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Bioethanol production using food waste fermentation by *Pichia stipitis* and *Saccharomyces cerevisiae*

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Abstract
Ethanol has become an immensely important chemical compound in the pandemic times. Its use has increased dramatically in the last few years because of its ability to eradicate the harmful viruses. The source of ethanol has so far been corn and other sugar based crops which has increased the pressure on food industries. The crops produced to eat are consumed in the formation of ethanol. Thus ethanol fermentation of food wastes like green coconut shell and jackfruit seeds can act as a sustainable alternative to other sugar based crops. A co-culture of microorganisms provides a higher yield of ethanol through fermentation as compared to a single culture of microbes. A right combination of microorganism can be an ideal for fermentation by producing a concentrated ethanol which can be used in alcohol based hand sanitizers.

Keywords: Ethanol fermentation, *saccharomyces cerevisiae*, *Pichia stipitis,* food waste, Hand sanitizers

Introduction
With a growing population, the demand for food commodities has increased many folds. There has been a change in the dietary lifestyle of people over the years. A large group of the population have adopted a vegan lifestyle. This makes them more dependent on fruits for nutrition [1].

Food processing industry is regarded as the second most waste producing industry after household sewage. The solid and liquid waste released from the fruit industries can pose a major contributor for land pollution [2].

Processing of fruits can yield residues in the form of pods, peel, pulp, stones, and seeds. Disposal of these residues without processing in landfills can further aggravate the situation by causing environmental pollution [3]. Moreover, the fruit wastes contain bioactive compounds which can be converted into useful products by anaerobic digestion and thermochemical treatments [4]. One of the organic compound that can be converted into a useful by-product is carbohydrates. Bioethanol can be produced by anaerobic fermentation of the carbohydrates present in the fruit residues [5]. I would like to thank Jozbiz technologies to give me an opportunity to think about this topic and develop an idea.

Ethanol based hand sanitizers in COVID-19 Pandemic
Till date, no medicine has been developed to treat COVID 19 infection by SARS-COV2. WHO has recommended the precautionary measures to fight against the viral infection? One of the precautionary measures includes the use of alcohol based hand sanitizer to maintain hand hygiene. Alcohol based hand sanitizers have become a part and parcel of one's life [6]. A market research by Nielsen suggested that the hand sanitizer sale boosted up after the onset of pandemic. Alcohol based sanitizers are preferred over the non-alcoholic sanitizers because of their greater efficacy and broader spectrum to combat the COVID 19 virus [7]. Centre for disease control and prevention (CDC) has recommended the use of 60% to 95% alcohol in Alcohol based hand rubs (ABHR) [8]. Due to superior virucidal activity of ethanol, it is the major constituent of ABHR. Also, the skin tolerance for ethanol is greater than propanol which makes it a superior choice as a constituent alcohol in ABHR [9].

Majority of ethanol produced for commercial use is manufactured using the process of fermentation. Sugarcane, corn and sugar beets are the major sources of sugar which are undergoing fermentation to produce ethanol. In the United States of America, ethanol is majorly produced from corn. The major concern in using sugar based food to produce ethanol is that the food that should be used to feed people especially when there is a large fraction of population belonging to the malnourished class, is now used to produce ethanol [10]. Thus, it is important to change the sugar source so that the food can be used to feed people instead of producing ethanol.

Fermentation
Fermentation refers to a process involving degradation of glucose or other organic molecules to produce energy in the form of ATP in anaerobic conditions. It was a widely used mechanism in ancient times as the organisms lived in an anaerobic environment during that time.

The basic schema of fermentation involves two steps:

1. Degradation of glucose or other sugar molecule to yield 2 molecules of pyruvate, 2 molecules of ATP and two molecules of NADH via Glycolysis.
2. Oxidation of pyruvate to end products like lactate or ethanol with a concomitant release of 2 molecules of NAD+.

This process of fermentation enables the cells to regenerate NAD+ since in anaerobic conditions the NADH cannot be oxidized to NAD+. According to the enzyme catalyzing the oxidation of pyruvate, different products can be formed from pyruvate. The products commonly formed are ethanol and lactate in alcoholic and lactate fermentation respectively.

