SYNTHESIS AND CHARACTERIZATION OF POLYOL BY BIOCONVERSION OF GLYCEROL

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OBJECTIVES

The core objective is the bioconversion of glycerol

The other objectives are

- Uses of E-coli, enterobacter aerogen and pseudomonas etc. for bioconversion of glycerol
- Separation of biologically converted products
- Characterization and use of 1,3-PDO for preparation of polyol
- Optimization of reaction parameter

INTRODUCTION

Glycerol was first discovered by Karl Wilhelm Scheele. He synthesized and characterized many other chemical compounds such as tartaric acid, citric acid and lactic acid. Glycerol is also known as 1,2,3-propanetriol as it contains three hydroxyl groups there so, it is also termed as polyol compound. It is the principal by- product obtained during trans-esterification of vegetable oils [1, 2, 3]. Glycerol is completely soluble in water and alcohol and slightly soluble in ether, ethyl acetate, and dioxane. It is insoluble in hydrocarbons. It has useful solvent properties which are similar to water and simple aliphatic alcohols [4, 5]. About 0.8 billion gallons glycerol was produced in India till 2013[6]. Glycerol is synthesized by various methods from propylene via acrolein route, via allyl chloride route, in fat splitting, saponification, ethanolic fermentation of glucose and major in biodiesel production.

Biodiesel is produced by transesterification of vegetable oils with methanol using sodium hydroxide as catalyst. Biodiesel is a mixture of methyl ester and fatty acids. Biodiesel can be used in the diesel engine motors. Germany is the largest producer and consumer of biodiesel in the world, which produces more than 2.5 billion litres annually [7]. Many countries use biodiesel as an admixture to diesel with different proportions. Brazil used 2% biodiesel till January 2008, which is now increasing to 5%. There are two reasons on the basis of which Brazil will become a major producer and consumer of biodiesel: Brazilian used alcohol in fuel cars since long and

second, the conditions for cultivating oleaginous plants are extremely favourable in many areas of the Brazil [8]. The availability of petroleum is limited in the future, so biodiesel use will continually grow. In 2010, the gradual declining of petroleum production was started, and it assumes petroleum reserves may completely deplete by 2050. On the other hand, the demand of biofuel is rising worldwide[9].

A major by-product of biodiesel industry is glycerol which is commonly known as glycerine. Glycerol is a trihydric alcohol, miscible with water, ethyl acetate and dioxane while immiscible with chloroform, benzene and ether. It is a colourless, odourless, viscous and hygroscopic liquid with a high boiling point. Pure glycerol is a versatile product and readily compatible with other substances. Glycerol finds applications in the pharmaceutical, paint, automotive, cosmetic, food, tobacco, leather, textile, paper and pulp industries.

The glycerol is a renewable resource produced as by-product in fat processing, ethanolic fermentation of glucose and biodiesel industry in a constantly increasing amount. Among which biodiesel industry have glut of crude glycerol as by-product which results in a serious environmental and disposal problems. Approximately 12.2 million metric ton biodiesel is produced which generates 1.22 million metric ton crude glycerol. The massive glycerol production also forces a collapse in its market price. On the other side, demand of petrol and diesel as a fuel in world is increasing day by day while the petroleum resources are decreases continuously. Fuel crisis has been affected the worldwide economy. In the present scenario, biodiesel which is obtained from 100% renewable resources provides an alternative fuel option for future. The biodiesel is a very important product for now and a future aspect and use of it helpful in protection of environment. The crude glycerol from biodiesel process can be utilized for further synthesis or application then biodiesel may available in economic price [22].

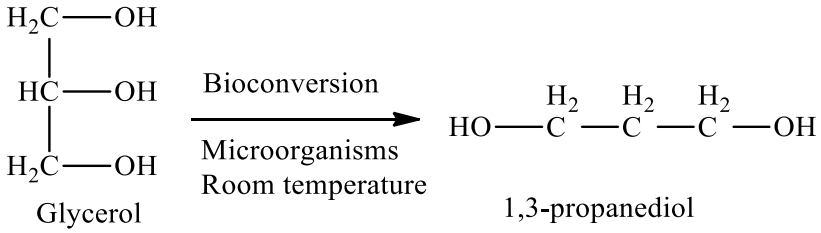
Crude glycerol obtained from the biodiesel industry contains impurity such as methanol, fatty acid and salt. Purification of crude glycerol can be done by distillation method. But this method is quite costly if it is compared to the production cost required for traditional synthesis of glycerol. This technology produces high purity glycerol at high yields. But the distillation of glycerol is an energy intensive process because of its high heat capacity and required a high supply of energy for vaporization[10]. Ion exchange has also been used to purify raw glycerol, but this technique is not economically viable from an industrial view of point due to the high content of salts present in crude glycerol [11].

