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Arthospira platensis: Growth and Biochemical Composition on Various Combinations of Fish Waste Silage and Seaweed Hydrolysate

C. Rajeswari[1]*, D. Manikandavelu[1], B. Ahilan[2], S. Aruna[1], N. Muralidharan[3] and P. Ruby[4]

[1]*Department of Aquatic Environmet Management, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Dr.M.G.R Fisheries College and Research Institute, Ponneri – 601 204, Tamil Nadu, India.*
[2]*Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Fisheries College andResearch Institute, Thoothukudi – 628 008, Tamil Nadu, India.*
[3]*Department of Fish Processing Technology, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Dr.M.G.R Fisheries College and Research Institute, Ponneri – 601 204, Tamil Nadu, India.*
[4]*Department of Aquatic Animal Health Management, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Dr. M.G.R Fisheries College and Research Institute, Ponneri – 601204, Tamil Nadu, India.*

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CR: Conducted the trial and analysis, analyzed the data and drafted the manuscript. DM, BA, SR, NM: Conceptualized and designed the study and corrected the manuscript. PR: carried out the statistical analysis part.

Key words
***Arthrospira platensis*, Fish waste manure, Seaweed manure, Cell density, Carbon sequestration**

ABSTRACT

Spirulina is a single-cell protein rich in all essential nutrients and vitamins and can be used to produce functional food. This study was conducted to evolve suitable organic media for algal culture by evaluating various combinations of fish waste and seaweed manure in supporting the bioperformance and carbon sequestration potential of a filamentous, Gram negative cyanobacterium, *Arthrospira platensis*. In the culture of *A. platensis* the peak value of cell density ($47.62 \pm 0.18 \times 10^3$ cells/ml) was found in 3:9 combination of 3% fish silage of mineral acid and 9% *Sargassum* sp. acid hydrolysate. Correspondingly biomass, 4.84±0.23 g/L; CO_2 sequestration, 8.71±0.14 g/l/d; protein,64.01±1.59%; carbohydrate,10.09±0.14%; lipid, 8.11±0.15%; and ash content 7.2±0.21% could be attained in 3% fish acid hydrolysate and 9% *Sargassum* sp. acid hydrolysate (S3). The combination of 3% fish silage of mineral acid and 9% *Sargassum* sp. acid hydrolysate was found to be better combo for the culture of *A. platensis*.

INTRODUCTION

The problem of climate change always has been concern of environmentalists and was addressed by terrestrial plants. Terrestrial plants able to reduce nine gigatons of carbon while algae in aquatic systems have been deemed to capable of consuming nearly 34 gigatons of carbon at a cost of displacing a sizable land meant for farming. The growth of microalgae continuously requires organic nutrients, which is usually expensive and hence the use of inexpensive nutrients need to be explore to reduce the overall cost of production at the same time growing these algae organically.

As the global fish production has witnessed a remarkable growth in recent past reaching 178.5 million tonnes in 2018, with 96.4 million tonnes from capture and 82.1 million tonnes from aquaculture and global production of seaweeds has been estimated at 32.4 million tons in 2018 with 97.1% of the total produced from culture and the remaining 2.9% from natural beds (SOFIA, FAO, 2020).

Global fish waste generation is estimated to be in excess of 100 mMT, and in the Indian scenario it is >4 mMT. During the post-harvest technology of finfish and shellfish the waste such as viscera, offal, skin, scales, shells and body parts are rich in variety of plant nutritive elements and devoid of hazardous contaminants and pathogens (Mathur, 1993). These wastes are a potential source of pollution and contamination of the environment, as they degrade rapidly in warm temperatures. On the other hand, they contain high amount of nutrients such as protein,

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* Corresponding author: rajeswarisekar1@gmail.com
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fat and minerals (Djazuli and Budiyanto, 1999) which is available in low cost So that, there is need for developing new methods for biotransformation of this fishery waste. The best alternative solution is to utilize the wastes for the production of by-products (Ramasubburayan *et al.*, 2013). One of the ways of utilizing fish waste and seaweed material is the production of manure. The product has a good nutritive quality and can be sufficient for animal feeding. This procedure is safe, cost effective, eco-friendly and has a good nutritive quality which can be adequate for animal feeding (Hanafy and Ibrahim, 2004). The seaweed liquid manure is mostly used in the field of agriculture plant growth. As one of the methods for utilizing the fish waste and seaweed is to get prepare manure for the development of microalgae.

