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Production Of Bioethanol From Starch Based Crops

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ABSTRACT

This study aimed to estimate the production of bio-ethanol from starch based crops (cassava, sweet potato, Palmyra sprout, taro and corn). The production of ethanol was done by fermentation method using *Saccharomyces cerevisiae* and palm wine yeast. Using this method ethanol samples were obtained. From the samples high concentration of ethanol were obtained in taro plant. It was determined by the OD values from colorimetric method and also by means of antimicrobial activity. *Saccharomyces cerevisiae* produced more concentration of ethanol than the palm wine yeast.

Keywords: palm wine, yeast, cassava, bioethanol, taro, Palmyra sprout, sweet potato, corn.

Increasing energy requirements and atmospheric contamination by combustion gases has opened avenues for new, safe, effective and more accessible energy sources. People livelihood diversification would require the understanding of society dynamics in terms of domestic energy consumption as well as investigating possible ways of producing energy from available resources (Amigun *et al.,*2008).Bio-ethanol can be used as an alternative fuel to gasoline.Bio-ethanol can be produced by fermentation from several renewable sources, such as from potatoes and corn.

Bio-ethanol is microbiological way of converting simple sugars into ethanol and carbon dioxide (Damaso *et al.,* 2004). The main sources of sugar required to produce ethanol come from fuel or energy crops (Kim *et al.,* 2005). These crops include cassava, corn, sweet potato and palmyra sprout. Ethanol is a high octane fuel and has replaced lead as an octane enhances in petrol (Oghgren *et al*., 2006). By blending ethanol with gasoline we can also oxygenate the fuel mixture so it burns more completely and reduces pollution emission. Starch based feedstocks include grains such as corn or wheat, and tubers such as (sweet) potatoes, cassava and palmyra sprout. These feedstocks contain long complex chains of sugar molecules. The starch can be easily be converted to fermentable sugars. The starch can be easily be converted to ethanol or drop-in fuels. The fibrous part of the plants (eg; wheat straw or corn stover) can be converted to advanced biofuels (cellulosic ethanol).

Cassava [*Manihot esculenta*] is a tropical root crops that serves as a food security and income generationcrop.Thisplant can grow in the less fertile land and harvest time can be done every

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year,cassava has high enough carbohydrate contents is 32-35%, and it is the most important carbohydrate source after rice,but advance in preparation technology of cassava make it not only for food.

Sweet potato [*Ipomoea batatas* L.] represents an important biomass resource for fuel alcohol production, because of its chemical composition and high density of starch, compared to other forms of biomass, and thus premise as an alternative bio resource for the production of ethanol through fermentation (Roukas, 1994).It is cheap, readily available in the local market and offers ease in product processing.

Corn [*Zea mays*] contains about 70% starch .Corn is the feedstock for more than 90% of ethanol production in the United States due its abundance and low price ; most ethanol is produced in the corn growing states of the Mid west.

Palmyra sprout (also known as palmyra tuber) is a sprout that grows on Palmyra palm or *Borassus flabellifer*. In the states of Tamil Nadu, Bihar and Andhra Pradesh, India, and in Jaffna, Sri Lanka, the seeds are planted and made to germinate and the fleshy stems (below the surface) are boiled or roasted and eaten. It is very fibrous and nutritious, known as Panai Kizhangu or Panamkizhangu in Tamil and Thegalu or Gaygulu or Gengulu (especially in Andhra Pradesh and Telangana areas) in Telugu.

Taro commonly refers to the plant *Colocasia esculenta*, the most widely cultivated species of several plants in the Araceae family which are used as vegetables for their corms, leaves, and petioles. Thus, this article describes the "dasheen" form of taro; another variety of taro is known as eddoe or *Colocasia antiquorum*. Other species of taro include giant taro (*Alocasia macrorrhizos*), swamp taro (*Cyrtosperma merkusii*) and arrowleaf elephant's ear (*Xanthosoma sagittifolium).*

Bioethanol is ethanol from biomass through hydrolysis and fermentation process, where can be produced from foodstuff that contain starch, such as cassava, sweet potato, sago, beside that the raw material to produce bioethanol can be sugary, starch, and fibrous material. In manufacturing industry, ethanol is commonly used as raw material to produce ethanol derivative, mixture of alcoholic liquor, and pharmaceutical and cosmetic raw material.

MATERIALS AND METHODS

Sample Collection

In the present study, the sample crops were collected from Vadasseri market, Nagercoil, Kanyakumari, Tamil Nadu. The sample crops are cassava, corn, sweet potato, taro and palmyra tubour (fig.1).