Pure glycerol is required for utilizing in different application viz. in food, drugs, creams, tobacco processing, wrapping and packaging of materials, pharmaceutical industry, gaskets and cork products, as lubricants. As glycerol is obtained as a by-product in the production of biodiesel and it is assumed that by the year of 2020, production of glycerol will reach six times

more [12]. The massive glycerol production forces a collapse in its market price and currently the market price of glycerol is reached to 60/-Rs. per Kg.

A company like Dow Chemicals, Procter and Gamble closes their glycerol producing facilities. Therefore, alternative uses of glycerol are required. It can be utilized for combustion, animal feeding, thermo-chemical conversions, composting and biological conversion methods. The combustion of crude glycerol has been used for disposal. But, this method is not economical for large producers of biodiesel [13]. The process also generates the toxic greenhouse gases like CO and CO_2, which also have an adverse effect to the atmosphere and living organisms. It has also been suggested that glycerol can be composted or used to increase the biogas production of anaerobic digesters but it requires only 1% glycerol so this method is not solution for disposal[14]. Biodiesel-derived glycerol was fed to dairy cows in order to prevent ketosis, but found that it was not useful. Glycerol can be thermochemically converted into propylene glycol. In which Raney nickel catalyst was used at 230^0C [15].

Crude glycerol can be converted to variety of products such as 1,3-propanediol (1,3-PDO), 1, 2-propanediol, succinic acid, ethanol, butanol using chemical and biological method. Especially when desired product is 1,3-PDO, it can be produced chemically by hydration of acrolein. But it required high energy consumption, toxic intermediates and expensive catalysts like Ag, Ir and Cr are required which leads to high costs of 1,3-PDO production. An attractive alternative for chemical synthesis is a bioconversion of waste glycerol to 1,3-PDO.The microbial route carried out at or slightly above room temperature and atmospheric pressure. Bioconversion of waste glycerol into 1, 3-propanediol can be carried out using microorganisms like Escherichia coli, Bacillus species, Pseudomonas, Enterobacter aerogen, Clostridiumbutyricum and Citrobacterfreundii in aerobic as well as anaerobic conditions. Glycerol is also used in the bioconversion process to obtain various products such as1,3-propanediol, ethanol, citric acid and succinic acid. These products also obtained by chemical synthesis too [16, 17].



When the desired product is 1,3-PDO, it can be produced chemically by two methods: the hydration of acrolein and the hydroformylation of ethylene [18]. Chemical synthesis of 1,3-PDO requires high energy consumption, toxic intermediates like 3-hydroxyl propyonaldehyde, expensive catalysts like Ir, Cr and Ag which leads to high costs of 1,3-PDO production. More than 0.1 million tons of 1,3-PDO are produced every year [19]. Currently, more than 2 million tons 1,3-PDO produced [20]. Consequently, chemical synthesis is

expensive, thus, 1,3-PDO still has a low market volume. Due to the environmental benefits and use of a renewable feed stock, the bioconversion of glycerol to 1,3-PDO is an attractive alternative to chemical synthesis[21]. Bioconversion of crude glycerol from the biodiesel process to value-added products is a driver towards higher cost efficiency of biodiesel production. Glycerol can be used by different microorganisms as an energy source. Microorganisms have the potential use in bioconversion of crude glycerol produced from biodiesel. During industrial fermentation processes, glycerol can be used as a substitute for carbohydrates, such as sucrose, glucose and starch. Bioconversion of glycerol adds significant value to the productivity of the biodiesel industry [22].

