International Journal of Agriculture Sciences
ISSN: 0975-3710&E-ISSN: 0975-9107, Volume 8, Issue 37, 2016, pp.-1763-1767.
Available online at http://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000217

Research Article

OPTIMIZATION OF GROWTH CONDITIONS AND DEVELOPMENT OF A LABORATORY MODEL RACE WAY POND FOR CULTIVATION OF *Spirulina platensis* IN ANAEROBICALLY DIGESTED CASSAVA SAGO FACTORY EFFLUENT

MANICKAM KARTHIK* AND KUMAR KRISHNAMURTHY

Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, 641003, Tamil Nadu, India
*Corresponding Author: Email-karthikm102@gmail.com

Received: May 02, 2016; Revised: May 25, 2016; Accepted: May 26, 2016; Published: September 18, 2016

Abstract- Sago effluent pose a serious environment pollution, if discharged on both soil and water bodies without proper treatment. In this study, we attempted cultivation of *Spirulina platensis* using anaerobically treated sago effluent diluted with water at different dilution levels *viz*., 80:20, 60:40, 50:50, 40:60, 20:80 and 100% (Undiluted) supplemented with different concentrations of $NaHCO_3$ and $NaNO_3$ sources as carbon and nitrogen respectively, based on Zarrouck's broth composition. Results showed that the most suitable dilution of sago effluent with water for maximum growth of *Spirulina platensis* was 80:20 dilution with addition of $NaHCO_3$ at 0.2 M (16.8 g L^{-1}) and $NaNO_3$ at 0.03 M (2.5g L^{-1}) concentrations. The lab model raceway pond (65 L working volume) was designed to study the feasibility of *Spirulina* cultivation in the anaerobically digested sago effluent, with the optimized dilution and nutrient supplementation levels. It was found that *Spirulina platensis* was able to produce a biomass of 0.4g L^{-1} on dry weight basis with in a period of 18 days. The population of *Spirulina platensis* was 5 ×10^4 cells mL^{-1} with OD value of 0.753 and protein content 57%. The BOD, COD, TSS, TDS and organic carbon content of sago effluent were also significantly reduced indicating that sago effluent is further purified by *Spirulina* cultivation, which also serves as the single cell protein for farm animals.

Keywords- *Spirulina platensis*, Sago effluent, Anaerobically digested sago effluent, Laboratory model raceway pond, Namakkal

Citation: Manickam Karthik and Kumar Krishnamurthy (2016) Optimization of Growth Conditions and Development of A Laboratory Model Race Way Pond for Cultivation of *Spirulina platensis* in Anaerobically Digested Cassava Sago Factory Effluent. International Journal of Agriculture Sciences, ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 8, Issue 37, pp.-1763-1767.

Academic Editor / Reviewer: V.C. Ingle, Dr Amee K Nair, Dr K Muthuselvam

Introduction

Cassava (*Manihot Esculenta*, Crantz) is a root crop largely used in human and animal nutrition, as well as raw material for several industrial products. In cassava cultivation, India ranks 25th in area, 11th in production and 1st in productivity (34.95 tonnes/ha). Sago is a processed edible starch available in granulated form, pearls or flakes and is valued as food for invalids and infants. Sago industries are considered to be one of the largest sources of food processing wastewater, since it includes washing and extraction process [Fig-1]. Sago wastewater from the cassava processing industries contributes significantly to environmental pollution and aesthetic nuisance. In the southern region of India, particularly in Tamil Nadu, nearly 500 units of sago industries discharge about 30,000 to 40,000 L of sago effluent per tonne of sago processed [1,2].

The raw sago effluent generally contains a higher amount of BOD and COD. [3] Showed a depletion of dissolved oxygen, depression in pH values, elevation of BOD, carbon and nitrogen content in the discharged wastes. The problem was that the sago effluent was released directly into the soil and river before proper treatment. The possible alternative is the use of effluent as raw material for anaerobic digestion. Anaerobic digestion of the wastewater not only results in a production of a useful form of energy, but also reduces the problem of environmental pollution caused by the sago effluent. The anaerobic digestion is capable of completely metabolizing the cyanides in the sago effluent [4]. Currently, anaerobic digestion systems such as the covered lagoon and plug flow digester or up-flow anaerobic sludge blanket (UASB) are widely used for the effluent treatment. The amounts of nutrients in the effluent from such bioremediation treatments are sufficient for microalgal growth. In recent years, many microalgae such as *Spirulina, Chlorella, Botyrocoous, Phormidium* and *Scenedesmus* have been cultivated in many kinds of industrial effluent to recycle and improve water quality [5-12].

