Production of Ethanol from Waste Potato Using Locally Available Biocatalyst

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Abstract Bioethanol is a renewable energy source produced from the resources which can be easily replenished. The bioethanol production through fermentation may provide an economically competitive source of energy by its incorporation into gasoline. Production of bioethanol from waste food crops like potatoes could be the better substrate, and the waste produced is also biodegradable. Lack of storage facilities and postharvest losses make potato a promising crop which can be used for production of ethanol. Moreover, the conversion of potato starch into glucose by *bakhar* is more cost-effective, and fermentation with baker's yeast *Saccharomyces cerevisiae* yields maximum amount of ethanol. This process of production of ethanol from waste potato would be promising and economically effective for the production of biofuel, called bioethanol.

Keywords Waste potato • Pretreatment • Saccharification • Fermentation • Bioethanol

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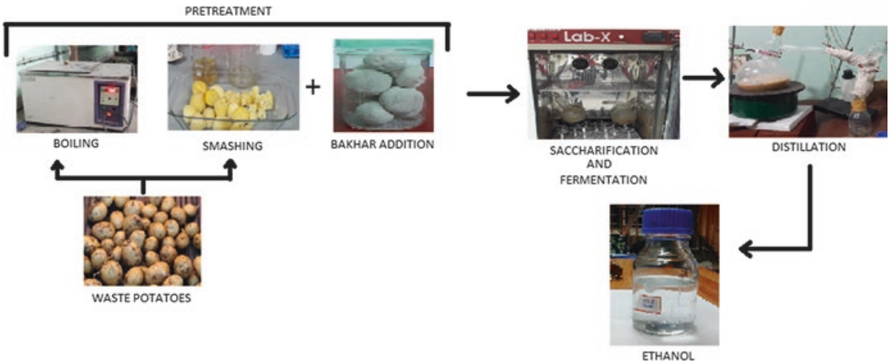


1 Introduction

The fossil fuel depletion and consequent increase in price encourage the study of alternative process and substrates to meet the global energy demand. Besides, fossil fuel also has the direct impact on the atmosphere. The increase in the use of fossil energy causes greenhouse gas emissions that have an adverse effect on the environment. The burning of petroleum-based fuels causes the increase of CO_2 level in the environment which is directly responsible for global warming. Methane, hydrogen and ethanol can be used as potential substitutes to fossil fuel as alternative sources of energy. Among these three, bioethanol is the most favourable alternative liquid fuel. Bioethanol can be derived from agricultural biomass resources or waste which has been considered as the cleanest liquid fuel alternative to the fossil fuel like gasoline. Agricultural material like sugarcane juice, rice, potato starch, molasses, cassava and bagasse can be used for the production of bioethanol. Apart from the known usage of ethanol as a fuel, about 45% of the produced ethanol is being used as portable alcohol and 40% in the industrial sectors, and only the remaining is available for blending with petrol. In the industrial sectors, ethanol is used by chemical, pharmaceutical industries, etc. It is also used to produce ethyl tertiary-butyl ether (ETBE) (Chandel et al. 2007). Bioethanol is made by fermenting from a variety of biomass from their sources. It is acknowledged that bioethanol is a unique transportation fuel with powerful economic, environmental and strategic attributes.

The ethanol from biomass-based waste materials from renewable sources for fuel or fuel additives is fermented to produce bioethanol (Ghosal et al. 2013). It is flammable and volatile, so it evaporates easily when left in an open container. The chemical formula of ethanol is C_2H_5OH. Bioethanol is a high-quality, stable liquid. It is an alternative renewable energy source produced through the anaerobic digestion process. Bioethanol is processed using either free or immobilised cell. Immobilised cells are more advantageous over free cells as it has the ability to separate cell mass and reduce the risk of contamination and better operational stability

Table 1 Potato composition (Rani et al.; Yekta and Ulgar 1994)

Composition	Content (%)
Starch	20
Moisture	80.28
Total protein	2.19
Ashes	0.65
Crude fibre	0.85
Total sugars	0.41
Total lipids	0.12

and cell viability for several cycles of operations enhancing yields (Ghosh et al. 2014; Gohel and Duan 2012). Currently, bioethanol production is based on the fermentation of starch- and sugar-based feedstocks, leading to the production cost fluctuation with the price of these food sources. Based on the utilisation of waste potatoes, ethanol is produced from potatoes. In potato cultivation, waste potatoes are produced from 5 to 20% of crops as by-products (Liimatainen et al.; Ghosal et al. 2013). However, depending on the biological waste for the production of bioethanol, potato and ethanol have some chemical composition which is required to know for the production of ethanol.