Alcoholic fermentation

Organisms like yeast can oxidize glucose or other sugar to ethanol and carbon dioxide in a reaction catalyzed by two enzymes Pyruvate decarboxylase and alcohol dehydrogenase. This type of fermentation is known as alcoholic fermentation.

Alcoholic fermentation is carried out in two steps:

1. Pyruvate is irreversibly Decarboxylated to yield acetaldehyde and carbon dioxide in a reaction catalyzed by pyruvate decarboxylase.

Acetaldehyde is then converted to ethanol in a reaction catalyzed by alcohol dehydrogenase. This reaction requires NADH as the reducing equivalent which is oxidized to NAD+ while acetaldehyde is reduced to ethanol.

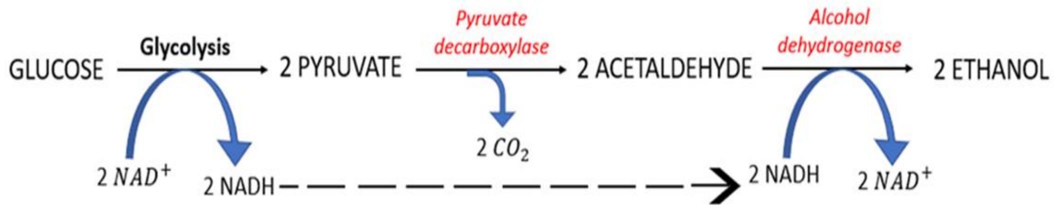

Fig 1: Represents steps of ethanol fermentation using Glucose as the starting sugar

Thus each molecule of glucose will produce two molecules of ethanol and two molecules of Carbon dioxide after oxidation via alcoholic fermentation.

Alcoholic fermentation is widely exploited by industries to produce useful products. Alcohol industry uses different carbohydrate rich food to produce ethanol. For example in beer brewing industry, the ethanol fermentation of sugar present in barley in the presence of yeast enzymes, yield beer [11].

Saccharomyces cerevisiae is commonly used to carry out alcoholic fermentation at the industrial level [12].

Composition of green coconut shells

The green coconut shell mainly comprises of cellulose (19.9%), hemicellulose (68.7%) and lignin (30.1%) [13]. There are a variety of sugars present in the green coconut shell including L-arabinose, galactose, L-rhamnose, xylose and glucose. Glucose forms 26% of green coconut shell [14].

Composition of jackfruit seeds

The jackfruit seeds are rich in Carbohydrates, Proteins and Minerals including Potassium, Sodium, Magnesium, Calcium and Phosphorus. Seeds from 100 g of edible portion contains approximately 25.8 g to 38.4 g Carbohydrates, 6.6 g to 7.04 g Proteins. 246 mg Potassium, 63.2 mg Sodium, 54 mg Magnesium, 50 mg Calcium and 38 mg to 97 mg Phosphorus [15].

Proposed Method

This research paper utilizes green coconut shell and jackfruit seeds as the sugar source. Alcoholic fermentation is carried out using *Saccharomyces cerevisiae.*

A. Collection of Raw material

The empty green coconut shell is taken and crushed using a green coconut shell grinding machine. A dry powder of particle size < 3 mm is obtained. For jackfruit seeds hydrolysate, the seeds are dried using a dryer and crushed in a laboratory mill before enzymatic assay. The particle size of powdered seeds should be less than 3 mm.

B. Pretreatment of food hydrolysate

The powdered food hydrolysate is mixed with 1 g sulphuric acid per 100g of powder. The mixture is kept at 120 degree celsius for 1 hour. The pretreated food hydrolysate is utilized for the fermentation process.

C. Isolation of Microorganisms

Saccharomyces cerevisiae is the commonly utilized microbe for fermentation of biomass because of its ability to ferment a wide variety of sugars and high ethanol production. Although it lacks the capability to ferment pentose sugars [16]. The Saccharomyces cerevisiae is bought from the market. 5 gram Saccharomyces cerevisiae is saturated using 100 ml of 20% sucrose. For 40 g of hydrolysate powder, 1 ml of saturated culture is required. The baker's yeast is kept overnight in a dark bottle to form saturated culture.

