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Waste-to-Energy: Tapioca Solid Waste-to-Ethanol

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Considerable attention has been given in recent years to the development of alcohol production technology due to increasing demand for petrol. Ethanol has been known for a long time, being perhaps the oldest product obtained through traditional biotechnology. Several renewable carbohydrate resources have been tested for the production of ethanol as a liquid fuel. It is expected that the demand for ethanol for transportation purposes will increase drastically in the near future. The usage of bio-based products and bio-energy will reduce the import of oil products. Sugarcane is the main raw material for ethanol production in India. Similar to ethanol production from sugarcane, the utilization of bio-waste as an alternate raw material can quench the demand for ethanol. By using biomass-derived ethanol, a net reduction in the levels of carbon dioxide (the main greenhouse gas) could range from 60–90% relative to gasoline consuming vehicles. This paper aims to quantify the availability of raw material and develop suitable metabolic engineering for the bio-ethanol production using microbial platforms from tapioca solid waste.

Key Words: Bio-ethanol, Tapioca solid waste, Energy, Waste.

INTRODUCTION

Tapioca (cassava), the commercial crop, is a major source for the production of starch. In India, tapioca is grown over an area of about 3 lakh hectares, with a production of 58–60 lakh metric tonnes. In Tamilnadu, tapioca is being cultivated over an area of about 1.4 lakh hectares providing employment for thousands of workers over fields and in 1000 processing units. In Salem region alone, about 1.1 lakh hectares of land is under tapioca cultivation. The composition of the tapioca root is given in Table 1.

TABLE-1
COMPOSITION OF THE TAPIOCA ROOT

Moisture	70%
Starch	24%
Fibre	2%
Protein	1%
Other	3%

Sago processing in industries

There are around 700 starch/sago industries in Tamilnadu, particularly in Salem region, based on tapioca roots, which are classified under small-scale industries (SSI) sector. They are engaged in processing raw tapioca into starch powder by peeling the tapioca, crushing, washing and settling the milk of starch and drying in solar evaporation pans and granulation. The processed material in the finished form as starch powder is utilized for the manufacture of sago. The remaining solid residue is called thippi. The solid waste contains 45–55% of un-extracted starch, which is mostly locked into the fibres.

The industries are engaged in production for about 7 months in a year. The starch produced by the medium scale and small-scale industries is marketed through Sagoserve, Salem (Trading Agency of Government of Tamil Nadu). The production capacities of these industries in Salem region are given in Table-2.

TABLE-2
PRODUCTION CAPACITIES OF SAGO INDUSTRIES IN SALEM REGION

Nature of sago industry	Number of units	Tapioca consumption per unit (mt/year)	Tapioca consumption (mt/year)	Starch/Sago production (mt/year)	Solid waste (dry) released (mt/year)
Large scale (automatized plants not classified under SSI by Govt. of TN)	5	80,000	4,00,000	1,00,000 (25%)	24,000 (5–7%)
Medium scale (classified under SSI by Govt. of TN)	100	3,000	3,00,000	60,000 (20%)	33,000 (10–12%)
Small scale (classified under SSI by Govt. of TN)	570	1,500	8,55,000	1,71,000 (20%)	94,050 (10–12%)
Total	**675**	—	**15,55,000**	**3,31,000**	**1,51,050**

The solid waste released from large scale automatized industries is about 24,000 mt/year and the unextracted/fibre-locked starch content is about 25–30%, whereas medium and small scale industries release about 1,30,000 mt/year and the unextracted/fibre-locked starch content is about 45–55%. The composition of the sago industry solid waste is given in Table-3.

TABLE-3
TYPICAL COMPOSITION OF THE TAPIOCA DRY SOLID WASTE

Content	Large scale industry	Small scale industry
Starch	25–30%	45–55%
Fibre	23–30%	16–20%
Protein	12–15%	8–10%
Other	30–35%	20–25%

Part of the wet solid waste (thippi—containing about 55–65% moisture) is sold for Rs. 150–450/mt for cattle as feed. Part of the dry waste is used for mixing with some food derivatives. The rest of the solid wastes is dumped in the nearby fields that causes health hazards and also affects the ecosystem.

For the production of ethanol from the solid wastes of tapioca, an efficient and cost effective technology is needed. Ethanol production processes using crops such as sugarcane and corn are well established. However, utilization of a cheaper substrate such as tapioca starch could make bio-ethanol more competitive with fossil fuel.

