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RESEARCH ARTICLE

OPTIMIZATION OF SAGO EFFLUENT FOR THE PRODUCTION OF *SPIRULINA PLATENSIS*

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ABSTRACT

One of the renewable sources of energy, microalgae (*Spirulina platensis*) is a good source of feedstock for the production of various biochemicals because of their unique properties. 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 *S. platensis* using raw sago effluent diluted with water at different dilution levels *viz*.,90:10, 80:20, 70:30, 60:40, 50:50, 40:60,30:70,20:80 (sago effluent : water) and 100% (Undiluted) supplemented with different concentrations of $NaHCO_3$,K_2HPO_4 and $NaNO_3$ sources as carbon, phosphorus and nitrogen respectively, based on Zarrouck's broth composition. The best cellular growth of *S. platensis* was observed in sago effluent medium was 60:40 dilution with $NaHCO_3$ (10. g L^{-1}) as carbon source, K_2HPO_4 (0.50 g L^{-1}) as a phosphorus source and $NaNO_3$ (3.00 g L^{-1}) as a nitrogen source, with trace of $FeSO_4$ and EDTA at pH 9.0. Parameters like, dry weight, chlorophyll, lipid and protein content were measured. 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. The use of sago effluent for the cultivation of microalgae is therefore an attractive economic venture and also a demonstration of Sustainable Resource Management (SRM).

Key words: Customer retention, Assurance, Reliability, Responsiveness, Tangibility.

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INTRODUCTION

Cassava (*Manihot esculanta*) is a root tuber crop that is widely cultivated in the tropical regions of the world. It 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. 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 (Savitha *et al.,*2009). The cassava effluent is the waste water generated from the processing of cassava or the liquid squeezed out of its mash. By its nature, cassava processing for extraction processes produces large amounts of effluent high in organic content, if untreated may be displayed in the form of stagnant effluent ponds from which strong odors emanate, its effect on the environment is significant as the air we breathe becomes contaminated with the odor emanating from it, resulting in adverse respiratory health problems (Eze, 2010). In many areas where traditional processing is practiced, the effluent is normally discharged beyond the "factory" wall into roadside ditches or fields and allowed to flow freely, settling in shallow depressions (Ehiagbonare *et al*., 2009). Eventually this will percolate into the subsoil through infiltration or flow into streams and other surface water sources thereby causing pollution of the underground water reservoir, agricultural surface water and the subsoil (Morenikeji, 2010). Both cassava peels and effluent contain a number of contaminating substances amongst which is cyanide. Cyanogenic glucosides in these cassava wastes are in various concentrations depending on the variety and growing conditions (Ehiagbonare *et al.*, 2009). Additionally, the presence of simple and complex cyanide and their break down products – cyanohydrins and hydrogen cyanide has been a

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cause of concern because of their possible effects on health and environment (Okunade and Adekalu, 2013). Therefore algal growth with cassava wastes is thus an added advantage because these wastes have proven very useful for the growth of microalgae because they contain the following essential compositions; Moisture 0.82%, Ash 2.71%, crude fibre 4.40%, crude protein 2.69%, crude lipid 3.92% and total carbohydrate 85.46% (Sarkiyayi and Agar, 2010), in addition to essential ions like nitrate, sulphate and phosphorus which are required for their growth. Microalgae are generally microscopic algae, found in fresh water or marine systems which have an extraordinary potential for cultivation as energy crops (Neboh *et al.*, 2014). Their common feature is their oxygenic photosynthesis similar to that in higher plants and they make large contributions to the equilibrium of the earth's atmosphere by producing oxygen and removing carbondioxide (Agwa *et al.*, 2011). Microalgae apart from been used as single cell proteins, are projected as living cell factories for the production of biofuels and various beneficiary biochemicals used in food, aquaculture, poultry and pharmaceutical industries (Anand, 2010). Microalgae have been discovered to have the highest oil or lipid yield among various plant oils, and the lipid content of some microalgae has up to 80% triglyceride that can be converted into biodiesel through transesterification (Chisti, 2007).