Spirulina consists of high protein (70-55%), carbohydrates (25-25%), essential fatty acids (18%), vitamins, minerals, and pigments such as carotene, chlorophyll A and phycocyanin (Anvar and Nowruzi, 2021). Apart from the high protein content, it also contains vitamins, especially B_{12} and β-carotenes, and minerals, like iron and phenolic acids, tocopherols and γ-linolenic acid (Dillon *et al*., 1995). Spirulina has been used as staple food as it can be easily digestible due to the lack of cellulose in their cell walls and it is a single-cell protein rich in all essential nutrients and vitamins and can be used to produce functional food. The goal of this study has been designed to create a low cost nutrient medium using fish waste and seaweed for the growth medium in order to ensure fairly high growth of Spirulina in an organic media.

MATERIALS AND METHODS

Fish market wastes were procured from Pazhaverkadu fish landing center. The collected fish wastes were washed with tap water, minced and stored at -4 °C until further used.

Preparation of fish waste silage using mineral acid

Silage production using mineral acid was prepared as of Mousavi *et al*. (2013). Minced fish waste (1 kg) of was added in a plastic container and 98% sulphuric acid at a weight percentage of 3.5% (v/w) and 65 mg of BHT were added to the plastic container. The mixture was stirred with sterile glass rod and kept in room temperature (28°C to 32°C) for 60 days and stirred 2 times per day. After 60 days the silage was filtered and stored.

Preparation of seaweed acid hydrolysate

The sargassum seaweed was collected, washed with freshwater, dried in hot air oven at 55°C for 72 h and ground into fine powder. Sea weed acid hydrolysate was prepared as described by Sarkar *et al*. (2018). 100 g of both the powdered sea weeds was mixed with 1 L of 1% HCl and sterilized for 10 min. The mixture was cooled and filtered using cheese cloth.

Proximate compositions evaluation

Proximate composition, i.e., moisture, crude protein, crude fat, ash, potassium and phosphorous of prepared fish waste manure and seaweed manure were determined as per standard methods of AOAC (2005).

Culture of microalgae

The prepared fish waste and seaweed manure were sterilized in an autoclave at 121°C for 20 min and cooled to room temperature prior to use. The fish waste and seaweed manure stock solution was diluted with sterilized fresh water to derive 3%, 6%, 9%, media. Zarrouk medium (Zarrouk, 1966) was kept as the control medium for *A. platensis,* to compare the growth and biochemical characteristics. The cultivation of algae was carried out in 3 L transparent plastic containers at room temperature and placed under white fluorescent light (light intensity of 2500 lux) with a light: dark cycle of 12:12 h. An amount of 10% inoculum containing 2.37×10^3 cells/ml *A. platensis* was added. Triplicate cultures were prepared for all the treatments. The fish manure was fish waste silage prepared by using mineral acid (F1) and seaweed manure was *Sargassum* acid hydrolysate (S1). The different combinations prepared were S1 (F1 3% + S1 3%), S2 (F1 3% + S1 6%), S3 (F1 3% + S1 9%), S4 (F1 6% + S1 3%), S5 (F1 6% + S1 6%), S6 (F1 6% + S1 9%), S7 (F1 9% + S1 3%), S8 (F1 9% + S1 6%) and S9 (F1 9% + S1 9%).

The cell concentration of each sample was measured by counting the cell number every third day. The cell density was determined by Sedgwick. Rafter chamber for *A. platensis* for 21 days of culture. The biomass was estimated at the end of culture period (21 day). The total protein of the sample was analyzed using Lowry's method (Lowry *et al*., 1951). Total carbohydrate was estimated using the phenol sulfuric acid method (Dubois *et al*., 1956). Total lipid was estimated using the modified Barnes and Blackstock (1973) method. Ash content was analyzed using the procedure of AOAC (2005).