The processing and utilization of this substrate is complex, differing in many aspects from crop based ethanol production. The process would be a two-stage process. The first step would be the conversion of tapioca-starch into glucose using suitable microorganism and the second step is to convert glucose to ethanol.

Conversion of starch to glucose

The fungus *Rhizopus oligosporus* is a prolific *amylase* enzyme producer and is known to be free of *mycotoxin* production, such as *aflatoxin.* From pilot plant experiments with tapioca tuber containing 65% starch it was calculated that 1 ha bearing 65 ton tapioca tuber can produce 3,500 kg of microbial protein with highly productive amylase enzymes to convert approximately 39,000 ton of grain or tapioca tuber into glucose, which is equivalent of 1200 ha harvest and a glucose yield required for the production of 15.6 million litres ethanol[1].

Single step biological hydrolysis of sago starch by *Aspergillus awamori* was carried out in batch and continuous processes[2]. For comparison, two-step enzymic hydrolysis, liquefaction and saccharification processes were also carried out in batch. The yield of glucose produced based on starch used and overall productivity obtained in continuous hydrolysis (0.58 g/g and 1.44 g/L.h respectively) were higher than those obtained in batch hydrolysis (0.42 g/g and 0.508 g/L.h respectively). A substantially high amount of biomass was also produced during biological hydrolysis using freely suspended cells, which can be used as nitrogen sources for media formulation. Cell free glucose production was achieved when cells immobilized in polyurethane foam were used in continuous hydrolysis. In comparison with freely suspended cells, the use of immobilized cells improved the production of fermentable sugars significantly in term of productivity (2.06 g/L.h), though the yield was more or less the same. The yield and overall productivity for biological hydrolysis were lower than for enzymic hydrolysis (0.88 g/g and 4.4 g/L.h, respectively). It is important to note that biological hydrolysis was carried out at a lower temperature (30°C) than that required in enzymic hydrolysis (60–90°C).

Raw sago starch and sago starch pretreated by heating at 60°C for 2 h in sodium acetate buffer (pH 3.5) were hydrolyzed using commercial *glucoamylase*—AMG (EC 3.2.1.3), α-*amylases*—BAN, Fungamyl and Termayl (EC 3.2.1.1), debranching *amylase—promozyme* (EC 3.2.1.41), and their mixtures in sodium acetate buffer, pH 5.0 at 35°C[3]. Raw sago starch was poor substrate for enzyme action compared to corn and tapioca starches tested under the same conditions, although pretreating the starch increased the extent and rate of hydrolysis. A strong

synergism between *glucoamylase* and α-*amylase* on the hydrolysis of both untreated and pretreated sago starch was observed. The hydrolysis products were characterized by high performance size-exclusion chromatography (HPSEC). The total carbohydrate concentration of hydrolyzed sago starch decreased but the *amylase* and *amylopectin* ratios in the residues remained the same.

The prospect of utilizing starch as an alternative carbon source for the production of energy (EtOH) and bioplastic (polyacetate) through enzymic hydrolysis of sago starch was studied under two sets of pH at 6.5 followed by 4.5 and at 6.0 followed by 4.3 for liquefaction and saccharification respectively[4]. The effects of pH were studied using four sets (15, 20, 25 and 30 wt./vol.%) of starch concentration. It was observed that both sets of pH were able to convert large amounts of reducing sugars but only the first set produced glucose (dextrose equivalent, DE 96%) with a 98.5% conversion after 4 h. The optimum starch concentration is 20 wt./vol.% producing over 196 g/L glucose after 4 h, a 98.4% conversion from starch. Higher starch concentrations exhibited a reduction in the level of maximum attainable dextrose.

Several microorganisms produce raw starch-digesting *amylase*. *P. brunneum* was isolated from a sago palm tree and was used as a source of starch-digesting *amylase*[5]. All the raw starch-digesting enzymes were effective for cereal starches, but root starches and sago starch were resistant to the enzyme reaction. Treatment of sago starch by heating to temperature below gelatinization temperature at lower pHs resulted in an increase in the ability of enzyme to digest sago starch granules. Heating to 60°C at pH 2.0 resulted in a conversion rate of sago starch granules to glucose near to the conversion rate of raw corn starch to glucose. At higher concentration, the degree of hydrolysis of treated sago starch granules was *ca.* 275% that of untreated sago starch granules. Addition of the enzyme in large amounts or small portions at various time intervals was effective in the hydrolysis of treated sago starch granules.