Microalgae, used as single cell proteins, are projected as living cell factories for the production of various beneficiary bio-chemicals used in food, aquaculture, poultry, energy and pharmaceutical industries [13]. Some algal species allow the use of organically rich sago factory effluents as substrate for production of microbial biomass rich in protein. *Spirulina*, due to its faster growth rate, ease of cultivation, harvesting and processing offers excellent scope for bioremediation of the sago factory effluent and concomitant production of animal feed for possible utilization in the poultry farms, which is a major commercial venture in the sago factory areas. The production of *Spirulina* as dietary supplements for animal feed utilizing the nutrients contained in sago effluent units offers several advantages, including a significant saving in the cost of culture medium. However, inorganic carbon and nitrogen sources are though present in anaerobically digested sago effluent, must often be provided to enhance the growth of *Spirulina*. The present work has been formulated to standardize dilution and nutrient supplementation levels of anaerobically digested sago effluent and design a laboratory model race way pond for *Spirulina* cultivation with the objective of purifying the sago effluent further.

Materials and Methods

Sago factory effluent:

Raw and anaerobically digested sago effluent was collected from Sri Selliamman Sago Industries at Alavaipatty, Rasipuam Taluk, Namakkal District, Tamil Nadu, India. The effluent samples were transported in a cooling box to the Tamil Nadu Agricultural University, Coimbatore, India and were stored in a cold room (+4°C). The raw and anaerobically digested effluent was collected and stored in the cold room until use. The sago effluents were physico-chemically characterised using standard procedures as detailed in [14].

Source of *Spirulina platensis* culture:

Spirulina platensis was locally isolated strain from the effluent storage ponds of sago factory. The isolate was purified by repeated streaking, identified based on their morphological characteristics and maintained in an Algal Growth Chamber at 27±1° C with a light intensity of 3000 lux and 12:12 hrs day/night cycle in the Zarrouk's liquid medium [15].

Experiment I: Standardization of dilution level of anaerobically digested sago effluent for cultivation of *Spirulina platensis*

Anaerobically digested sago effluent was diluted with distilled water at different dilution levels *viz*., undiluted effluent, 90:10, 80:20, 60:40, 40:60, 20:80 and 10:90 in conical flasks. *Spirulina platensis* (OD_{540nm} at 1.0) was inoculated into the flasks at 5% initial inoculum. The experiment was replicated three times in a completely randomized block design. The culture was maintained in Algal Growth Chamber at 27±1° C with a light intensity of 3000 lux and 12:12 hours light and dark periods. The visual observation on growth, changes in pH, OD_{540} nm, population and dry weight was recorded after 30 days of incubation.

Experiment II: Optimization of level of dilution and nutrient supplementation with $NaHCO_3$ and $NaNO_3$ nutrient for cultivation of *Spirulina platensis*

Based on the above experiment, a laboratory experiment was conducted to evaluate three different dilution levels *viz*., undiluted effluent, 4:1 and 1:1 diluted with distilled water and addition of $NaHCO_3$ and $NaNO_3$ at different concentration as carbon and nitrogen source. The flasks were incubated in the algal growth chamber and physico-chemical parameters were analysed. The growth of *Spirulina platensis* in anaerobically digested sago effluent was evaluated with the following treatments replicated thrice.