Estimation of carbon dioxide assimilation

CO_2 fixation rate =1.88 × P

Where P is the overall biomass productivity in ($g\ L^{-1}d^{-1}$) (Barahoei *et al*., 2020). The all above parameters were estimated at three days intervals.

Statistical analysis

The data have been subjected to one-way ANOVA analysis followed by Duncan tests using statistical software SPSS version 22 (SPSS Inc., Chicago, USA). The values with the probability less than $P < 0.05$ were considered significant.

RESULT AND DISCUSSION

Proximate manure value of fish waste and seaweed liquid manure

Fish silage would be a good potential source for animals in arid regions as it contained high protein (Al-Abri *et al*., 2014; Pagarkar *et al*., 2006), reflecting the nitrogenous ingredients. Table I shows the composition of fish wate liquid manure and seaweed liquid manure. The nitrogen in fish waste silage is 2.84%, in accordance with the values (fish mineral silage 2.1%) of Palkar *et al*. (2017). Reduction of protein content in the ensilage may be due to break down of protein (FAO, 2007). The phosphorus content in fish waste mineral silage is 0.22%. The availability of phosphorus in silage preparations may be because of enzymatic action (Muck *et al*., 2018). The potassium in silage of fish waste was 0.24%. Close by values are reported in other fish silage 0.11% by De *et al*. (2020), and 1.51% by Sahu *et al*. (2016). The minerals as ash content in fish waste mineral silage was 2.29%. Palkar *et al*. (2017) reported the ash content values of 5.12%, in mineral silage silage.

Table I. Composition of fish waste liquid manure and seaweed liquid manure.

Components (%)	FWS (MA)	S (Ac.H)
Nitrogen	2.84 ± 0.39^b	1.93 ± 0.02^b
Phosphorous	0.22 ± 0.01^a	0.03 ± 0.00^a
Potassium	0.24 ± 0.01^a	1.94 ± 0.03^b
Ash	2.29 ± 0.05^b	5.02 ± 0.24^c
Moisture	88.73 ± 0.42^c	85 ± 2.00^d

Values were mean±stdev. Superscript a, b, c, d each column showed the significance difference, ($p<0.05$). FWS(MA), Fish waste silage production using Mineral acid, S(Ac.H), *Sargassum* sp., acid hydrolysate.

The nitrogen (1.93 %), phosphorous (0.03%), potassium (1.94 %) and ash content (5.02 %) of acid hydrolysates of *Sargassum* sp., was found to be congruent with earlier investigations of Alvarado *et al*. (2008), Ramya *et al*. (2010), Sutharsan *et al*. (2014), Uthirapandi *et al*. (2018), Malik *et al*. (2018). Probably the filtration mechanisms and differential moisture also could have contributed for the slight variations of percentages in various components. Nevertheless, with this nutrient status, in combination they can prove to cater to the needs of algal growth as in terrestrial higher plants.

Effect on cell density of A. platensis

In 21 days of spirulina culture A fairly better cell density of $47.6\pm0.18\times10^3$ cells/ml (S3) could be seen in 3:9 shown in Figure 1, fish waste silage using mineral acid and *Sargassum* sp., acid hydrolysate, closely followed by S2-3:6 ($43.8\pm0.23\times10^3$ cells/ml) and S4-6:3 ($34.45\pm0.26\times10^3$ cells/ml). The control and other combinations had less than $20.24\pm0.56\times10^3$ cells/ml. The lower value at higher fish concentrations implies inhibitory mechanisms. In *A. platensis* cell density evaluation, over a period of 21 days culture, fish and seaweed at a ratio of 3:9 provided optimal nutrients for sustaining the climax population, indicative of higher requirement of minor elements by *Spirulina*. This also, confirms higher osmolar demands of *Spirulina*. Strikingly, all these organic combinations unanimously evinced far higher pay off than control. Here also turbidity impact hampering growth at higher concentrations could be discerned. Nonetheless, this also merits further study as a growth around 272,505 cells/ ml at 30 ppt salinity and 36 watts light intensity could be achieved (Piu *et al*., 2022).