Conversion of glucose to ethanol

The next step is the conversion of glucose into ethanol by fermentation technology. There are two technologies available at present, the old traditional yeast (*Saccharomyces cerevisiae* or others) fermentation and a newly developed bacterial ethanol fermentation technology using *Zymomonas mobilis* isolated from tropical fruits. One important requirement is an efficient microorganism able to ferment glucose as well as to tolerate stress conditions. The literature[6–9] indicated that *Zymomonas mobilis* bacterium has several advantages over yeast for industrial alcohol production.

The yeast technology converts approx. 90% of all glucose carbons into ethanol with the bacterium increasing this to up to 98%[10]. The use of enzymically (α-*amylase*, followed by a mixture of *glucoamylase* and *pullulanase*) hydrolyzed sago starch for ethanol production by *Zymomonas mobilis* was carried out by Bujang *et al.*[11], utilizing a non-aerated bench-top fermentor. Comparative studies were performed between *Z. mobilis* grown on 10% (w/v) of either commercial glucose (CG) or hydrolyzed sago starch (HSS), with the pH either controlled at (initial) 5.5 or uncontrolled through the experiment. Uncontrolled pH generated

higher ethanol production (after 18 h) in both substrates, at 44 and 50.5 g/L compared to 41.5 and 49.0 g/L for commercial glucose and hydrolyzed sago starch respectively. However, hydrolyzed sago starch is preferred by the organism over commercial glucose in either controlled or uncontrolled pH conditions.

Ishizaki *et al.*[6] investigated the feasibility of using sago starch hydrolyzate (enzyme saccharification) as a carbon source instead of pure glucose and natural rubber serum powder (NRSP) as a nutritional source instead of yeast extract for the medium of ethanol fermentation by *Zymomonas mobilis*. The results showed that sago starch hydrolyzed with *amylase* and NRSP either as it is or hydrolyzed with *proteinase* has the potential to be used as a fermentation medium resource. It was found that specific glucose uptake rate, specific ethanol production rate and ethanol yield on sago starch hydrolyzate with NRSP hydrolyzate-Mieki medium (10% initial glucose concentration) are comparable to those values on pure glucose-yeast extract medium. The continuous fermentation in 1 litre jar fermentor with working volume of 500 mL at pH 5.5, 30°C, was accomplished by pH-stat with separate inflows of alkali and feed medium controlled through a digital set point control (DSC) with two pH set points: a high limit and a low limit. A small difference between the higher pH set point and a lower pH set point and a short control interval time resulted in high ethanol productivity, 11.1 g/l h, with high dilution rate, 0.27 L./h. Higher ethanol productivity for *Z. mobilis* using the medium consisting of sago starch hydrolyzate and rubber industry waste is expected if the measures to release the product inhibition are adopted.

Treatment of sago granules before incubation with the enzyme by heating to below gelatinization temperature at low pH was effective to improve the hydrolysis[12]. Glucose could be produced from treated (at 60°C, pH 2.0) sago starch granules by using the raw sago starch by digesting the enzyme from this strain. Alcohol fermentation from treated sago starch granules using a raw starch digesting enzyme from Aspergillus sp. No. 47 and *Saccharomyces cerevisiae* No. 32, under simultaneous process, was investigated; the optimum ethanol production was achieved at pH 4.8, 35°C and under yeast concentration of 1.8–10 cells/mL. Addition of raw starch digesting enzyme of 15 IU/g sago starch enzyme was sufficient. After 120 h incubation 7.3% ethanol was obtained from 15% sago starch granules; conversion yield of starch to ethanol was 48.4%.

Lee *et al.*[13] studied the simultaneous saccharification and EtOH fermentation (SSF) of sago starch using *amyloglucosidase* (AMG) and *Z. mobilis*. The optimal concentration of AMG and operating temperature for SSF process were 0.5% and 35°C, respectively. Under these conditions, with 150 g sago starch/litre as substrate, the final EtOH concentration was 69.2 g/litre and EtOH yield was 0.50 g/g (97% theoretically). Sago starch in the concentration range of 100–200 g/L was efficiently converted to EtOH. When compared to a two step process involving separate saccharification and fermentation stages, SSF reduced the total process time by half.

Also, immobilization of *amyloglucosidase* and *Z. mobilis* cell was studied by them in order to produce EtOH from sago starch economically[14]. Among various immobilization methods tested a co-immobilized system using chitin and sodium alginate appeared most promising with respect to EtOH productivity and opera-

tional stability. When the system was run in the continuous simultaneous saccharification and fermentation mode, maximum EtOH productivity was 72.2 g/L-h at a dilution rate 3.28/h. This system was stable for more than 40 days with a steady-state EtOH concentration of 44 g/L and an EtOH conversion yield 93%.

