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Challenges in Biochemical Engineering and Biotechnology for Sustainable Environment

Comparative Analysis of Saccharification of Cassava Sago Waste Using *Aspergillus Niger* and *Bacillus Sp.* for the Production of Bio-Ethanol using *Saccharomyces Cerevisiae*

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Abstract : Among the various environmental issues, waste management is a foremost area where effectual and exhaustive consumption of wastes and attaining useful products in natal ways are concentrated. Cassava the tuber crop, exploited for starch and sago production.The aim of this work is management of cassava sago industrial waste, because its availability is vast particularly in salem. Bio ethanol- high octane fuel that produced from renewed sources such as sugary (or) starchy agricultural crops. As a point of interest, it was affirm the disposed waste consists 55-60% of starch by biochemical characterization. On degradation, starch breaks up into simpler sugar units that simple sugars consumed for ethanol production by action of fermentation through analyzing various parameters, showing the excellent efficiency of the method is isolation of native starch degrading microorganism, instead of using enzymes for starch simplification. The starch simplifying organisms were isolated from the sago industry waste(*Bacillus sp*)as well as isolated frompotato(*Aspergillus niger*) and fruitfully established into the fermentation as separately in batch fermentation by co-cultivation. It was further accompanied by ethanol production using *Saccharomyces cereviseae.* The highest product yield was attained in fungal saccharification of sago waste which was pretreated by acid pretreatment method. The maximum ethanol production was 3.02 g/L from 5 g/L of initial sago waste concentration.
Key words: Sago waste, Starch degrading microorganism, *Saccharomyces cereviseae*, SSF, Bio ethanol.

Introduction

Tapioca Cassava (ManihotEsculentaCrantz) was introduced in India during the later part of the 17th century by the Portuguese lived in the state of Kerala. Cassava, a tuber crop which also known as manioc, sago, yucca and tapioca is one of the potential root crops with great potential for bio ethanol production.It is the most important survival food and industrial crop for the developing countries. The Salem region also houses the Tamil Nadu largest number of Sago industries which are engaged in the production Sago Foods and Starch. In Salem District alone, 34000 hectares of land is under tapioca cultivation which is the raw material for the sago industries and there are 650 units engaged in tapioca processing all over Tamil Nadu.Sago Industry waste is a profuse waste that can contaminate the ground water tables owing to improper disposal. Ethanol is a clean-burning, high-octane fuel that is produced from renewable sources. The later fact is vast magnitude, especially during recent times of grossly rising oil prices and gasoline prices at the pump. But, ethanol is produced from source which renewed each and every year. Ethanol also known as grain alcohol, can be made from agricultural crop.The project deals with the management of sago industrial waste. Because, disposed waste consists of 55-

60% starch[1] and cassava waste can be utilized to produce ethanol due to its containing cellulose and hemicelluloses at levels of 24.99% and 6.67% (w/w) respectively[2].

Materials and Methods

1. Collection of sago waste

The waste has been collected from a cassava sago industry in Salem. The waste was taken in the solid form that called as Residual pulp (Thippi). Collected sample were dried in sun light and stored at 4^0C in the Refrigerator.

2. Biochemical characterization

Table.1 Biochemical composition of cassava sago waste

S. No	Parameters	% Dry Weight
1	Starch	57.4
2	Cellulose	16.7
3	Hemi cellulose	13.58
4	Lignin	3.82
5	Reducing sugars	1.53

***not limited to and values may vary**

Starch is the major constituent in the waste of sago industry. Because, Cassava is the tuber crop which is one of the supplement of starch and Sago production. Cassava sago waste consists 55-60% of starch[1].It has negligible amount of Lignin, Hemi cellulose and reducing sugars and it were estimated by suitable methods[3].

3. Pretreatment of Sago waste

In this study, the sago waste (5 g) was treated with 1% of alkaline and acid solution[5]. The pretreated materialswere used for subsequent saccharification and ethanol fermentation. Dilute acid pretreatmentusing Hcl or $H_2SO_4$is the most-widely used method.Alkaline pretreatment is moreeffective in lignin removal whereas dilute acid pretreatment ismore efficient in hemi- cellulose solubilization[4].

4. Isolation of starch degrading organisms

4.1 Isolation of *Bacillus sp.* from sago waste

Sago waste wasused for the isolation. Serial dilution was made and was plated on nutrient agar by spreading 0.1ml of the diluted sample. Thenthe plates were kept for incubation at 37°C for overnight[5].