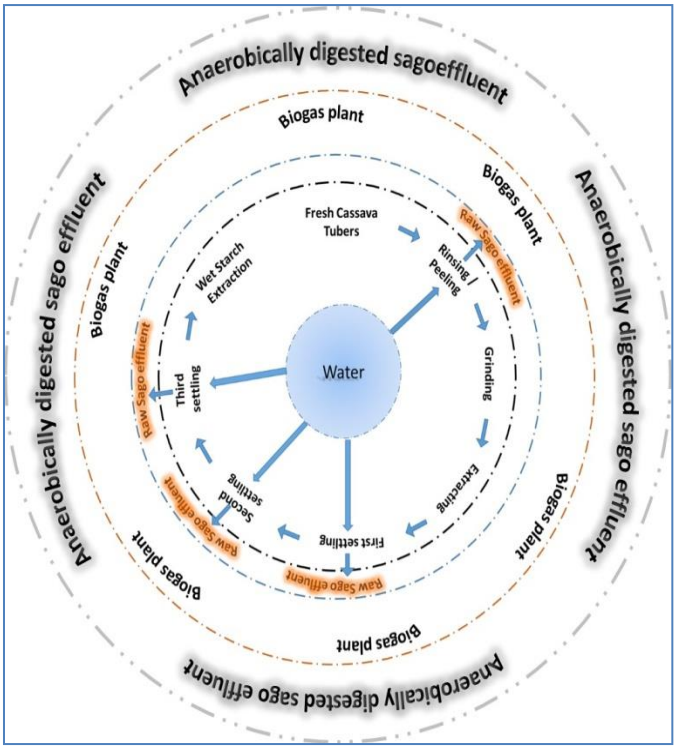


Fig-1 Processing of sago and generation of raw and anaerobically digested effluent obtained from sago processing industry

Nutrient supplementation	Dilution levels			
	Undiluted effluent (No dilution)	Diluted effluent (4:1)	Diluted effluent (1:1)	Zarrouck's broth
	T1 (C)	T2 (C)	T3 (C)	T4 (S)
0.05 M $NaHCO_3$	T5	T14	T23	C – Control, S - Standard
0.1 M $NaHCO_3$	T6	T15	T24	
0.2 M $NaHCO_3$	T7	T16	T25	
0.0075 M $NaNO_3$	T8	T17	T26	
0.015 M $NaNO_3$	T9	T18	T27	
0.03 M $NaNO_3$	T10	T19	T28	
0.05 M $NaHCO_3$+0.0075 M $NaNO_3$	T11	T20	T29	
0.1 M $NaHCO_3$+0.015 M $NaNO_3$	T12	T21	T30	
0.2 M $NaHCO_3$+0.03 M $NaNO_3$	T13	T22	T31	

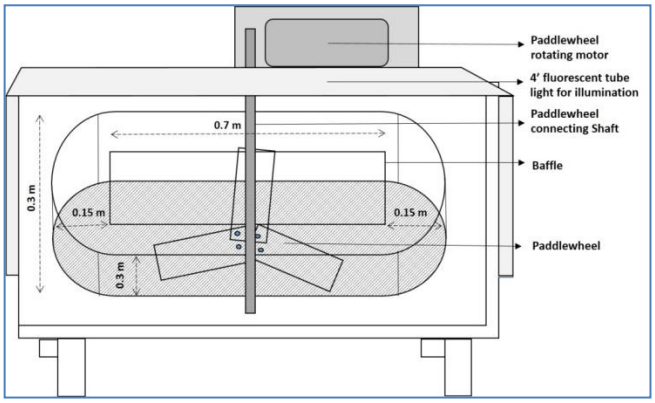


Fig-2 Design of Lab model raceway pond with dimensions for *Spirulina* cultivation in anaerobically digested sago effluent

Experiment III: Designing and evaluation of a Lab model raceway pond (LMRP) for *Spirulina* cultivation in anaerobically digested sago effluent

A oval shape lab model raceway pond with a total volume of 80 litres was constructed with a dimension of 1m x 0.3m x0.3m (length x breath x height) [Fig-2]. The paddle wheel was fixed in raceway pond with a rotating speed of 12 rpm. A 4' fluorescent tube light was fixed above the raceway pond for illumination. A volume of 65 litres of anaerobically digested sago effluent diluted with water at 4: 1 ratio and supplemented with 0.2 M $NaHCO_3$and 0.03 M $NaNO_3$ was added to the raceway pond and the starter culture of *Spirulina platensis* was inoculated at 5% level (3.25 L). Zarrouck's liquid medium was used as Control. Observations on pH, EC, $OD_{540\ nm}$, population of *Spirulina platensis* by direct microscopic count, BOD, COD, Organic carbon and TSS were recorded at every 3 days interval till 18th day after inoculation. The *Spirulina platensis* biomass was harvested on 18th day after inoculation and analysed for the total protein, carbohydrate, chlorophyll-a, reducing and non-reducing sugar contents according to [16]. The percentage reduction in physico-chemical parameters *viz*., TSS, TDS, TS, BOD and COD was calculated using the following formula,

$$\% \text{ reduction} = \frac{\text{value (before treatment)} - \text{value (after treatment)}}{\text{value (before treatment)}} \times 100$$

Statistical analysis: Data were analysed using descriptive statistics. Analysis of variance (ANOVA) was used to test for significant differences at $P \leq 0.05$. The treatment means were separated using Duncan's multiple range test (DMRT)

