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Synthesis of 1, 3- Propanediol from Sorghum

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Abstract: 1, 3-Propanediol (PDO) is the commonest diol used in polyester synthesis, polyurethanes and cyclic compounds. Starch was prepared from white sorghum. Dual enzyme technique was used to convert starch to sugar. Then glycerol was formed by fermentation of the sugar by yeast after 72 h. Mixed culture of *Escherichia coli* and *Klebsiella* specie was used to inoculate the glycerol obtained for final conversion to 1, 3-propanediol. The mixture was left for 72 h to ensure complete conversion. The reaction temperature for fermentation was 37°C. The expression of *Klebsiella* sp-diol dehydratase in E-*coli* catalyzed the conversion of glycerol to 1, 3-propanediol. The sample was then re-filtered, distilled and condensed to obtain the pure, bio-PDO which is colourless and odourless. Biologically produced 1, 3-propanediol was characterized and the result is as follows: specific gravity = 0.9992, purity 99.92%, boiling point 121-124°C. The PDO synthesized was found to be of comparable purity to chemically produced PDO.

Key words: 1, 3-Propanediol, bacteria, starch

INTRODUCTION

1, 3-Propanediol (PDO) is one of the commonest diol used in polyesters, manufacture of polyurethane and cyclic compounds. Recently, it has found application in cosmetics as a preservative (WIPO WO/2008/061187). 1, 3-propanediol could be biologically synthesized from cereals such as maize, millet, sorghum etc. Chemically synthesized PDO generally contain impurities from the chemical processes used to generate them. Many of such impurities are known to be harmful irritants and even toxic in some cases. In polyester synthesis, the use of chemically synthesized PDO and a chemically based diacid will not yield biodegradable polyester. These days, the concern for the disposal of non-degradable synthetic polymers has lead to increased interest in Biobased monomers and polymers which are biodegradable.

Biologically synthesized PDO has higher purity than chemically synthesized PDO. The production of PDO through fermentation was first discovered because of a major impurity in glycerol. In 1895, Noyes and Watkins discovered that glycerol that was unsuitable for certain applications contained greater than 1% of an unknown impurity. The onset of First World War and the need for high purity glycerol for trinitro glycerine for explosives made the identification of the impurity a major concern. Voisenet identified the compound as PDO in 1914 and it was determined to be product of anaerobic fermentation of the glycerol.

PDO could be prepared from hydrocarbons such as glycerol, reacted in the presence of carbon monoxide and hydrogen over periodic table group catalyst (US Patent 6,140,546; US patent 6,284,930).

Some conventional chemical routes to PDO include The Degussa process which consists of the following three steps:

1. Oxidation of propylene to acrolein

$$CH_2 = CH\ CH_3 + O_2 \longrightarrow CH = CH\ CHO$$

2. Selective hydration to 3-hydroxypropionaldehyde (3-HPA)

$$CH_2 = CHCHO + H_2O \longrightarrow OCHCH_2CH_2OH$$

3. Catalytic hydrogenation to 1, 3-propanediol (PDO)

$$OCH\ CH_2CH_2OH + H_2 \longrightarrow HO\ (CH_2)_3\ OH$$

Shell chemical uses a two step process:

1. Hydroformylation of ethylene oxide to 3-HPA

$$OCH_2\ CH_2 + CO + H_2 \longrightarrow OCHCH_2\ CH_2OH$$

2. Catalytic hydrogenation to 1, 3-propanediol (PDO)

$$OCHCH_2\ CH_2\ OH + H_2 \longrightarrow HO\ (CH_2)_3\ OH$$

These methods, apart from generating waste streams are expensive. As a result, several processes for biological production of PDO have been reported (EP 1204755; EP 107, 6708; WO 0112833; WO 0111070).

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Glucose from cereals such as maize and sorghum could be converted to glycerol using dual enzyme technique. Bacterial strains which are able to convert glycerol into 1, 3-propanediol are found in the species of *Klebsiella*, *citrobacter*, *clostridium* and *lactobacillus*. The conversions of glucose to PDO occur in nature in two stages; first by yeast to an intermediate product, glycerol then by bacteria to PDO or 3G.

Kurian *et al*. (2005) utilized maize as their renewable sugar source for PDO synthesis. They reported the use of genetically engineered *Klebsiella pneumonia* and *E-coli* for the conversion of glycerol to PDO. In this research, sorghum has been used as the renewable sugar source. There has been no report on the use of sorghum for PDO synthesis. *Klebsiella* species and *E-coli* have been used in this work. Sorghum is a genius of numerous species of grasses which belong to the family poaceae. The most popular specie is sorghum bicolor.