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 (Ungsethaphand *et al.,* 2009). *Spirulina platensis*, 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. The present work has been formulated to standardize dilution and nutrient supplementation levels of sago effluent for the production of *S. platensis.*

MATERIALS AND METHODS

Raw sago effluent: Raw sago effluent was collected from Sri Thirumalaivasan Sago and Starch industries, Thoppapatty, Rasipuam Taluk, Namakkal District, Tamil Nadu, India. The raw effluent was collected and stored in the cold room until use. The sago effluent was physico-chemically characterised using standard procedures as detailed in (APHA, 1992)

Source of *Spirulina platensis* culture: In the present study, the growth of *S. platensis* (filamentous) was used to cultivate on the formulated sago industry effluent medium. The *S. platensis* CAS10 was obtained from C.A.S in Botany, University of Madras, and Tamilnadu, India. The culture was maintained in Zarrouk's medium in a 1000ml Erlenmeyer flask in the normal room temperature with a light intensity of 3000 lux and 12:12 hrs day/night cycle (Zarrouk,1996).

Standardization of dilution level of sago effluent for cultivation of *Spirulina platensis* : Raw sago effluent was diluted with distilled water at different dilution levels *viz viz*.,90:10,80:20, 70:30, 60:40, 50:50, 40:60,30:70,20:80 and 100% (Undiluted) in conical flasks. *Spirulina platensis* (OD540 nm 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, OD540 nm, population and dry weight was recorded after 30 days of incubation

Optimization of level of dilution and nutrient supplementation with $NaHCO_3$ and $NaNO_3$ nutrient for cultivation of *S. platensis* : Based on the above experiment, a laboratory experiment was conducted to evaluate three different dilution levels *viz*., undiluted effluent and 40:60 diluted with distilled water and addition of $NaHCO_3$, K_2HPO_4 and $NaNO_3$ at different concentration as carbon, phosphorus and nitrogen source. The flasks were incubated in the algal growth chamber and physico-chemical parameters were analyzed. The growth of *S. platensis* in raw sago effluent was evaluated with the following treatments replicated thrice.

Mass cultivation of *S. platensis* in the outdoor cultivation in cement pond : Mass cultivation of *S. platensis* in the outdoor is possible under optimal conditions of nutrients, light (sunlight), pH (9.5), temperature, agitation, culture depth and initial inoculum concentration. The production unit has to be located in areas with suitable climatic conditions and places where all culture conditions are optimum. The technological factors involved in the mass cultivation of *S. platensis* CAS10 are presented in Table (5). The Parameters like dry weight, chlorophyll, lipid and protein content were recorded after 30 days of incubation. The methodology followed to record the above parameters is presented in Table (3).

RESULTS AND DISCUSSION

Physico-chemical characteristics of the raw and raw sago effluent: The physico-chemical characteristics of the raw and raw sago effluent are shown in Table (1).

Table 1. Physico-chemical characteristics of raw cassava sago effluent

S. No.	Parameters	Raw sago effluent
1.	pH	4.41(±0.22)
2.	EC (dSm-1)	3.69(±0.19)
3.	TDS (mg/L)	2275(±90.07)
4.	TSS (mg/L)	1542(±37.76)
5.	TS (mg/L)	3817(±127.68)
6.	OC (%)	1.86(±0.07)
7.	BOD (mg/L)	4973(±241.33)
8.	COD (mg/L)	9925(±562.05)
9.	Nitrogen (mg/L)	64.25(±3.46)
10.	Phosphorous (mg/L)	12.58(±3.46)
11.	Potassium (ppm)	231.58(±82.20)
12.	Sodium (ppm)	34.28(±10.57)
13.	Cyanide (CN) (mg/L)	4.48(±0.32)

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), EC was 3.69 dSm^{-1}. The TS, BOD and COD of the raw sago effluent were 3817, 4973 and 9925 mg/L respectively. The cyanide (CN) content of raw sago effluent was 4.48 mg/L.