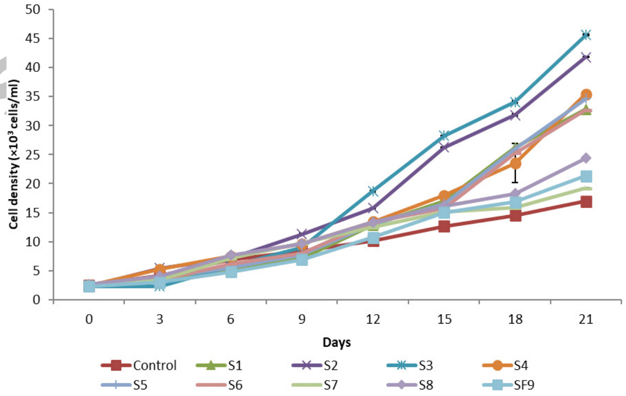

Fig. 1. Effect of fish waste silage combinations using mineral acid and *Sargassum* sp., acid hydrolysate on cell density of *A. platensis*.

S1= F 13% + 513%; S2 = F 13% + 516%; S3= F 13% +519%; S4 = F 16% + 513%; S5= F 16 % + SI 6 %; S6 = F1 6%+519%; S7= F 19%+ 513%; S8 = F 19% + 516%; S9= F 19% + S19%.

Effect on biomass in A. platensis

In the combination of fish waste silage using mineral acid and *Sargassum* sp., acid hydrolysate the highest biomass shows in Figure 2 at the 21st day were found to be 3.78 ± 0.28 and 4.16 ± 0.26 g/l in S2 and S3, respectively. The final biomass obtained over a period of 21 days is reflective of potential productivity, in these combinations. Following the above trend, fish waste at 3% and sea weed at 9% level rendered a fairly double the production of control. The encompassing levels of this biomass production could be discerned in the investigations of Aouir *et al.* (2015), Lima *et al.* (2018) and Lu *et al.* (2020). The reports of 5.71 g/l of production in spirulina culture using cassava waste as carbon source (Agwa *et al.*, 2014) is of worthy consideration for comparison and evolving out strategies for enhancement.

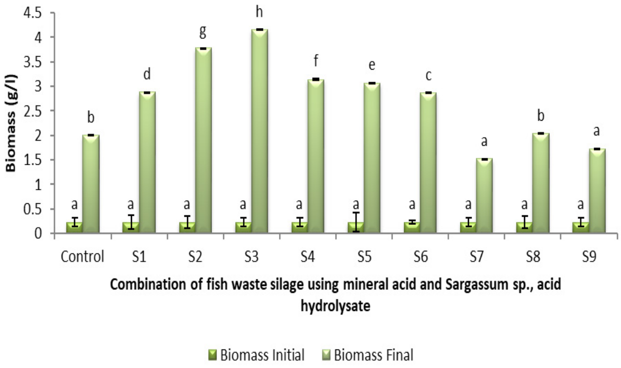

Fig. 2. Effect of different combinations of fish waste silage using mineral acid and *Sargassum* sp., acid hydrolysate on biomass of *A. platensis*. For details of combinations of fish waste silage, see Figure 1.

Effect of CO_2 sequestration of A. platensis

Figure 3 shows the CO_2 sequestration potential of *A. platensis* cultivated in various combinations of fish waste and seaweed fertilizers. In combination of fish waste silage using mineral acid and *Sargassum* sp., acid hydrolysate the CO_2 sequestration at the 21st day were found to be 7.11±0.19 and 7.83±0.6 g/l/din SF2 and SF3, respectively. The carbon dioxide sequestration potential of *spirulina* appears to be quiet high because the biomass generation gives an implied picture of higher potential of this alga around 8 g/l/d in all different combinations of 3% fish and 9% seaweed. Here again the supercilious performance of this organic manure over conventional media is emphatically established. This gives a lead that this algae merits cultivation in all spatial and temporal probabilities, in the context of regional and global climate changes. Badger and Price (1994) reported that the carbon fixation activity supported by enzyme rubisco in microalgal cells. The amount of CO_2 that can be absorbed by 1 kg of dry *spirulina* is 1.83 kg of CO_2 (Zhu, 2020; Prinajati, 2021). A corroborative picture of this theoretical paradigm (4.84 g/l of biomass to– 8.71 g/l/d of co_2) could be seen in this study during climax biomass productivity level of this alga. The current level of observation appears to be far stretched higher values of erstwhile observations of (414.15 mg/l/d) Zhu *et al.* (2021), and (600 mg/l/d) Zhu *et al.* (2021).