Results and Discussion

The physico-chemical characteristics of the raw and anaerobically digested sago

effluent are shown in [Table-1]. The raw effluent was acidic in nature and pale white in colour, rich in total suspended solids and with high BOD and COD values. A considerable amount of nitrogen, phosphorus, potassium and sodium were present in the effluent. The results showed that pH of raw sago effluent was highly acidic (4.41) and the pH raised towards a neutral (7.03) in anaerobically digested sago effluent. The EC was 3.69 dSm^{-1} in raw effluent, where as in anaerobically digested sago effluent EC was reduced 2.81 dSm^{-1}. The TS of the raw sago effluent was 3817 mg/L, and the anaerobically digested effluent was 2781 mg/L. The BOD_5 of raw sago effluent was 4973 mg/L. In the anaerobically digested sago effluent, 3592 mg/L of BOD_5 was observed. The COD of raw and anaerobically digested sago effluent were 9925 and 7726 mg/L respectively. In anaerobically digested sago effluent, a maximum COD and BOD_5 removal of 22.15 and 27.76 per cent was observed. The cyanide (CN) content of raw sago effluent was 4.48 mg/L. [Table-1].

Table-1 *Physico-chemical characteristics of raw and anaerobically digested cassava sago effluent*

Parameters	Raw sago effluent	Anaerobically digested sago effluent
pH	4.41(±0.22)	7.03(±0.42)
EC (dSm^{-1})	3.69(±0.19)	2.81(±0.18)
TDS (mg/L)	2275(±90.07)	1713(±47.63)
TSS (mg/L)	1542(±37.76)	1068(±65.82)
TS (mg/L)	3817(±127.68)	2781(±112.79)
OC (%)	1.86(±0.07)	1.39(±0.09)
BOD_5 (mg/L)	4973(±241.33)	3592(±227.19)
COD (mg/L)	9925(±562.05)	7726(±325.63)
Nitrogen (mg/L)	64.25(±3.46)	17.18(±3.41)
Phosphorous (mg/L)	12.58(±3.46)	7.25(±0.80)
Potassium (ppm)	231.58(±82.20)	97.31(±117.51)
Sodium (ppm)	34.28(±10.57)	42.35(±15.65)
CN (mg/L)	4.48(±0.32)	BDL

Values followed by different letters in a column were significantly different ($P<0.05$)

Standardization of dilution level of anaerobically digested sago effluent for cultivation of *Spirulina platensis*:

The growth of *Spirulina platensis* as a measure of pH, OD_{540nm} and dry weight of biomass in the different dilution levels of sago effluent is furnished in [Table-2]. It was found that *Spirulina platensis* was able to grow in low dilution of anaerobically digested sago effluent (80:20), because the *Spirulina platensis* was able to adapt itself to the high amount of organic nutrients present in anaerobically digested sago effluent. The *Spirulina platensis* showed the maximum growth in the dilution level of 80:20 on 30th day after inoculation with OD_{540nm} (1.761) and dry weight of *Spirulina platensis* (3.77 g mL^{-1}) than the other treatments. However, the results clearly indicated that *Spirulina platensis* was able to grow in anaerobically digested sago effluent diluted with water at 80:20 ratio without much inhibition compared to Control (Zarrouck's broth). These results are in confirmity with the findings of [17], who found that *Spirulina platensis* grown in anaerobically digested distillery effluent recorded maximum growth in 50% dilution of anaerobically digested effluent over standard Zarrouck's medium. However, the biomass production by *Spirulina platensis* can be further increased by supplementation of inorganic carbon and nitrogen nutrients in the sago effluent.

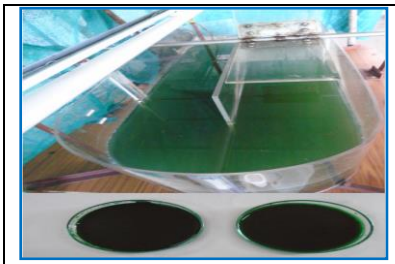
Table-2 *Effect of Spirulina platensis cultivation in different dilutions of anaerobically digested sago effluent on its pH and growth of Spirulina*