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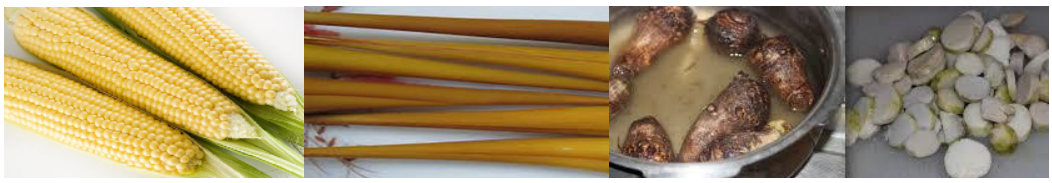
Cassava [*Manihot esculenta*] Sweet Potato [*Ipomoea batatas* L]



Corn [*Zea mays*] **Palmyra sprout Taro [*Colocasia esculenta*] [*Borassus flabellifer*]**

Fig.1. Starch based crops

Fresh palm wine was collected from Cheran Mahadevi, Thirunellveli, Tamil Nadu. It was obtained in pre-sterilized 100ml container and immediately transported to the laboratory for the isolation of test organism. The sample was allowed to stand for 12 hours before the isolation procedure commenced. One millilitre of sample was withdrawn aseptically and diluted using the serial dilution technique and the pour plate method was employed in inoculating 0.1ml of dilutions up to10^{-5}dilution.

Isolation of Palm Wine Yeast

One milliliter of 10^{-1} and10^{-2} dilutions of palm wine was inoculated on Rose Bengal Agar.Rose Bengal agar or Sabouraud dextrose agar using pour plate technique. The plates were prepared in duplicates and incubated at 28°C for 28 hours.

Identification of isolates

The isolates were purified by sub culturing and characterized based on cellular morphology and staining.

Identification test

Gram's Staining

The smear was prepared, air dried and heat fixed. The smear was flooded with Gram's crystal violet for 1 minute. The slide was washed with tap water to remove excess stain.Again it was flooded with Gram's iodine for 1 minute. It was flooded with 95% ethanol for 20 seconds and

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then counter stained with saffranin for 30 seconds. Rained the slide briefly with tap water and examined under microscope.

Preparation of Juice Samples for Fermentation

The crop samples such as corn, cassava, palmyra sprout, sweet potato and taro were sterilized by thorough washing with water. After that the crops were cut into small pieces and dried under sunlight. The dried crops were grinded with distilled water and sieved. The mash were collected, dried and kept in the form of dried powder.100 ml of distilled water were taken in 10 conical flasks and add 20 grams of powdered samples in two conical flasks.

Preparation of Starter Culture

Saccharomyces cerevisiae and palm wine yeast were inoculated into 100 ml of sample solution. Then inoculate the first five samples with *Saccharomyces cervisieae* and another set of the same five samples with a loop ful of yeast isolated from palm wine. And kept it for 7 days incubation at room temperature 28°C. This period of incubation is called fermentation period.

Distillation

Each of the samples were poured into the pressure cooker. One end of the tube was connected instead of whistle in pressure cooker. The other end of the tube was placed into a collection bottle. Middle portion of the tube was dipped in cold water containing vessel for condensation. Then the steam coming from the cooker was converted into water vapour. As a result, the end product was collected in collection bottle. After cooling kept it in the plastic bottles (fig. 2).

Fig. 2. Distillation apparatus

Estimation of Ethanol Content

Per cent of ethanol in alcoholic samples was estimated by the method of Caputi *et al.* (1968).

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Standard stock solution of ethanol

Ethanol standards were made by using ethanol-water solution in the range of 0 – 20 % ethanol (v/v).

Preparation of potassium dichromate solution

Potassium dichromate solution was prepared by adding 325 mL conc. H2SO4 to 400 mL distilled water in 1-liter volumetric flask. After mixing and cooling (80-90^{o}C), 33.768 g $K_2Cr_2O_7$ was added and then final volume of 1 litre was made with distilled water at 20°C.

Preparation of standard curve

Standard curve was prepared by taking 1 ml of each concentration of the standard solution [0-20% (v/v)] in a 100 ml volumetric flask containing 25 ml of potassium dichromate solution. The samples were heated at 60°C for 20 minutes in a water bath and then cooled and diluted to 50 ml with distilled water. Absorbance was recorded at a wavelength of 620 nm using SPECTRONIC® GENESYS TM5 Spectrophotometer.

Estimation of alcohol in the samples

One ml of alcoholic sample was added directly to the distillation flask, diluted to 30 ml with distilled water and then distilled. Distillation was carried out at 70+ 2 °C and 20 mL of distillate was collected in a 50 mL volumetric flask containing 25 mL of potassium dichromate solution. The contents in the volumetric flask distilled water. After mixing and cooling the contents of the flask, the absorbance was recorded at were heated at 60°C in a water bath for 20 minutes and final volume was made to 50 mL with 620 nm. The amount of ethanol in each sample was determined by using the standard curve of ethanol.

Antimicrobial Activity Test

Disc Diffusion Agar Method

The disk diffusion agar method tests the effectiveness of antibiotics on a specific microorganisms. An agar plate is first spread with bacteria, then paper discs of antibiotics are added. The bacteria is allowed to grow on the agar media, and then observed. If an antibiotic stops the bacteria from growing or kills the bacteria, there will be an area around where the bacteria have not grown enough to be visible. This is called a zone of inhibition. Here wells were made on nutrient agar plates using gel puncture and pour 0.1ml of distilled ethanol samples in each wells. After 24 hours incubation at 37°C,results were observed.