In Nigeria and South Africa, sorghum is industrially used for the production of lager beer (Doggett, 1988; Dufour *et al*., 1992). Sorghum grain comes in three major varieties: white, yellow and red. Agu *et al*. (1995) reported that the white variety has better brewing properties. Sorghum starch has been successfully applied for the production of bio-ethanol. In India and other places, sweet sorghum stalks are used for producing bio-fuel.

MATERIALS AND METHODS

Preparation of starch: White sorghum purchased from a local market was sorted and the foreign bodies and half grains removed. It was winnowed to remove the husks and dusts. The clean grains were steeped for twelve (12) hours changing the water every six (6) hours. The steep water was drained off, crushed and soaked in fresh-water. The germs which float were skimmed off. The endosperm was then milled to amorphous sized particles and sieved with nylon cloth to separate the raw starch from the fiber.

The starch was then allowed to settle and the clear water skimmed off. The starch was then spread evenly on a thermos hot air oven trays and dried overnight at sixty five (65) degree Celsius. The dried starch sample was used for the work.

Synthesis of 1, 3-Propanediol (PDO): 50 grams of starch was dissolved in 450 ml of process water. pH of the slurry was checked with a Hanna digital table pH meter and adjusted to 6.4 with 1 gram of calcium hydroxide. 0.2 grams of calcium sulphate was added and the starch hydrolyzed using dual enzyme conversion technique by the infusion-decoction method of mashing. 2 mls of protease was pipetted into the mash and its temperature maintained at 50°C for the protein content of the mash (if any) to be converted to amino acids. 2 mls of termamyl (a bacterial alpha amylase) was pipetted into it. The mash was heated up gradually to 95°C. While raising the mash temperature to 95°C, the starch gelatinized and the gel liquefied by the bacterial alpha amylase added.

At 95°C, the mash was rested for 5 min for the liquefaction to be completed.

200 mls of chilled process water was added gradually into the mash, reducing the mash temperature to 60°C. The pH was adjusted to 5.6. The mash was rested at 60°C for 15 min. Within this period of rest the rate of starch degradation to simple sugar was monitored. After saccharification, the mash was heated up to 78°C and rested at that for 3 min for the exogenous enzymes used to be inactivated. It was then filtered, cooled to 20°C and the original gravity measured with a saccharometer. It was cooled further to 10°C and after measuring the volume, it was pitched with yeast slurry (saccharomyces uvarum) and fermented at 37°C for 72 h under constant shaking with orbital shaker. The fermented "must" or" wort" as it is called was then filtered and inoculated with cultures of *Escherichia Coli* (*E. coli*) and Klebsiella specie. The entire mixture was stirred with orbital shaker set at 150 rpm for 72 h. At the end 72 h of agitation, the PDO synthesized was filtered. The filtrate was then distilled out with heat supplied by a heating mantle and the PDO condensed with a spiral condenser. The distillate was cooled further to 20°C and its specific gravity measured with a 25 ml specific gravity bottle.

Characterization of 1, 3-Propanediol

Boiling point: This was determined using fusion tube attached to a thermometer. The temperature is noted at the point the liquid begins to boil.

Gravity: This was determined using a 20 to 30cp Saccharometer.

Specific gravity: Specific gravity bottle was employed for the determination of gravity.

Determination of purity: Determination of purity of PDO was done using ultraviolet spectrophotometric method of world intellectual patent organization WIPO, 2008. The spectrophotometer was set at different wavelengths namely; 220 nm, 240 nm and 275 nm. The samples were read and the optical density noted.

Table 1: Purity of the samples under different wavelengths

Wavelengths (nm)	Expected absorbance	Absorbance observed
220	<0.200	-0.291
250	<0.075	-0.294
275	<0.075	-0.298

RESULTS AND DISCUSSION

The boiling point of PDO synthesized was found to be 121-124°C. This is comparable with 1, 3-Propanediol synthesized from maize (122°C). The gravity was found to be 24.34° P. Both gravity and specific gravity determines the solid content of the PDO sample before and during fermentation. They measure the level of solid in the sample that is available for fermentation. The specific gravity was 0.9992. The purity of the sample as read under different wavelengths is given in Table 1.

These values of absorbance observed helps to confirm the purity level of PDO synthesized. Sorghum-based PDO has similar properties with maize based PDO. Sorghum based PDO has been found to be of comparable purity to chemically synthesized PDO with purity level of 99.96%. White sorghum has been found to give better appearance (colour) than red and yellow varieties. Sorghum is commercially available in Nigeria and the prize is affordable. Therefore, the study recommends the use of sorghum in place of maize which has various uses and exorbitant in price, in biological synthesis of 1, 3-Propanediol.

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