	Treatments	pH	OD (540nm)	Dry Weight (gm/l)
T1	Undiluted effluent (100% wastewater)	7.81	0.352^{g}	1.39^{f}
T2	10:90 Water + Wastewater	7.76	0.893^{e}	1.68^{e}
T3	20:80 Water + Wastewater	7.52	1.761^{b}	3.77^{a}
T4	40:60 Water + Wastewater	7.63	1.359^{d}	2.07^{d}
T5	60:40 Water + Wastewater	7.70	1.427^{c}	2.31^{c}
T6	80:20 Water + Wastewater	7.42	0.932^{e}	1.21^{g}
T7	90:10 Water + Wastewater	7.51	0.446^{f}	2.02^{d}
T8	Zarrouck's Broth (control)	9.50	2.012^{a}	3.64^{b}
	SEd	1.623	0.021	0.040
	CD(0.05)	3.442	0.045	0.085

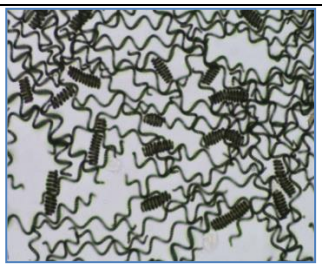
Values in ach column are mean of three replications. Values in each column followed by different letter(s) are statistically different

Optimization of dilution level and nutrient supplementation with $NaHCO_3$ and $NaNO_3$ of anaerobically digested sago effluent for cultivation of *Spirulina platensis*:

The *Spirulina platensis* growth in different dilutions of anaerobically digested sago effluent with the addition of different concentrations of $NaHCO_3$ and $NaNO_3$ as carbon and nitrogen sources was assessed [Table-3]. The dilution of sago effluent with water at 4:1 ratio and addition of 0.2 M concentration of $NaHCO_3$ and 0.03 M concentration of $NaNO_3$ recorded higher growth in terms of OD_{540nm} (2.207) and dry weight (3.82 g L^{-1}) on 30 days after incubation than Control (Zarouck's broth) and other treatments. This might be due to $NaHCO_3$ and $NaNO_3$ serving as good source of carbon and nitrogen, particularly $NaHCO_3$, which is preferred carbon source for *Spirulina* inducing its growth [18, 19]. When *Spirulina* is provided with $NaHCO_3$ as a source of carbon, the released carbon dioxide is used for photosynthesis. The optimum concentration of $NaHCO_3$ for *Spirulina platensis* is 0.2 M (16.8 g L^{-1}) and the resulting NaOH from the process could help in increasing the pH value to 9 enabling *Spirulina platensis* to adapt to higher pH [20-22]. Hence, dilution of anaerobically digested sago effluent with water at 4:1 ratio and nutrient supplementation with 0.2 M conc. $NaHCO_3$ and 0.03 M conc. $NaNO_3$ were standardized as the optimum conditions for achieving maximum growth and biomass production by *Spirulina* in anaerobically digested sago effluent.



a. Lab model Raceway pond for *Spirulina* cultivation in anaerobically digested sago effluent
b. Harvested wet biomass of *Spirulina*



Microphotograph of *Spirulina* grown in anaerobically digested sago effluent (Magnification x 20X)

Fig-3 Lab model raceway pond (LMRP) for *Spirulina* cultivation in anaerobically digested sago effluent

Table-3 *Optimization of dilution level and nutrient supplementation with $NaHCO_3$ and $NaNO_3$ of anaerobically digested sago effluent for cultivation of Spirulina platensis*