Potato is grown almost everywhere in the world. Potato is a tuberous crop from the perennial nightshade *Solanum tuberosum* L. Potato is a cheap substrate for alcohol fermentation which is rich in starch (Table 1) and production with higher starch yield per unit land cultivated. After China and Russian, India is the third largest potato producer. The amount of waste and by-products of potato is estimated to be around 12–20% of their total production. Good-quality alcohol can be produced from potato which can be used as a good fuel. Therefore, it is a need to explore also an attempt to utilise the possibility of ethanol production with maximum efficiency with suitable processing and low-cost materials for conversion of waste potatoes into ethanol.

2 Materials and Methods

2.1 Biomass Feedstocks and Materials

The substrates for ethanol production are recommended to be cheap. Ethanol has been produced from varieties of the substrate. The current industrial ethanol output was derived from the input of sugar- and starch-based feedstock such as sugar cane, sugar beet, sorghum, corn, wheat, cassava, rice and others (Sanchez and Cardona 2008; Mussatto et al. 2010; Hill et al. 2006). Ethanol production from wastes has two major advantages. On the one hand, it reduces or eliminates the cost of waste disposal. On the other hand, wastes are cheap and it reduces the cost of ethanol production. The amount of substrate depends on the waste potato and broken rice

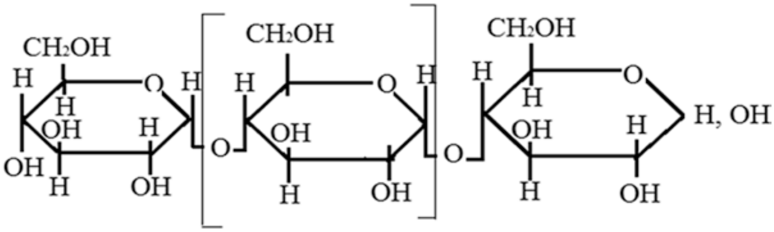


Fig. 1 Structure of starch

which is wasting per year. Waste potatoes and broken rice as biomass are alternative raw materials that have attracted the considerable attention of both the ethanol industry and academic research owing to its large availability, low cost and lack of competition with food production.

In bioethanol production, starch is the main component in potato. Starch (Fig. 1) is a polymeric carbohydrate composed of glucose units. The properties of starch vary with the plant source. Starch is made up of two components, amylose and amylopectin, which are polymers of glucose. Amylose is a linear polymer of glucose units joined by alpha-1, four bonds which consist of up to 20–25% of starch weight. The linear polymer contains up to 6000 glucose units. Amylopectin is a branched polymer in which the glucose units are joined by alpha, 1–4 bonds in the linear molecule sections and by alpha, 1–6 bonds at the branching point which typically occur at every 20–30 glucose units and contain up to 75–80% of most starch types. Amylopectin molecule is one of the largest molecules in nature and has about two million glucose units (Anonymous 1981; Batum 1993; Hoek et al. 1998).

Bakhar plays an important role in fermentation and is an amylolytic enzyme which directly converts the starchy raw material to fermentable sugars and finally alcohol. The principal ingredient of bakhar which is mixed with a traditional starter is low-grade boiled rice (*Oryza sativa* L.). Bakhar is a mixture of old ferments (containing microbial inoculums), parts of different plants (fresh root sand leaves) and rice dust (Dhal et al. 2010; Lin et al. 2012). In the eastern states of India like West Bengal, tribal people prefer rice-based beverages called haria. The traditional starter used for the haria preparation is bakhar or ranu tablet.

