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# Bioconversion of waste glycerol to 1,3-propanediol and its application

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## ABSTRACT

The excess waste glycerol generated during biodiesel production leads environmental concern, as it cannot be disposed of in the environment. The waste glycerol was utilized as carbon source for microbial growth. In this present study, bioconversion of crude glycerol was carried out using aerobic microorganisms. Pseudomonas was isolated to obtained 1,3-propanediol (1,3-PDO) from waste glycerol using bioconversion method. 1,3-PDO was used for synthesize unsaturated polyester resin using maleic anhydride and phthalic anhydride in the presence of zinc acetate, zinc chloride and p-toluene sulfonic acid (PTSA) catalyst. Resulting 1,3-PDO and polyester resin were characterized by Infrared spectroscopy and Gas chromatography.

## KEYWORDS

Bioconversion;
Pseudomonas;
Waste glycerol;
1, 3-propanediol;
Unsaturated polyester.

## INTRODUCTION

Glycerol is a colourless compound which contains three hydroxyl groups in its structure. It is generally miscible with all alcohols, ethyl acetate and dioxane. It is immiscible with ether, benzene and chloroform. Glycerol finds applications in the pharmaceutical, paint, automotive, cosmetic, food, tobacco, leather, textile, paper and pulp industries[1-3].

The glycerol is a renewable resource produced in a constantly increasing amount as a by-product from fat processing, ethanolic fermentation of glucose and rapidly developing biodiesel industry, becomes a serious problem and the massive glycerol production also forces a collapse in its market price[4]. Biodiesel is a very important product for now and a future aspect, but the production cost of biodiesel is very high and disposal of this waste glycerol may add some additional cost. As the petroleum resources are decreases day by day, the use of biodiesel may helpful not only in protection of environment but also alternative for petroleum products[5,6]. If the waste glycerol from biodiesel industry shall be utilized for further application then cost cutting in biodiesel production is possible which may lead to make use of biodiesel as substitute of petrol and diesel[7,8]. Crude glycerol can be use to obtain various products like 1,2-propanediol, 1,3-propanediol succinic acid, ethanol, xylitol, propionate. The same can be carried out by chemical as well as biological route[9-12]. During chemical synthesis of 1,3-PDO high energy consumption and expensive catalysts like Ag, Ir and Cr are required which leads to high costs of 1,3-PDO production[13,14]. An attractive alternative for chemical synthesis is a bioconversion of waste glycerol to 1,3-PDO[11,12]. The microbial route carried out at or slightly above room temperature and atmospheric pressure[15,16]. Bioconversion of waste glycerol into 1,3-propanediol can be carried out using microorganisms like yeast and E. Coli in aerobic as well as anaerobic conditions.

1, 3-propanediol can be formulated into composites, adhesives, laminates, powder coating, UV-cured coatings, mouldings, solvents and as an anti-freeze agent[17,18]. It can be used in manufacturing of polyester, polyurethane and polyol. 1,3-propanediol can be utilized for development of a biodegradable polymer poly (trimethylene terephthalate), which having unique physiochemical properties in the fiber industry and other applications in cosmetics, foods, lubricants and medicines[19,20].

There so in the present work bioconversion of waste glycerol into 1,3-PDO was carried out using Pseudomonas and the resulting 1,3-PDO was utilized for the synthesis of unsaturated polyester resin. 1,3-PDO was characterized by FTIR and gas chromatography (G.C.).

## MATERIALS AND METHODS

### Materials

Phthalic anhydride, maleic anhydride, zinc acetate, p-toluene sulfonic acid (PTSA), zinc chloride, potassium hydroxide (KOH), hydroquinone and neutrient broth medium were purchased from Sigma Aldrich while solvents viz. Xylene, chloroform, methanol and styrene were purchased from Merck India Private Ltd. Any other solvent were used of A.R grade.

### Methods

#### Bioconversion of waste glycerol to 1, 3-propanediol

Bioconversion of waste glycerol was carried out using Pseudomonas. The neutrient broth medium (25gm) (composition shown in TABLE1) and waste glycerol (30, 40, 50 and 60gm) was dissolved in 1liter distilled water. The prepared medium was sterilized in an autoclave at 121$^{0}$C at 15lb/in$^{2}$ for 30 minutes. Pseudomonas was added in each 250 ml flasks containing 150 ml medium. The flask was incubated at 30$^{0}$C for 3,6,9,12,15,18,21….105,108,114hrs.