Table2. Effect of different dilutions of raw sago effluent on the growth of *S. platensis*

Treatments	pH	OD (540nm)	Dry Weight (gm)
T_1 - Undiluted effluent (100% raw sago effluent)	4.41	0.352	0.79
T_2 -90:10 (raw sago effluent +water)	4.76	0.793	1.08
T_3 -80:20 (raw sago effluent +water)	5.32	0.961	1.27
T_4 -70:30 (raw sago effluent +water)	5.69	1.159	1.47
T_5 -60:40 (raw sago effluent +water)	6.40	1.427	1.61
T_6 -50:50 (raw sago effluent +water)	7.02	1.532	2.61
T_7 -40:60 (raw sago effluent +water)	7.91	1.905	3.35
T_8 -30:70 (raw sago effluent +water)	8.51	1.761	3.07
T_9 -20:80 (raw sago effluent +water)	9.03	*1.623*	2.81
T10- Zarrouck's broth (control)	9.50	2.012	3.64
CD(0.05)	3.442	0.045	0.085
SEd	1.623	0.021	0.040

Table 3. Optimization of nutrient supplementation with $NaHCO_3$, K_2HPO_4 and $NaNO_3$ of raw sago effluent for cultivation of *S. platensis*

Treatment	Additional nutrient	Different Concentration(gL^{-1})	pH	OD (540nm)	Dry Weight (gm)
T_1 - Undiluted effluent (100% raw sago effluent)	$NaHCO_3$	5.0	7.53	0.621	0.79
		10.0	8.36	0.724	1.47
		15.0	8.98	0.713	1.49
	K_2HPO_4	0.10	5.31	0.532	0.64
		0.25	6.06	0.556	0.99
		0.50	6.83	0.610	1.01
	$NaNO_3$	1.0	6,29	0.593	0/.6
		2.0	6.77	0.674	0.98
		3.0	7.84	0.710	1.04
T_2 - 40:60 (raw sago effluent +water)	$NaHCO_3$	5.0	8.28	1.320	1.95
		10.0	8.91	1.874	2.94
		15.0	9.28	1.801	2.82
	K_2HPO_4	0.10	7.29	1.232	1.67
		0.25	7.96	1.535	2.15
		0.50	8.57	1.611	2.42
	$NaNO_3$	1.0	7.84	1.231	1.87
		2.0	8.45	1.456	2.34
		3.0	8.96	1.576	2.56
T_3 - Zarrouck's Broth (standard)			9.50	2.012	3.64
CD(0.05)			0.399	0.036	0.099
SEd	0.018	0.034	0.196	0.018	0.046

Table 4. Methodology adopted for estimation of biomass, chlorophyll, protein, lipid, total carbohydrates and phycocyanin content of *S. platensis*

S.No.	Parameters estimated	Methodology adapted
1.	Biomass	Pandey *et al.* (2010)
2.	Chlorophyll content	MC Kinney (1941)
3.	Protein content	Lowry *et al.* (1951)
4.	Lipid content	Foich and Lees(1957)
5.	Total carbohydrates	Hedge and Hofreiter (1962)
6.	Phycocyanin content	Horvath *et al.*,(2013)

Table 5. The technological factors for mass cultivation of *S. platensis* in raw sago effluent enriched medium during Sept., 2017

Cultivation system	Cement tank
Nutrients	Sago industry effluent medium supplemented with $NaHCO_3$ (10 g L^{-1}), K_2HPO_4 (0.5 g L^{-1}), $NaNO_3$ (3.0 g L^{-1}), $FeSO_4$ (0.01 g L^{-1}) and EDTA (0.08 g L^{-1})
Light	Sunlight
pH	9.5 adjusted by sodium bi carbonate addition
Temperature	39°C
Agitation	Manual stirring by plastic stick (30 min/day)
Culture depth	15 cm to 20 cm
Seeded culture	*Spirulina platensis*- CAS10 strain inoculam dose 0.25 g L^{-1}
Culture period	30 days
Harvesting	Filtration through muslin cloth
Drying period	Sun drying on plastic sheets
Season	Sept., 201 7
Location	:partment of Microbiology, Faculty of Agriculture, Annamalai University, Annamalainagar, Chidambaram,Tamilnadu

Table 6. Biochemical analyses of *Spirulina platensis* biomass grown in diluted raw sago effluent (40:60) with selected conc. of $NaHCO_3$ (10. g L^{-1}), K_2HPO_4 (0.50 g L^{-1}) and $NaNO_3$ (3.00 g L^{-1}) and Zarrouk's basal medium

Parameters	Spirulina biomass grown in Zarrouck's broth (Standard)	Spirulina biomass grown in diluted raw sago effluent with nutrients
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
Lipid Content (mg ml^{-1})	0.315	0.298
Dry Weight (gm)	3.63	3.45
Total phycocyanin content (mg g^{-1})	117.25	115.09