4.1.1. Morphological and Biochemical Characteristics

Gram staining, motility, indole production, methyl red, Vogues Proskauer's, citrate utilization, triple sugar iron,nitrate reduction, catalase, oxidase, urease, hydrolysis of casein, hydrolysis of starch werecarried out[5]. Finally it confirmed as *Bacillus sp.* by Gram staining, and also confirmed the ability of starch degradation of the organism by starch hydrolysis test[5].

4.2.Isolation of starch degrading fungi from potato

Fungal colonies were isolated form potato enriched for amylase producing microorganisms wherein PDA (potato dextrose agar) media was prepared, autoclaved and poured in sterile Petri-plates. The inoculated Petri-plates were incubated at 28°C for 48 hours. It has ability to degrade the starch by secreting α-amylase enzyme[6].

4.2.1. Screening of Fungal Isolates for Amylase Production

Fungal isolate were screened for amylase production efficiency in starch agar media comprising the following in gm L-1yeast extract 1.5, peptone 0.5, sodium chloride 1.5, starch 10, agar 15, pH 5.6. Fungal isolate were streaked centrally on sterile solidified starch agar plates, a blank without inoculation was also maintained for comparison. Platewas incubated at 28°C for 48 hours after that the plate along with blank were flooded with iodine and observed for zone of hydrolysis[6].

5. Fermentation

Table.2 Composition of fermentation medium

Constituents	Grams/Liter
Substrate	5
Yeast extract	3
Peptone	4
Malt extract	3
$MgSO_4$	1
KH_2PO_4	2
KCl	0.5
Ammonium sulphate	2
Manganese sulphate	0.1

***For starch degrading bacteria (*Bacillus sp.*)**

Constituents	Grams/Liter
Substrate	5
Yeast extract	3
Peptone	5
Malt extract	3
Dextrose	10

***For starch degrading fungi (*Aspergillus niger*)**

Figure-1 Starch degrading test (on bacteria)

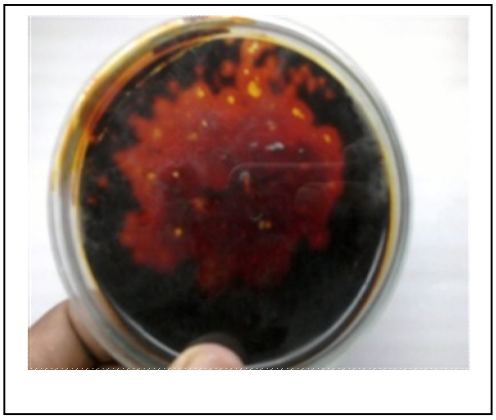


Figure-2 Starch degrading test (on fungi)

Fermentation is a metabolic process that converts sugar(Glucose) to acids, gases and alcohol.

$$C_6H_{12}O_6 \longrightarrow 2CH_3CH_2OH + 2\ CO_2$$

5.1 Collection of Microorganisms

The organism *Saccharomyces cerevisiae*was achieved from MTCC, Chandigarh, India. The MTCC No-180 that has particular prospects on ethanol production.

5.2 Inoculation and Incubation terms

The plan of fermentation is the co-cultivation of starch degrading bacteria and ethanol producing yeast in the fermentation medium. On the zeroth day, the isolated strain was introduced at 5% load into 250ml conical flaskas a batch fermentation with production medium (Table.2) and were incubated.On the 2nd day, the yeast was inoculated at 1% load into the production medium. The pH is 7.3. The condition maintained in 150 rpmat 35^{0}C. For the starch degrading fungi pH is 7 and the situation maintained in 150 rpm at 28^{0}C. On 5th day the biomass and the ethanol were estimated.

5.3 Confirmation test for ethanol

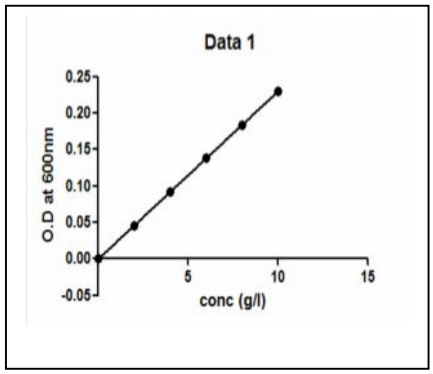
- The Iodoformtest was carried out to confirm the presence ethanol in the production medium on the 5th day. The yellow precipitation is a positive and a white precipitation is a negative result for the Iodoform test.
- The litmus test also carried out to confirm the presence of ethanol. The litmus blue color paper altered into red color caused by presence of ethanol.