**TABLE 1 : Nutrient broth medium composition**

| No. | Composition | Gms/liter |
|---|---|---|
| 1. | Peptone | 10 |
| 2. | Beef extract | 10 |
| 3. | Sodium chloride | 05 |

#### Separation

The biomass was separated by centrifugation at 5000rpm. Then 1,3-PDO was isolated by chloroform as solvent in extraction process. The chloroform was recovered by distillation.

#### Gas chromatography

The resulting 1,3-PDO was characterized by Gas chromatograph (GC) using Perkin Elmer auto system XL instrument using capillary column.

#### Synthesis of polyester resin

Phthalic anhydride (7.40gm), 1, 3-propanediol (8.97ml), catalyst (0.2gm) and xylene (40ml) as solvent were added in three neck flask. The temperature of reaction mass was raised to 130$^{0}$C till the reaction mass becomes clear. At this stage maleic anhydride (4.90gm) was added and the temperature was raised to 180$^{0}$C. The reaction carried out until acid value was reached to below 25. Water formed was continuously removed using azeotropic distillation using xylene as solvent. Xylene was recovered at the end of reaction by distillation. The product was allowed to up to cool 160$^{0}$C, and then hydroquinone (25mg) was added as inhibitor. The further cooling was carried out up to 140$^{0}$C, 30% styrene was added as diluent.

#### Acid value determination

Acid value of polyester was determined at regular interval. Pre-weigh sample of polyester was dissolved in acetone and titrated against standard alcoholic KOH solution, using phenolphthalein indicator.

$$\text{Acid value} = \frac{\text{B. R. X N X 56.1}}{\text{Weight of the sample}}$$

Where, N = Normality of the alcoholic KOH solution.

#### FTIR spectroscopy

The polyester was characterised by FTIR spectroscopy using Perkin Elmer spectrum GX instrument.

## RESULT AND DISCUSSION

Bioconversion was carried out with 30gm/L, 40gm/L, 50gm/L and 60gm/L glycerol concentration for 3,6,9,12,15,18,21….105,108,114hrs using pseudomonas strain. Optical density (OD) was kept constant at 1.0. Among various concentrations, 50gm/L glycerol concentration in nutrient broth medium was give the best

TABLE 2 : 1, 3-PDO yield

| Sr. No. | Hrs | Yield(mol/mol) | | | |
|---|---|---|---|---|---|
| | | 30gm/L | 40gm/L | 50gm/L | 60gm/L |
| 1 | 3 | 0.010 | 0.033 | 0.047 | 0.050 |
| 2 | 6 | 0.011 | 0.034 | 0.049 | 0.051 |
| 3 | 9 | 0.089 | 0.093 | 0.094 | 0.094 |
| 4 | 12 | 0.100 | 0.105 | 0.109 | 0.111 |
| 5 | 15 | 0.120 | 0.125 | 0.124 | 0.127 |
| 6 | 18 | 0.152 | 0.153 | 0.157 | 0.160 |
| 7 | 24 | 0.160 | 0.162 | 0.170 | 0.171 |
| 8 | 30 | 0.200 | 0.210 | 0.221 | 0.221 |
| 9 | 36 | 0.256 | 0.260 | 0.290 | 0.293 |
| 10 | 42 | 0.264 | 0.269 | 0.331 | 0.332 |
| 11 | 45 | 0.270 | 0.275 | 0.354 | 0.354 |
| 12 | 48 | 0.287 | 0.292 | 0.384 | 0.383 |
| 13 | 51 | 0.300 | 0.310 | 0.403 | 0.401 |
| 14 | 57 | 0.320 | 0.324 | 0.438 | 0.439 |
| 15 | 60 | 0.325 | 0.329 | 0.445 | 0.445 |
| 16 | 66 | 0.332 | 0.335 | 0.488 | 0.490 |
| 17 | 69 | 0.353 | 0.351 | 0.498 | 0.498 |
| 18 | 72 | 0.390 | 0.400 | 0.514 | 0.514 |
| 19 | 75 | 0.390 | 0.400 | 0.513 | 0.512 |
| 20 | 81 | 0.391 | 0.399 | 0.514 | 0.513 |
| 21 | 90 | 0.391 | 0.399 | 0.510 | 0.512 |
| 22 | 99 | 0.390 | 0.400 | 0.514 | 0.513 |
| 23 | 105 | 0.391 | 0.398 | 0.514 | 0.512 |
| 24 | 108 | 0.391 | 0.400 | 0.514 | 0.514 |
| 25 | 114 | 0.391 | 0.400 | 0.514 | 0.513 |