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RESULTS AND DISCUSSION

Raw material

Bioethanol is ethanol produced from biomass through fermentation process by microorganisms. Raw material of bioethanol are sugary, fibrous and starch material. The raw materials such as corn, cassava, sweet potato, Palmyra sprout and taro were used.

Isolation of palm wine yeast

In present study palm wine yeast was isolated in Rose Bengal Agar (Fig. 3) and also in Sabouraud Dextrose Agar. SimilarlyAyernor and Matthews in 1971, developed various microbial species from palm wine were isolated in Rose Bengal agar.

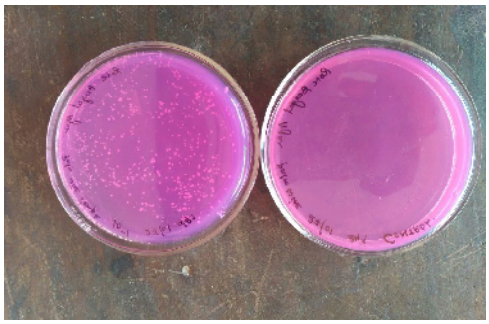

Fig 3: Yeast on Rose Bengal Agar Plates

Gram staining

The species that predominates on fermentative process for wine production is *S. cerevisiae* (Ivorra *et al*., 1999). During the fermentation the yeast produces ethanol, carbon dioxide and other secondary products important for flavour, taste and quality of wine serving as a reference on strain isolation (Lilly *et al.,* 2000).

Distillation

After 7 days of fermentation the samples were distilled and the obtained ethanol samples were kept in small bottles.

Ethanol concentration

Using colorimetric method, OD values at 620nm, ethanol in different concentration were obtained Caputi *et al.,* in 1968 used the same technique for the estimation of alcohol in percentage.

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Crops	OD values at 620 nm	
	S.cerevisiae	Isolated palm wine yeast
Taro	0.75	0.47
Cassava	0.44	0.16
Sweet potato	0.26	0.24
Palmyra sprout	0.15	0.16
corn	0.51	0.22

Table 1. OD values of the ethanol samples

The table showing, *Saccharomyces cerevisiae* produces more bio ethanol than the palm wine yeast. According to the OD values of taro, relatively the sample which was inoculated with *Saccharomyces cerevisiae* (0.75 OD value at 620 nm) produced more concentration of ethanol than palm wine inoculated sample. In all the remaining crops like corn, cassava, sweet potato and palmyra sprout, *S. cerevisiae* produced more ethanol than the palm wine yeast. Kordylas (1990) reported that the fact that the higher the concentration, the shorter the fermentation period required to achieve maximum yield. Togarepi *et al.,* in 2010 reported that for the ethanol concentration the rates increased rapidly with the increase in the amount of yeast concentration of (8g/20g) fruit pulp.

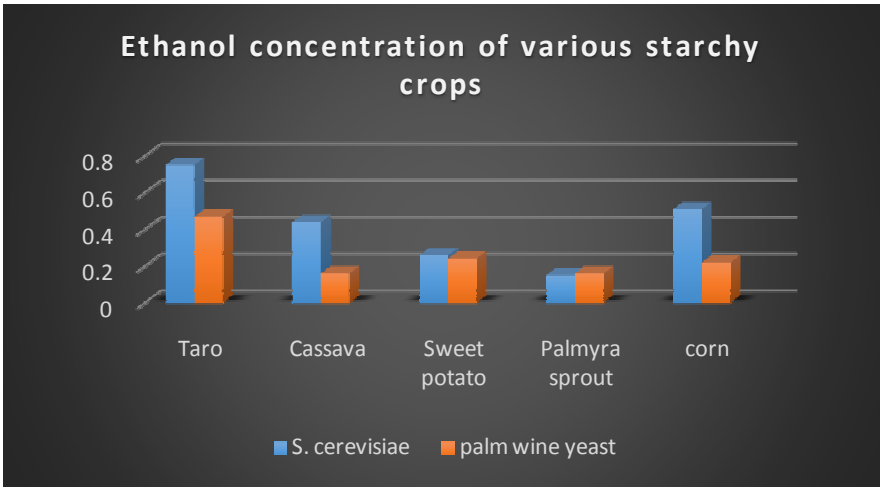

Fig. 5. Graphical representation of various concentration of ethanol two yeast species in starch based crops

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From the graph (fig. 5) sugary and starch based crops produces ethanol in different concentrations. Two strains of yeast were used, *S. cerevisiae* and palm wine yeast. It was observed that *S. cerevisiae* produces more ethanol than thepalm wine yeast.

Among these samples, taro produces high concentration of ethanol. It showed antimicrobial activity for *Klebsiella* (9mm diameter).

Ethanol or ethyl alcohol is a clear colourless liquid, it biodegradable, low in toxicity and causes no environmental pollution. From this present study it was conclude that bio-ethanol can be produced from very commonly available cheap starch based crops.

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