al.*[25] reported that the large-scale production of *Spirulina* biomass depends on many factors, like nutrient availability, alkaline condition and light. These factors influence the changes in metabolism which leads to the growth of *S. platensis* and the composition of the biomass.

The nitrogen sources like nitrate, ammonia and urea are relatively good for algal growth. Richmond[26] reported the utilization of these nitrogen compounds and other organic sources like amides and amino acids, nucleic acids and peptones by *S. platensis.* According to him, the growth of the alga was not inhibited up to 5 mg N L^{-1} of ammonia. In the present study the nitrite level was found to decrease in the tank manured with cephalopod compost when compared to other composts like trash fish, crustacean, mangrove plant and kitchen waste composts. The values of nitrite level recorded during the 1^{st} day to 60^{th} day, ranged between 31.76 and 24 µg NO_2-N L^{-1}. When compared to the 1^{st} day, the nitrite level was more or less decreased during the 60^{th} day. But, the nitrate level, in the cephalopod compost tank, was higher than the other compost tanks, which did not show any marked difference comparatively. Similarly, the utilisation of phosphate derived from waste media was high in cephalopod compost media. These high phosphate levels indicate that *S. platensis* is being produced in large quantities. Phosphate levels were found to be very low in other media manured with compost, such as crustacean waste. In the trial tanks, the other waste compost media did not support the growth of this species. Phosphorous is a major nutrient required for the growth of algae and determines the primary productivity as reported by Mostert & Grobbela[27], who indicated the essential role of phosphorous in maintaining high production rates of *S. platensis* mass cultures. According to Goldman *et al.*[28], the sewage is deficient in available carbon for algal growth. But in the present study, different types of compost media were used as nutrients for *Spirulina* culture. The cephalopod waste has a high source of carbon and nitrogen, and these sources induced more *Spirulina* production.

The *S. platensis* production was more in the tanks manured with the cephalopod waste compost, followed by other culture media like trash fish waste compost, crustacean waste compost, mangrove plant litter compost and kitchen waste compost treated tanks. Manimaran & Srinivasan[9] reported the same opinion and according to them, the molluscan waste contains more nitrogen source but the chicken manure is superior to the seafood waste compost in terms of biomass production. The analysis of proximate composition indicated that the *Spirulina* contains a high amount of protein to the tune of about 60 %. It contains a sufficient amount of other nutrients as well. Braun[29] analysed and reported the nutritive values of *S. platensis*. Owing to its high content of quality protein ranging from 55 to 70 %, it's been regarded as the food for the future and as a dependable source to alleviate human malnutrition. The current study also confirms the high protein content in this blue-green alga. Besides, *Spirulina* was also found as an important, adequate and nutritious food source that increases the growth of the fish species *C. carpio* and also serves as one of the valuable feed supplements for Indian major carps[30]. The protein efficiency ratio was more favourable in *Spirulina* based diets than in the control diet in sturgeon fish[31]. As a conclusion, the compost made by cephalopod waste, and trash fish waste has more nitrogen and phosphate content which clearly indicated superiority among the waste compost in terms of biomass production and profitably be recycled for the production of protein-rich *Spirulina* under homestead condition.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical Statement

The use of *S. platensis* for this experiment was carried out according to the guidelines of animal ethical procedures of Tamil Nadu Veterinary and Animal Sciences University, Chennai.

Author Contributions

PG designed and performed the experiments; MR took the lead in writing the manuscript and contributed to the final version of the manuscript. MV & AS supervised the project.

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