	Treatment	Additional Source	Different Concentration	pH	OD (540nm)	Dry Weight (g/l)
			0.2M	9.38	0.816 ^{n}	1.69 ^{k}
		$NaHCO_3$	0.1M	9.14	0.555 ^{q}	1.00 ^{q}
			0.05M	9.06	1.342 ^{e}	1.84 ^{j}
			0.03M	8.81	1.000 ^{k}	1.65 ^{k}
T1	Undiluted effluent	$NaNO_3$	0.015M	8.78	1.101 ^{h}	1.38 ^{n}
	(100% wastewater)		0.0075M	8.73	1.178 ^{f,g}	1.00 ^{q}
			0.2M+0.03M	9.25	1.396 ^{d}	3.28 ^{e}
		$NaHCO_3$ + $NaNO_3$	0.1M+0.015M	9.13	1.574 ^{c}	2.50 ^{f}
			0.05M+0.0075M	9.04	1.170 ^{g}	1.34 ^{n,o}
			0.2M	9.31	0.512 ^{r}	2.41 ^{g}
		$NaHCO_3$	0.1M	9.18	1.359 ^{d}	3.56 ^{c}
			0.05M	9.13	0.257 ^{u}	0.95 ^{q}
	20:80 (1:4 ratio)		0.03M	8.85	0.899 ^{l}	1.50 ^{m}
T2	Water + Wastewater	$NaNO_3$	0.015M	8.82	1.036 ^{i,j}	1.14 ^{p}
			0.0075M	8.86	1.171 ^{g}	1.10 ^{p}
			0.2M+0.03M	9.30	2.207 ^{a}	3.82 ^{a}
		$NaHCO_3$ + $NaNO_3$	0.1M+0.015M	9.25	1.389 ^{d}	2.31 ^{h}
			0.05M+0.0075M	9.15	0.856 ^{m}	1.30 ^{o}
			0.2M	9.34	0.389 ^{t}	2.38 ^{g}
		$NaHCO_3$	0.1M	9.43	0.446 ^{s}	1.38 ^{n}
			0.05M	9.27	0.504 ^{r}	1.50 ^{m}
	50:50 (1:1 ratio)		0.03M	9.05	1.071 ^{h,i}	1.37 ^{n}
T3	Water + Wastewater	$NaNO_3$	0.015M	9.09	1.208 ^{f}	1.57 ^{l}
			0.0075M	9.01	0.605 ^{p}	0.97 ^{q}
			0.2M+0.03M	9.36	1.018 ^{j,k}	3.43 ^{d}
		$NaHCO_3$ + $NaNO_3$	0.1M+0.015M	9.37	1.403 ^{d}	2.14 ^{i}
			0.05M+0.0075M	9.36	0.680 ^{o}	1.49 ^{m}
T4	Zarrouck's Broth (standard)	media composition	-	9.6	2.079 ^{b}	3.63 ^{b}
	SEd			0.149	0.018	0.034
	CD(0.05)			0.299	0.036	0.069

Table -4 *Effect of Spirulina cultivation in the anaerobically digested sago effluent using lab model race way pond on its growth and physico-chemical characteristics of sago effluent*

Different Intervals	pH	EC (dsm^{-1})	OD (540nm)	Population Cells / ml (10^4)	BOD (mg/L)	COD (mg/L)	OC (%)	TSS (mg/L)	Dry weight (gm/L)
Initial (0^{th}day)	8.73	19.74^{a}	0.233^{e}	1.50^{e}	3241^{a}	5128^{a}	1.35^{a}	941 mg/l	
3^{rd} day	8.63	18.82^{b}	0.270^{e}	3.00^{d}	2412^{b}	4317^{b}	0.97^{b}		
6^{th} day	8.58	18.78^{b}	0.300^{d}	3.50^{c}	1612^{c}	3671^{c}	0.95^{c}		
9^{th} day	8.39	18.26^{d}	0.340^{d}	3.50^{c}	1414^{c}	3152^{c}	0.95^{c}	437 mg/l	26 gm / 65 litre
12^{th} day	8.46	18.43^{c}	0.454^{c}	4.00^{c}	872^{d}	2363^{d}	0.92^{d}	(18 DAI)	(0.40gm
15^{th} day	8.53	18.37^{c}	0.574^{c}	5.00^{b}	478^{e}	1828^{e}	0.94^{c}		/litre)
18^{th} day	8.47	18.21^{d}	0.753^{b}	5.00^{b}	245^{f}	1052^{f}	0.88^{e}		
Zarrouck's broth* (18^{th}day)	9.52	19.92^{a}	1.143^{a}	8.50^{a}					0.59 gm / litre
SEd	1.62	0.04	0.021	0.14	0.01	0.13	1.12	-	-
CD(0.05)	3.44	0.09	0.045	0.29	0.03	0.28	2.25	-	-

DAI – Days after incubation, OD – Optical density, BOD – Biological oxygen demand, COD – Chemical organic demand, OC – Organic carbon.
*Grown in flask under *in vitro* conditions in the Algal growth chamber. Values in each column are mean of three replications. Values in each column followed by different letter(s) are statistically different.