Pichia stipitis is a yeast isolated from decayed woods and newborn larvae of insects living in wood. It has the capability to ferment hexoses like galactose and glucose, pentoses like xylose and disaccharides like cellobiose in anaerobic condition [17]. It is grown in a growth media containing 10 g/l xylose,3 g/l1 yeast extract, 3 g /1 malt extract and 5g/l peptone at pH 4.5 at a temperature of 30 degree celsius.

D. Ethanol Fermentation

The preatreated food hydrolysate is hydrolyzed using alpha amylase at 85 degree celsius for 30 minutes which is subsequently exposed to glucoamylase at 65 degree celsius for 30 minutes and cellulases. The hydrolyzed mixture is kept for 6 hours at 50 degree celsius on orbital shaker at 200 rpm [18].

The fermentation takes place in an orbital shaker at 80 rpm with temperature of 30 degree celsius at pH 5.0. The hydrolyzed food mixture is taken in a Erlenmeyer filter flask. 1 ml of saturated baker’s yeast culture is added for every 40 g of original hydrolyzate powder. After the addition of baker’s yeast, 5% (v/v) *Pichia stipitis* is added. The flask is covered to provide anaerobic conditions. The mixture is kept for 4-7 days.

Result

A co-culture *Pichia stipitis* and *Saccharomyces cerevisiae* of to ferment sugar is a better alternative to a single yeast culture. A larger amount of ethanol with a higher concentration is produced in the former case. Baker’s yeast can ferment hexose sugar and Pichia stipitis is capable of fermenting hexose as well as pentose sugars. A layer of ethanol is formed at the top which can be further distilled to produce a higher concentration of alcohol. At the bottom, the residues are obtained which can be useful as fertilizers.

Discussion

Waste accumulation has always been a problem in populated, developing countries. The waste generation has far exceeded the waste disposal.

The conventional methods of waste management have become so ineffective due to increased waste, that waste has started piling up in the dumpsters.

Burying food waste is not a healthy practice since it can produce foul smells and act as a breeding ground for flies and rodents.

Thus the novel approach to transform this waste (specifically kiwi peel, banana peel, and date seed) into a useful product is the need of the hour.

Fermentation of the jackfruit seeds and green coconut shell produces three major fractions that can prove to be useful products. They are: ethanol, carbon dioxide and residues.

Ethanol is a major constituent of alcohol-based sanitizers, which are capable of killing virus and bacteria. During COVID times ethanol produced from waste produced in kiwi, banana and date processing units can be put to use for producing ethanol-based hand sanitizer instead of using other starch-based crops.

60% to 95% (v/v) ethanol in alcohol based hand sanitizers, has deemed to be effective and efficient for disinfection including SARS-COV2 [19].

Moreover, the liquid residue formed at the bottom of the container after fermentation, contains a high proportion of proteins, minerals (for example iron, zinc, manganese, calcium), dietary fibre. Thus, this residue can be used as a fertilizer for plants that promote their growth by providing necessary nutrients. The presence of yeast culture in the liquid residue improves its qualities as a fertilizer.

Yeast increases the soil acidity which promotes better absorption of nutrients by the plant. Also, yeast can stimulate the conversion of insoluble phosphorus ion into soluble phosphorus which increases the uptake of phosphorus by plants by enhancing it's availability in soil. Yeast also contain trehalose 6- phosphate synthase which catalyzes the formation of trehalose. Trehalose improves the stress tolerance in plants and promote its development [20].

Also the solid residue left after juice extraction from kiwi and banana peels can be utilized as manure for promoting plant growth. Carbon dioxide released as a by-product can be directed to greenhouses where it promotes plant growth, doing so can prevent combustion of natural gas usually used to increase yield of greenhouse crops.

Conclusion

Bioethanol production using food waste is a sustainable alternative as it reduces the pressure on sugar based food industries and also promotes the utilization of waste products. In COVID-19 pandemic, the use of ethanol based hand sanitizers has increased exponentially. The food products like corn and sugarcane which should be used to feed people have been used for years to synthesize ethanol. Ethanol fermentation using co-culture of *Pichia stipitis* and *Saccharomyces cerevisiae* yields high quality ethanol with a greater yield.

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