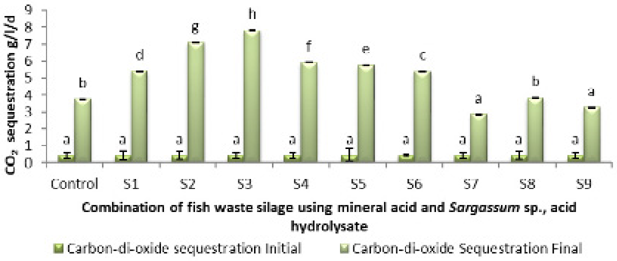

Fig. 3. Effect of different combinations of fish waste silage using mineral acid and *Sargassum* sp., acid hydrolysate on CO_2 sequestration of *A. platensis*. For details of combinations of fish waste silage, see Figure 1.

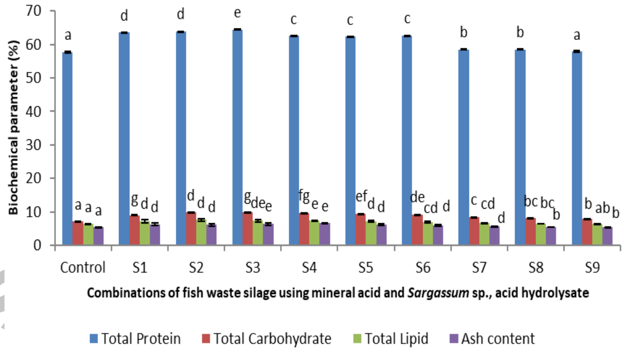

Fig. 4. Effect of different combinations of fish waste silage using mineral acid and *Sargassum* sp., acid hydrolysate on proximate composition in *A. platensis*. For details of combinations of fish waste silage, see Figure 1.

Effect on proximate composition of A. platensis

The proximate composition of this category is shown in Figure 4. The representative peaks of protein, carbohydrate, lipid and ash content could be witnessed in S2 (63.87 %) and S3 (64.37 %), S2 and S3 (~9.8 %), S2 (7.6%) and S3 (7.37 %) and S2 and S3 (6.3 %) correspondingly. The protein in *Spirulina* of this inquest is remarkably high with nearly 65%. The protein content observed here is either a little higher or on par with earlier studies with values of 45.31 – 55.15% (Lu *et al.*, 2020), 55.52%, (Khandual *et al.*, 2021), 64.2% (Pandey and Tiwari, 2010). Carbohydrate content is an index for algal biomass energy potential assessment. The total carbohydrate contents of erstwhile studies in *Spirulina* depicts ranges of 13.58-15.63% (Aouir *et al.*, 2017), 15-25% (Quillet, 1975), 8.53%–12.32% (Lu *et al.*, 2020). On a collation it renders a lesser picture, especially control indicates, a very poor scenario, offers scope for further inquest with improvements in other ecofactors. The lipid content of around 8% observed here falls between the earlier impressions of 7.28% (Bensehaila *et al.*, 2015), 11% (Hudson and Karis, 1974) and 10.22%–11.49% (Lu *et al*.,2020). Culture media as an essential determinant in bioaccumulation of minerals in spirulina, aside temperature, pH and salinity has been bespoken by Habib *et al*. (2008).

CONCLUSION

The present study concluded that the various combination of fish waste and seaweed manure, the combination of 3% fish silage of mineral acid and 9% *Sargassum* sp., acid hydrolysate was found to be better combo for the culture of *A. platensis* in terms of cell density, biomass and biochemical parameters such as protein, carbohydrate and lipids. Organic cultivation of these algae is of great economic value, incorporating carbon trading component as environmental cost of the enterprise in the context of climate change.

DECLARATIONS

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Ethical statement

The study was performed in compliance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The ethical committee of Tamil Nadu Dr. J. Jayalalithaa Fisheries University (TNJFU, 2020), Nagapattinam, Tamil Nadu, India, has also approved the study.

Statement of conflict of interest

The authors have declared no conflict of interest.

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