1,3-PDO yield as shown in TABLE2. During initial hours low yield of 1,3-PDO were obtained in case of all glycerol concentration which was used. Optimum yield was obtained during 72hrs of bioconversion process, while almost same yield was noted in further hours. 30gm/L and 40gm/L glycerol concentration in nutrient broth medium were give lower yield compare to 50gm/L. While using 60gm/L concentration almost same yields were obtained as obtained using 50gm/L concentration. So it was concluded that during 72hrs process, 50gm/L concentration of waste glycerol gives best results which were tabulated in TABLE2.

## Effect of reaction time

Effect of reaction time on the synthesis of polyester was optimized. High acid value was obtained during 8hrs reaction time. If the reaction time was increased low acid value was obtained using zinc acetate, zinc chloride and PTSA as catalyst. Almost same acid values were obtained during 12hrs and 15hrs reaction time so 12hrs reaction was economical. In the case of zinc acetate catalyst low acid value was obtained compare to other catalyst. The results were tabulated in TABLE3.

TABLE 3 : Unsaturated polyester resin

| No. | Raw Materials | Mole Ratio | Catalyst | Reaction Time (Hours) | Acid Value |
|---|---|---|---|---|---|
| 1 | Phthalic anhydride, 1,3-PDO, Maleic anhydride, Xylene, catalyst | 0.50:1.25:0.50 | Zinc chloride | 8 | 90.23 |
| | | | | 12 | 55.27 |
| | | | | 16 | 54.78 |
| | | | Zinc acetate | 8 | 76.63 |
| | | | | 12 | 47.08 |
| | | | | 16 | 46.94 |
| | | | PTSA | 8 | 86.35 |
| | | | | 10 | 51.90 |
| | | | | 15 | 50.02 |
| 2 | Phthalic anhydride, 1,3-PDO, Maleic anhydride, Xylene, catalyst | 0.50:1.25:0.50 | Zinc acetate | 12 | 39.27 |
| 3 | Phthalic anhydride, 1,3-PDO, Maleic anhydride, Xylene, catalyst | 0.50:1.25:0.50 | Zinc acetate | 12 | 24.60 |
| 4 | Phthalic anhydride, 1,3-PDO, Maleic anhydride, Xylene, catalyst | 0.50:1.25:0.50 | Zinc acetate | 12 | 18.34 |

## Effect of catalyst

Effect of catalyst on the synthesis of polyester was optimized and the results were tabulated in TABEL3. Catalyst plays a very important role for any reaction. Zinc acetate, PTSA and zinc chloride were used as a catalyst for the unsaturated esterification process. Low acid value (18.34) was obtained using zinc acetate as catalyst during 12hrs reaction time. While in case of other catalyst comparatively higher acid value were obtained. So zinc acetate proves best as a catalyst for manufacturing unsaturated polyester resin.

## Gas chromatography

The chromatograph is shown in Figure A. The sample was run into the GC instrument. Peak for 1,3-PDO was observed at 5mV (12 min). As chloroform was used as solvent for sampling in GC, its peak was