Haska[12] studied the simultaneous fermentation of raw sago starch granule to ethanol by *Saccharomyces cerevisiae* and its hydrolysis at a low pH and temperature (below that for gelatinization) using amylase obtained from *Aspergillus* strain No. 47. Under optimum conditions for ethanol production at an enzyme activity of 15 IU/g, sago where pH = 3.5, conversion yield of 48.4% was obtained. Raw sago starch was hydrolyzed by enzymes synthesized by *Aspergillus niger* N-10 in a medium at an initial pH of 3.5; high stability and activity also exhibited at lower pH. Direct ethanol production in a mixture of raw sago starch *S. niger* N-10, and *Saccharomyces cerevisiae* IFO 0309 was successful. Under non-sterile conditions 30 g/L was produced equivalent to a yield of 0.4 g ethanol/g starch[15].

Rice *amylase* and *glucoamylase* produced by *Saccharomyces cerevisiae* YKU 107 and YKU 131, respectively, were partially purified using anion exchange chromatography and characterized[16]. The optimum active condition for amylase was at pH 5.0 and temperature 50°C. It was capable of hydrolyzing more than 85% of potato, rice, cassava and sago starch; however, it could only hydrolyze 64% of cornstarch. *Glucoamylase* was optimally active at pH 5.5 and temperature 35°C. *Glucoamylase* could hydrolyze more than 70% of cassava, sago and potato starch, it also hydrolyzed more than 65% of corn and rice starch. Both the enzymes were not severely affected by the changes of ionic strength of acetate buffer at molarity lower than 0.20 M.

Fermentation status of starch in india

In India, some basic research works were carried out on the direct fermentation of sweet potato flour to ethanol by mixed cultures of *Endomycopsis fibuligera* and *Zymomonas mobilis.* But, no research work related to this new idea of converting sago starch waste into ethanol is reported to have been carried out in India. The Indian literature about the tapioca research work is discussed below.

Gunasekaran *et al.*[17] have reported the ethanol fermentation of cassava starch hydrolysate (CSH) by *Z. mobilis*. Their studies revealed the strain NRRL B-4286 of *Z. Mobilis* to be superior to the already-established efficient strain, ZM4, in the fermentation of glucose, fructose and sucrose up to a concentration of 200 g/L. NRRL B4286 also proved to be the best strain for fermentation of cassava starch hydrolysate. Their results showed that adaptation of the cells to the higher concentration of sugars in CSH could help to achieve maximal ethanol concentrations in relatively shorter period of time. With the culture adapted to the concentration of sugars in CSH, fermentation was completed in 28 h with a maximum concentration of 80.1 g/L ethanol. In contrast to this, a maximum concentration of alcohol of 78.5 g/L, after 40 h of fermentation was obtained with the non-adapted culture. Supplementation of the CSH with various additives did not result in higher concentration of ethanol.

A mixed culture of *Endomycopsis fibuligera* NRRL 76 and *Zymomonas mobilis* ZM4 could directly and more efficiently ferment cassava starch (22.5% w/v) to ethanol (10.5% v/v) than the monocultures[18]. The combination of culture filtrate of *E. fibuligera* containing amylases and *Z. mobilis* simultaneously saccharified and fermented the cassava starch to ethanol equally well. *Glucoamylase* (0.01%) added to the fermenting medium improved ethanol (13.2% v/v) production by the above mixed culture to almost the theoretical level (98%) indicating that this enzyme is a rate-limiting factor in *E. fibuligera*. *Z. mobilis* alone converted the enzyme-hydrolyzed starch only to almost theoretical level (98%).

Despite these, no detailed information is reported for the production of bio-ethanol from sago industry solid waste (Thippi) using *Zymomonas mobilis*/ *Saccharomyces cerevisiae* and other related bacteria.

Solid state fermentation

In recent years, considerable interest has been shown in using agricultural byproducts such as sweet sorghum, corn, apple, grape, sugarcane and sugar beets for fuel ethanol production. Due to the complex composition and insolubility of these agro-substrates, solid-state fermentation of these sources would be economical. Very few reports are available regarding the production of ethanol by solid state fermentation. Amin[19] has described ethanol fermentation in solid state by *Z. mobilis* grown on sugar-beet. Ethanol yield of 0.48 g/g sugar, volumetric productivity of 12 g/L/h and final ethanol concentration of 130 g/L showed good perfoemance of *Z. mobilis* in a solid-state fermentation. Furthermore, Amin *et al.*[20] reported that during solid-state fermentation fewer byproducts were produced, compared to conventional submerged fermentation. At optimum fermentation temperature of 35°C, an ethanol yield of up to 95% of the theoretical value with final ethanol concentration of 142 g/L was obtained.