Baker's yeast (*Saccharomyces cerevisiae*) can produce a high concentration of ethanol and can grow under the optimum condition without causing harm in its growth (Nigam 2000). *S. cerevisiae* is the microorganism which is used for ethanol production due to its well-known fermentative capabilities (Cot et al. 2007; Rani et al. 2010). Yeast requires nutrients for their growth like minerals, peptides and vitamins and magnesium for glucose conversion. Nitrogen deficiency retards yeast growth and metabolism resulting in arrested sluggish fermentation (Gohel and Duan 2012; Rani et al. 2010). An adequate amount of yeast is required for fermentation of raw materials (rice or potato) for maximum yield of ethanol. Moreover, scientific knowledge about *S. cerevisiae* allows a great potential of metabolic engineering modifications with the aim of boosting its fermentation efficiency.

2.1.1 Temperature

The yeasts at high temperature are ideal for bioethanol productions which are active and tolerant. The enzymatic activity and membrane turbidity of yeast cells are greatly affected by the temperature. The optimum temperature for the fermentation of the *Saccharomyces cerevisiae* is 30–40 °C with a pH range of 4–6. The exponential phase of yeast cells shortened, and ethanol production reduced considerably at a higher temperature (more than 50 °C). Due to change in transport system, accumulation of toxin increases including ethanol in the cell.

2.1.2 pH

For the culture of *Saccharomyces cerevisiae*, 4–6 is the range of optimum pH. The incubation period was continuing for a long time though the ethanol concentration was not reduced significantly due to a decrease in pH below 4, and when the pH was above 6, the concentration of ethanol diminished substantially. So, before fermentation, the pH of the feedstock should be checked or should be optimised in the range of 4–6 for maximum yield of bioethanol.

2.2 Pretreatment

Pretreatment is a very important step before saccharification. Preparation of waste potatoes and boiling of the wastes are required before mixing of bakhar. The starch can easily be broken down with the biocatalyst, bakhar into glucose.

Waste potatoes were collected; 500 g of waste potatoes was boiled for 1.5 h in a water bath. The boiled potato was peeled, mashed and mixed very well in 200 ml of water and allowed to cool down. Further, 25 g of bakhar was weighed and mixed in the starch suspension of potato and incubated.

2.3 Saccharification

The process of breaking down of a complex carbohydrate such as starch into monosaccharide components such as glucose is termed as saccharification. A saccharifying enzyme such as bakhar provides maximum starch conversion used on a liquefied starch-containing substrate to produce sugars for fermentation. The liquefied

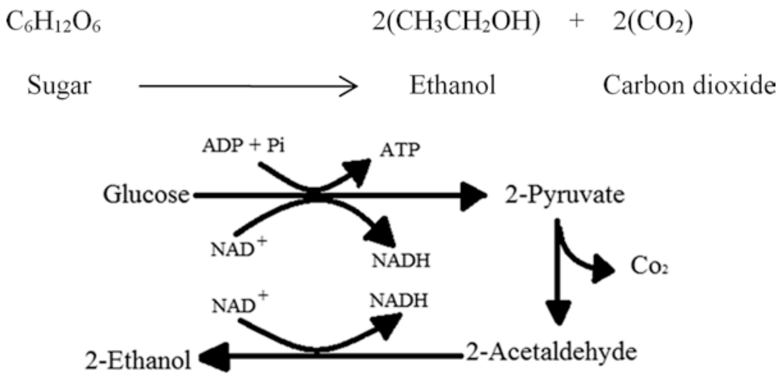


Fig. 2 Biosynthesis process for fermentation of glucose into ethanol

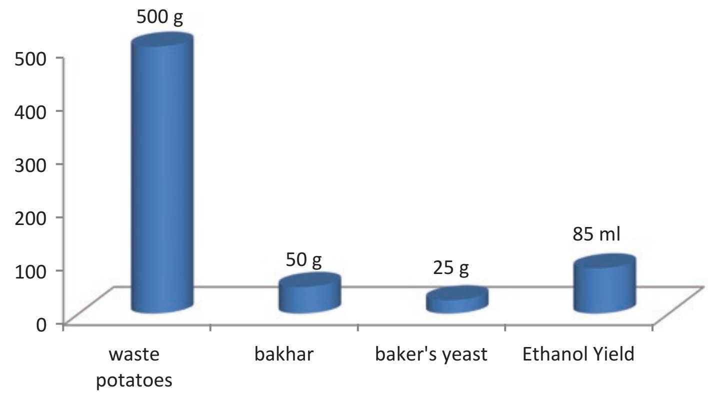
starch-containing substrate was incubated for 48 h at 35 °C in an incubator for the conversion of glucose.