Table-5 *Biochemical analyses of Spirulina platensis biomass grown in anaerobically digested sago effluent and Zarrouk's basal medium.*

Parameters	*Spirulina* biomass grown in Zarrouck's broth (Standard)	*Spirulina* biomass grown in anaerobically digested sago effluent (LMRP)
Color and appearance	Dark blue-green fine powder	Dark blue-green fine powder
Total Protein (%)	60.40	57.20
Total carbohydrate (%)	27.90	28.69
Chlorophyll (µg / l)	57.46	51.77
Reducing (%)	0.87	0.75
Non reducing sugar (%)	21.30	25.23

Evaluation of laboratory model raceway pond for *Spirulina* cultivation in anaerobically digested sago effluent:

The laboratory model raceway pond was evaluated for *Spirulina platensis* cultivation in anaerobically digested sago effluent at 4:1 dilution and enriched with 0.2 M $NaHCO_3$ and 0.03 M $NaNO_3$ [Table-4]. The results indicated that there was gradual increase in OD_{540nm} (0.753) and population of $5.0x10^4$ cells mL^{-1} and dry weight of 0.40 g L^{-1} on 18^{th} day after inoculation [Fig-3]. [23] found that the *Spirulina platensis* cultivated in anaerobically treated swine wastewater with added nutrients consisting of 8 g L^{-1} $NaHCO_3$ and 1.5 g L^{-1} $NaNO_3$ with a retention time of 12 days resulted in a *Spirulina* cell count of 17.8×10^4 cells mL^{-1}, OD_{540nm} value of 1.09 with 56% protein content. It was also observed that the BOD and COD levels were reduced from initial 3241 mg l^{-1} to 245 mg L^{-1} and 5128 mg L^{-1} to 1052 mg L^{-1} respectively. The results confirmed that carbon and nitrogen supplementation was necessary to produce good biomass. The bioremediation

efficiency was calculated as percentage reduction in TSS, TDS, TS, BOD_5 and COD from the initial level of the anaerobically digested sago effluent and depicted in [Fig-4]. The treatment of diluted sewage water with alginate beads, resulted in increase of the pH of the sample from 7.6 to 9.3 and 94.6% reduction in BOD_5 [24]. In the present study, organic carbon was also reduced to 0.88 % due to *Spirulina platensis* cultivation on 18th day after inoculation. It has been reported earlier that in spite of photoautotrophic nature of *Spirulina*, it can utilize organic carbon present in effluent under illuminated conditions [25].

[Table-5] gives the biochemical analyses of the *Spirulina platensis* grown in Zarrouck's broth and anaerobically digested sago effluent. In general, there was not much reduction in protein content of *Spirulina platensis* grown in the anaerobically digested sago effluent compared to the *Spirulina platensis* growth in Zarrouck's broth. The colour and appearance of *Spirulina platensis* biomass produced from both Zarrouck's broth and anaerobically digested sago effluent was dark blue-green fine powder. The *Spirulina* cultivated in digested sago effluentrecorded a protein, carbohydrate and lipid content of 63%, 17% and 9%, respectively [9]. With this biochemical composition, the *Spirulina platensis* biomass has the potential to be utilized as a high quality animal feed especially for poultry, aquaculture and also as a source of useful biochemical. This is in support with the studies carried out by [26, 27] that cassava wastes can be used for the production of microalgal biomass, which can be used as single cell protein

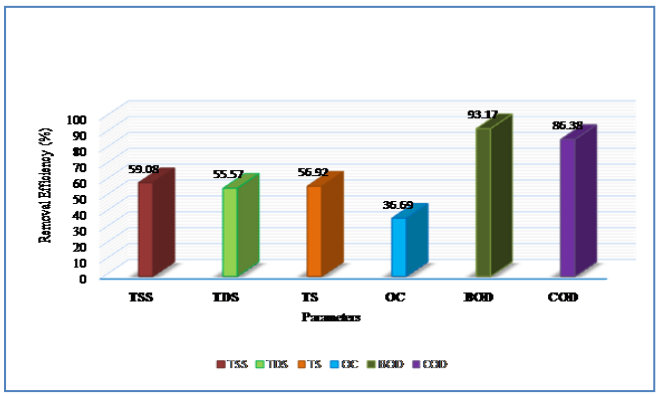


Fig-4 The percentage reduction of physic-chemical parameters due to *Spirulina platensis* cultivation in anaerobically digested sago effluent cultivation of 18 days after

Conclusion

It is evident from this study that anaerobically digested sago effluent serves as very good sources of nutrients required for the cultivation of *Spirulina platensis.* However, the *Spirulina platensis* through their photosynthetic machinery, were able to convert effluent into organic macromolecules (carbohydrate, lipids, and proteins) stored in the cell as biomass. In lab model raceway pond, the most suitable conditions for biomass production of *Spirulina platensis* in anaerobically treated sago effluent was standardized as 4:1 dilution with water and addition of nutrients consisting of 0.2M $NaHCO_3$ and 0.03M $NaNO_3$, with a retention time of 18 days. The *Spirulina platensis* biomass is a protein-rich animal feed material. The demonstration of its successful production at laboratory level is an incentive to develop an effective large-scale method (High Rate Algal Pond) for the treatment of cassava sago factory effluent.