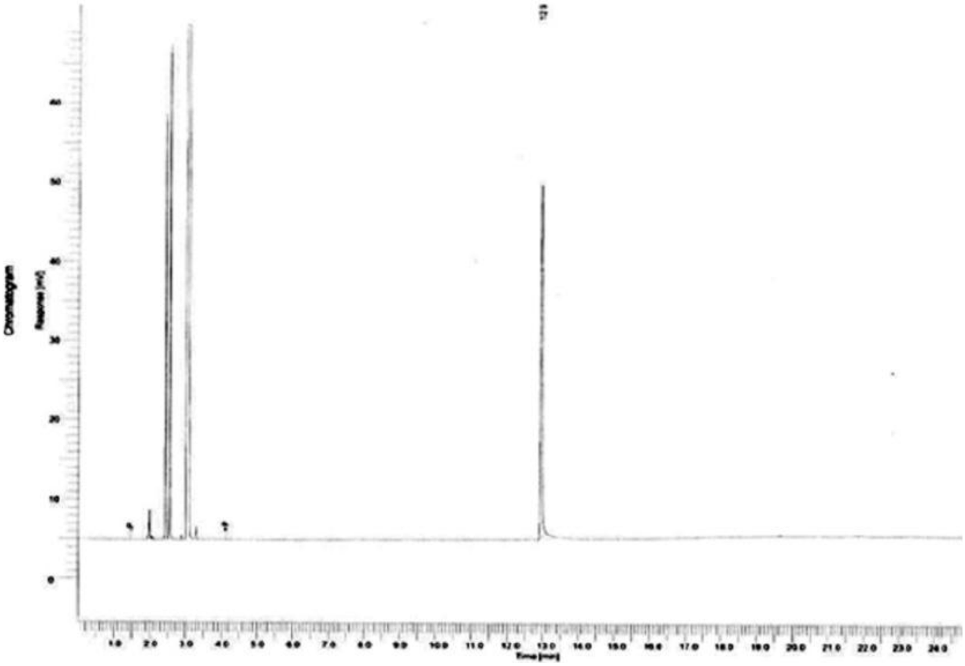

Figure 1 : Gas chromatography

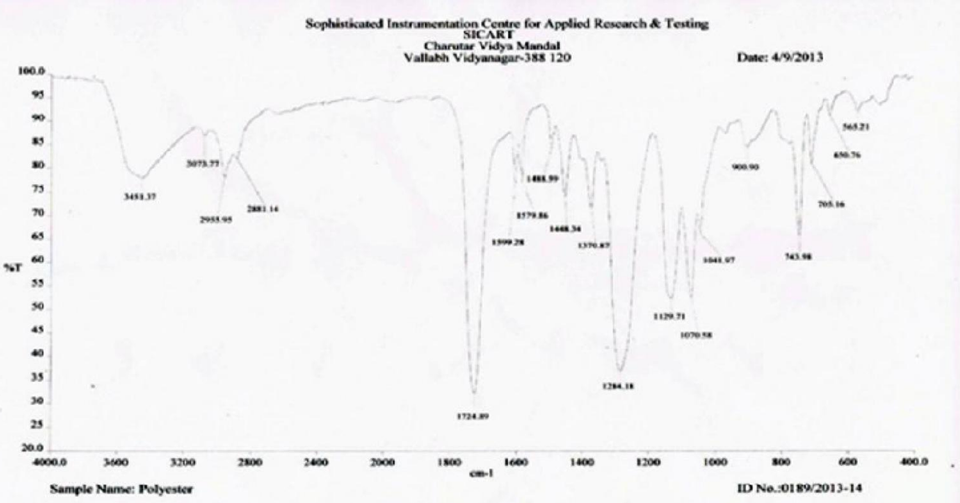

Figure 2 : FTIR spectroscopy

observed at 70mV (2-4min).

### FTIR spectroscopy

The FTIR spectrum of polyester resin is shown in Figure B. The main structure of the polyester resin sample had ester, aromatic ring, alcohol and anhydride because they may remain unreacted in the sample. The strong vibration observed at 900.90 cm$^{-1}$ which confirms the present of benzene group. The C=O streching observed at 1724.89 cm$^{-1}$ which confirms the carbonyl group. The bending observed at 705.16 which confirms the C=C group. Due to meta substituted benzene deformation, vibration observed at 705.16 & 743.98 cm$^{-1}$. While incorporation of 1,3-PDO into polyester confirms by strong absorption band at 1070.58 cm$^{-1}$.

## CONCLUSION

Waste glycerol obtained during the biodiesel production, was converted to the 1,3-PDO using Pseudomonas strain. Best results were obtained in 72hrs process with 50gm/L glycerol concentration. This 1,3-PDO was utilized in the unsaturated polyester resin manufacturing.

In polyester manufacturing, there are so many parameters that have to keep in mind like the molar ratio of monomers, reaction temperature and time, catalyst etc., to be best unsaturated polyester manufacturing.

So from result, best parameters for manufacturing unsaturated polyester were, 0.5:1.25:0.5 molar ratios of phthalic anhydride, 1,3-propanediol and Maleic anhydride in the presence of zinc acetate catalyst for 12 hours at $180^0$ C where the 18.34 acid value was obtained.

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