2.4 Fermentation

Fermentation is a process of bioconversion of sugars into ethanol using yeast *Saccharomyces cerevisiae*. In this process, 50 g of baker's yeast was added in the saccharified starch suspension and allowed to ferment for 96 h maintaining a pH range of 5–6. Although there are over 150 amylolytic yeast species, their industrial use is limited because of their low ethanol tolerance. The fermentation of glucose into ethanol includes the following biosynthesis process in the form of flow chart given in Fig. 2.

2.5 Distillation

Distillation is a process of separating chemical components from a liquid mixture in different boiling points by selective evaporation and condensation. In this process, the ethanol is separated from the fermented starch suspension. The fermented potato starch suspension was distilled for 4–5 h for the yield of ethanol. For further purification, the liquid was further distilled for maximum concentration of ethanol.



Graph 1 Graphical representation of the ethanol with fermentative materials and feedstocks

3 Results and Discussions

As alternatives to fossil fuels, the use of renewable biofuels is developing in many countries to decrease greenhouse gas emissions and to reduce the dependence on petroleum (Hill et al. 2006; Lin and Tanaka 2006; Zabed et al.). Fermentation of waste potato by baker's yeast (*Saccharomyces cerevisiae*) and sugar production by saccharifying the liquefying starch suspension produce the maximum amount of ethanol. The results showed that starch utilisation, amylolytic activity and ethanol yields are high in *S. cerevisiae*. Starch utilisation of up to 94.8% is observed in 48 h to convert into glucose. By simultaneous saccharification and fermentation at different concentration of enzymes, yeasts and parameters, maximum yield of ethanol was observed. During fermentation, at 96 h maximum yield is observed rather than 24 h and 48 h. Detailed studies and experimentation revealed that bakhar has the potential to convert the starch into glucose within 48 h, but a further extension of saccharification degrades the yield of ethanol. Five hundred grammes of waste potatoes and 25 g of bakhar and 50 g of baker's yeast produce 85 ml of ethanol after saccharification and fermentation. The graphical representation of the ethanol yield with fermentative materials and feedstocks is shown in Graph 1. The yield of ethanol from waste potato could be a turning point to be used as fuel and can also reduce the cost of the ethanol. Fermentation and yield conditions for ethanol production of waste potatoes are described (Table 2).

Table 2 Fermentation and yield conditions for ethanol production

Name of the organism	Temperature (°C)	pH	Bakhar (g)	Time of saccharification (h)	Baker's yeasts (g)	Time of fermentation (h)
Baker's yeast (*Saccharomyces cerevisiae*)	35	5–6	50	48	25	96

4 Conclusion

Currently, a lot of attention has been given for the production of biofuels in order to contribute the rising demands of fuels. Renewable biomass such as sugar, starch and lignocellulosic materials is expected to be one of the dominating renewable biofuels in the transport sectors within coming 20 years. The use of waste potato in biofuel production seems to be potential, as the biofuel production from waste potato is a well-known process. Due to high starch content in potato, it has been proposed as a feedstock for bioethanol production. The analysis reveals that production of ethanol from potato could meet the need of global demand. According to the study and analysis with experiments, it can be concluded that with the help of bakhar, large amount of ethanol can be produced. The experimental result shows 0.17 ml/g of ethanol is produced from waste potato. Bakhar reduced the cost of the ethanol, and the material is also biodegradable. With today's increasing demand and cost of the fuel, this process of production of ethanol could be a breakthrough to meet the need of social, economic and environmental problems.

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