Standardization of dilution level of raw sago effluent for cultivation of *S. platensis*: The growth of *S. platensis* as a measure of pH, OD540nm and dry weight of biomass in the different dilution levels of sago effluent is furnished in Table (2). It was found that *S. platensis* was able to grow in low dilution of raw sago effluent (40:60), because the *S. platensis* was able to adapt itself to the high amount of organic nutrients present in raw sago effluent. The *S. platensis* showed the maximum growth in the dilution level of 40:60 on 30th day after inoculation with OD540nm (1.905) and dry weight of *S. platensis* (3.35 g mL^{-1}) than the other treatments. However, the results clearly indicated that *S. platensis* was able to grow in raw sago effluent diluted with water at 40:60 ratio without much inhibition compared to Control (Zarrouck's broth). These results are in conformity with the findings of (Kaushik *et al*.,2006) ,who found that *S. 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 *S. platensis* can be further increased by supplementation of inorganic carbon, phosphorus and nitrogen nutrients in the sago effluent

Optimization of dilution level and nutrient supplementation with $NaHCO_3$, K_2HPO_4 and $NaNO_3$ of raw sago effluent for cultivation of *S. platensis*: The *S. platensis* growth in different dilutions of raw sago effluent with the addition of different concentrations of $NaHCO_3$, K_2HPO_4 and $NaNO_3$ as carbon, phosphorus and nitrogen sources was assessed (Table- 3). The dilution of sago effluent with water at 40:60 ratio and addition with concentration of 10 g $L^{-1}$of $NaHCO_3$ recorded higher growth in terms of OD540nm (1.874) and dry weight (2.94.65 g L^{-1}) followed by 0.5 g /L K_2HPO_4 and 3.0 g / L $NaNO_3$ OD540nm values of 1,611 and1.576 and dry weight of 2.42 and 2.56 g L^{-1}respectively on 30 days after incubation. These were on par with Control (Zarouck's broth) and other treatments were recorded lower values. This might be due to $NaHCO_3$, K_2HPO_4, and $NaNO_3$ serving as good source of carbon, phosphorus and nitrogen, particularly $NaHCO_3$, which is preferred carbon source for *Spirulina* inducing its growth (Abu *et al*., 2007),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 *S. platensis* is 10.0 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. Hence, dilution of raw sago effluent with water at 40:60 ratio and nutrient supplementation with 10 g/ L of $NaHCO_3$, 0.5 g /L K_2HPO_4 and 3.0 g / L $NaNO_3$ were standardized as the optimum conditions for achieving maxim um growth and biomass production by *Spirulina* in raw sago effluent.

Mass production of *S. platensis* in outdoor condition: Mass production of *S. platensis* in outdoor condition using of raw sago effluent diluted with water at 40:60 ratio and nutrient supplementation with 10 g/ L of $NaHCO_3$, 0.5 g /L K_2HPO_4 and 3.0 g / L $NaNO_3$ were studied with comparison of Zarrouck's broth Table (6). In general, there was not much reduction in protein content of *S. platensis* grown in the sago effluent compared to the *S. platensis* growth in Zarrouck's broth. The colour and appearance of *S. platensis* biomass produced from both Zarrouck's broth and sago effluent was dark blue-green fine powder. The *Spirulina* cultivated in diluted raw sago effluent recorded protein, carbohydrate, dry weight, phycocyanin, chlorophyll and lipid content were on par values with Zarrouck's broth. With this biochemical composition, the *S. 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 (Budiyono and Kusworo , 2011) that cassava wastes can be used for the production of microalgal biomass, which can be used as single cell protein.

Conclusion

It is evident from this study that raw sago effluent serves as very good sources of nutrients required for the cultivation of *S. platensis*. However, the *S. platensis* through their photosynthetic machinery, were able to convert effluent into organic macromolecules (carbohydrate, lipids, and proteins) stored in the cell as biomass mass. Production of *S. platensis* in raw sago effluent was standardized as 40:60 dilution with water and addition of nutrients consisting of 10 g/ L of $NaHCO_3$, 0.5 g /L K_2HPO_4 and 3.0 g / L $NaNO_3$, with a retention time of 30 days. The *S. platensis* biomass is a protein-rich animal feed material.

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