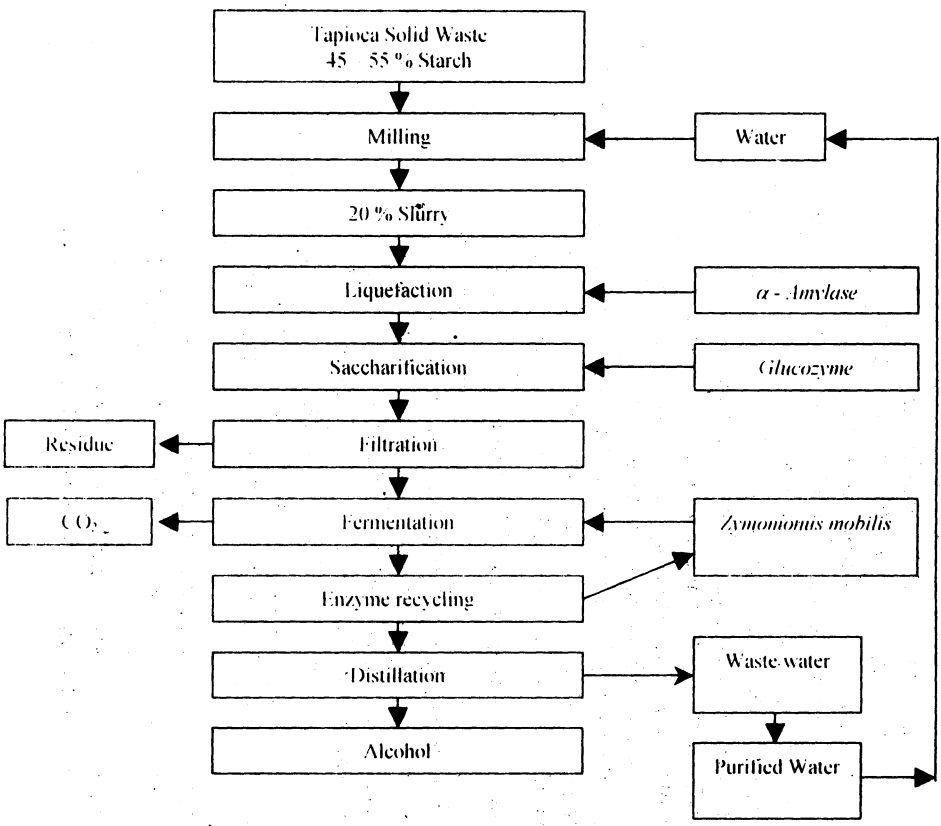
Conversion of tapioca solid waste to ethanol

The tapioca solid waste was dried, powdered and 20% slurry was prepared in water. The pH of the suspension was adjusted to 6.5 by the addition of 1 M sodium hydroxide. Liquefaction was carried out by adding 0.2% (v/v) *α-Amylase* to the slurry and incubating at 80°C for 3 h.

Saccharification was carried out by adding 4 units of *Glucozyme* per g of sago solid waste to the liquefied slurry at pH 5.5 and incubating at 50°C for 24 h. After saccharification it was filtered and the solid residue was removed. Glucose concentration was determined with a polarimeter.

Fermentation was carried out with *Zymomonas mobilis* (10 g/L for 100 g/L glucose) in a jar fermentor with an agitation speed of 500 rpm at 35°C and pH 5.5.

Ethanol was estimated by gas chromatography (Netel Omega-QC$^+$, Netel Chromatographs, India). The ethanol yield, based on glucose, was found to be 91%.



Conclusion

In recent years, keen attention has been given to effective utilization of agro-byproducts and industrial bio-waste to produce fuel using suitable fermentation technologies. As seen from previous reviews, a trial of ethanol production from tapioca solid waste by *zymomonas mobilis* has been made

Owing to the various advantages of *zymomonas mobilis*, it is possible to make use of this organism in industrial production of ethanol from not only tapioca solid waste but also from other bio-waste. Thus, it is time that the industrialists collaborate with academicians to apply the laboratory findings in science for the benefit of society.

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