Result and Discussion

Cassava is the third most important source in the tropics, after rice and maize. An effective nutrient composed of sago waste and several inorganic salts was consumed successfully for batch fermentation for ethanol production. In batch fermentation of 250ml volume in conical flask was used. Bacterial load- 5% and Yeast load – 1% were adequate for getting high yield[7]. The modifications are necessary for the effective production of Bio ethanol, in the form changing parameters such as pH, temperature, aeration, shaking and other minor parameters. The work has been done with one set of parameters values i.e., for starch degrading bacterial load, Temperature – 35^0C, no aeration, pH – 7.8, shaking at 150rpm. For starch degrading fungal load, Temperature – 28^0C, no aeration, pH – 7, shaking at 150rpm.The saccharification carried out by both bacteria and fungi as individually on the sago industry waste which has been pretreated through acid and alkali pretreatment method[7].

Table.3 O.D for Ethanol concentrationGraph.1 O.D curve for various Ethanol conc.

Ethanol concentration g/L	Optical Density (O.D) at 600nm
0	0.000
2	0.046
4	0.092
6	0.138
8	0.184
10	0.230



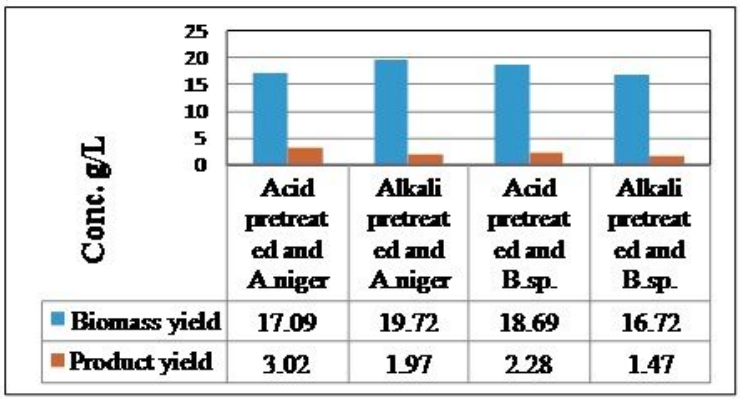


Figure-3 Comparative analysis of biomass yield and product yield

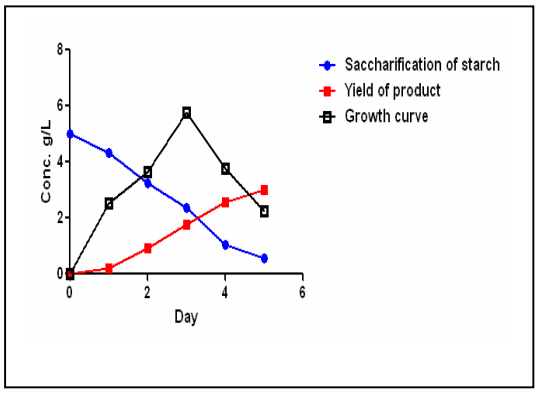
(X axis: Pretreated substrate along with fungal and bacterial saccharification (*Aspergillus niger*&*Bacillus sp.*); Y axis: Concentration of Biomass and Product in g/L)

- For fungal saccharification,After 5 days of fermentation period the Biomass was calculated and the yield of biomass 17.09 g/l.The maximum ethanol production was 3.02 g/l, on behalf of acid pretreated sample. As well as, after 5 days of fermentation period the biomass was calculated and the yield of biomass 19.72 g/l.The maximum ethanol production was 1.97 g/l, on behalf of alkali pretreated sample (Figure-3).
- For bacterial saccharification,After 5 days of fermentation period the Biomass was calculated and the yield of biomass 18.69 g/l.The maximum ethanol production was 2.28 g/l, on behalf of acid pretreated sample. As well as, after 5 days of fermentation period the biomass was calculated and the yield of biomass 16.72 g/l.The maximum ethanol production was 1.47 g/l, on behalf of alkali pretreated sample (Figure-3).
- The highest ethanol yield was attained in fungal saccharification of sago waste which was pretreated by acid pretreatment method. The maximum ethanol production was 3.02 g/l from 5 g/l of initial sago waste[8].

The major constituent of sago industry waste is starch (Table.1).Sago industry waste could be the upcoming solution for clean, efficient and economically-feasible carbon source for the bio production of ethyl alcohol.

Table.4 &Graph-2 Saccharification of sago waste by *Aspergillus niger* for the response of product yield on behalf of acid pretreated substrate.

Day	Saccharification of Starch g/L	Product yield g/L	Growth curve
0	5.00	0.00	0
1	4.32	0.21	2.54
2	3.24	0.94	3.64
3	2.37	1.70	5.78
4	1.04	2.58	3.75
5	0.57	3.02	2.24



***** The highest product yield was attained in fungal saccharification of sago waste which was pretreated by acid pretreatment method. The maximum ethanol production was 3.02 g/L from 5 g/L of initial sago waste concentration.

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