Acknowledgement

The authors would like to gratefully acknowledge the support extended by the authorities of Tamil Nadu Agricultural University, Coimbatore to carry out the research work availing the lab facilities in the Department of Agricultural Microbiology and Department of Bio-energy and also the financial support extended by awarding DBT fellowship.

Conflict of Interest: None declared

References

[1] Saravanane R., Murthy D.V.S. and Krishnaiah K. (2001) *Wat. Air Soil Poll.,* 127,15–30

[2] Savitha S., Sadhasivam S., Swaminathan K. and Feng Huei L. (2009) *J. Cleaner Prod.,* 17,1363– 1372.

[3] Arimoro F.O., Iwegbue C.M. and Enemudo B.O. (2008) *Acta Zool. Lituanica.,* 18, 147-156.

[4] Ranjithkumar R., Soora M., Sujatha K., Balachandar D., Kumar K. and Benckiser G. (2011) *Int. J. Sustain. Eng.,* 1–11.

[5] Gantar M., Obreht Z. and Dalmacija B. (1991) *Bioresour. Technol.,* 36, 167–171

[6] Anaga A. and Abu G.O. (1996) *Bioresour. Technol.,* 58, 93-95.

[7] Blier R., Laliberte G. and DeLa Noue J. (1996) *Process. Biochem.,* 31, 587–593

[8] Olguin E.J., Galicia S., Camacho R., Mercado G. and Perez T.I. (1997) *Appl. Microbiol. Biotechnol.,* 48, 242–247

[9] Phang S.M., Miah M.S., Yeoh B.G. and Hashim M.A. (2000) *J. Appl. Phycol.,* 12, 395-400.

[10] Travieso L., Benítez F., Sánchez E., Borja R., Martín A. and Colmenarejo M.F. (2006) *Ecol. Eng.,* 28, 158–165

[11] Jongkon P., Siripen T. and Richard D.L. (2008) *Int. J. Agric. Biol.,* 10, 437–441

[12] Ungsethaphand T., Peerapornpisal Y. and Whangchai N. (2009) *Int. J. Sci. Technol.,* 3, 379–387

[13] Anand M.N. (2010) *M.Sc. Thesis*, submitted to Graduate school of the Missour University of Science and Technology. pp 3-78.

[14] APHA (1989) 17th edition. American Public Health Association, Washington, DC.

[15] Zarrouk C. (1966) *Ph.D. Thesis*, Université de Paris, Paris.

[16] Chu W.L., Phang S.M. and Goh S.H. (1996) *J. appl. Phycol*., 8, 389–396.

[17] Kaushik R., Prasanna R. and Joshi H.C. (2006) *J. Sci. Ind. Res.,* (65), 521-525.

[18] Huertas E., Montero O. and Lubián L.M. (2000) *Aquac. Eng*., 22, 181–197

[19] Abu G.O., Ogbonda K.H. and Aminigo R.E. (2007) *Afr. J. Biotechnol.,* (6), 2550–2554

[20] Venkataraman L.V. (1983) Department of Science and Technology, India and the Indo-German algal project CFTRI, Mysore, p 100.

[21] Materassi R., Tredici M. and Balloni, W. (1984) *Appl. Microbiol. Biotechnol.,* 19, 384–386.

[22] Binaghi L., Borghi A.D., Lodi, A. Converti A. and Borghi M.D. (2003) *Process. Biochem.,* 38, 1341–1346

[23] Cheunbarn S. and Peerapornpisal Y. (2010) *Int. J. Agric. Biol.,* 12, 586–590

[24] Patnaik S., Sarkar R. and Mitra A. (2001) *Ind. J. exp. Boil.,* 39, 824-826.

[25] Ogawa T. and Terui G. (1972) *In*: Terui, G. (*ed.*) Society of Fermentation Technology, Tokyo, pp 543–549

[26] Ehiagbonare J.E., Adjarhore R.Y. and Enabulele S.A. (2009) *Afr. J. Biotechnol.,* 8(12), 2816-2818.

[27] Budiyoro and Kusworo T.D. (2011) *Int. J. Sci. Eng.,* 2(1), 4-8.