Bacterial fermentation has been known for almost 120 years in which glycerol is converted to 1,3-PDO. The 1,3-PDO is the main product obtained through bioconversion of glycerol. 1,3-PDO is the oldest fermentation product and was first observed as a product in 1881 in fermentation of glycerol. Then in 1914, production of 1,3-PDO by Bacillus spices was described. Microbiology School of Delft was analysed 1,3-PDO using different Enterobacteriaceae in 1928. The 1,3-PDO is an emerging speciality chemical. 1,3-PDO can be used to produce polyesters, polyethers and polyurethanes. It is also used as a solvent and lubricant [23].

The bioconversion of glycerol to 1,3-PDO has been demonstrated for several bacteria, such as E. coli, Pseudomonas, Lactobacillus, Citrobacter freundii, Klebsiella pneumonia, Clostridium pasteurianum(C. pasteurianum), Ennterobacteragglomerans (E. agglomerans) and E. aerogen. As an additional reducing equivalent required so complete conversion of glycerol to 1,3-PDO is not possible [24]. Glycerol is converted to 1,3-PDO by two steps, using any of the microorganisms. The first one is the conversion of glycerol to 3-hydroxypropionaldehyde and water and then 3-hydroxypropionaldehyde is reduced to 1,3-PDO by NAD^+ linked oxido-reductase . The production of 1,3-PDO from glycerol is carried out under aerobic as well as anaerobic conditions where glycerol is used as a carbon source, In Citrobacter, Klebsiella and Clostridium strains, a parallel pathway for glycerol conversion is used. In which glycerol is oxidized to dihydroxyacetone (DHA) by NAD^+ followed by phosphorylation of the dihydroxyacetone gives dihydroxyacetone phosphate. This is an oxidative pathway [25].

Bioconversion of crude glycerol provides substrates for the production of biodegradable polymers which directly benefit to the environment. An interesting example is a polytrimethylene terephthalate (PTT) production in which 1,3-PDO is used as monomer. PTT has unique physiochemical properties in the fibre industry and other applications in cosmetics, foods, lubricants and medicines. Also 1, 3-PDO can be formulated into laminates, composites, adhesives, powder coating and as an anti-freeze agent. It can be used in manufacturing of polyol polyester and polyurethane [26].

The polyol is a polyhydroxy compound. It is an important building block of polyurethanes and polyesters that are useful in wide range of applications such as construction, coatings agents, adhesives, sealants, elastomers, resins as well as in food science and polymer chemistry. Polyol are traditionally produced from petroleum. However, the production of polyols from petrochemicals is costly, requires a great deal of energy and also has adverse effects on the environment. Research in recent years has thus focused on alternative, non-petroleum based sources of polyols that are renewable, less costly and more ecofriendly. The bio-polyols synthesized from 1,3-propanediol are an attractive alternative for this purpose and has therefore drawn considerable current attention [27-30].

Polyol is the second primary component of a polyurethane formulation. In the polymer chemistry, polyol are polymers or monomers with hydroxyl functional groups available for organic reactions. Polymeric polyol may be polyethers, such as polyethylene glycol, polypropylene glycol or polytetrahydrofuran. Another class of polymeric polyol is the polyesters [31]. A specialist class of polyol is the hydroxyl-terminated polybutadienes. When polyol are combined with many different additives or materials according to a formulation, the resulting polyol is called a polyol blend [32].

Polyester polyol based on aliphatic and aromatic dicarboxylic acids are valuable materials in polymer technologies. Among them are low molecular weight oligomeric derivatives of phthalic and terephthalic acids that are widely used in high strength and rigidity polyesters and polyurethane foams. The use of aromatic acids offers many advantages to polymer properties including good mechanical characteristics, high thermal stability, resistance to major chemical solvents, and low flammability [33-35].

Terephthalate-based polyester polyol are readily prepared by the reaction of terephthalic acid (TPA) with glycols, such as diethylene glycol (DEG), at temperatures above 220^0C. This equilibrium process involves esterification reactions with evolving water, hydrolysis of ester links, and transesterification reactions and results in a complex mixture of oligomers with a wide range of molecular weights [36].

The polyol produced in India are glycols of high molecular weight of polyether, polyester and hydrocarbon types. Polyethene glycol is primarily produced to meet the demand of emulsifiers and surfactants and hardly any of it is used in the manufacture of polyurethane. But, 98% of the other polyethene polyol like polypropylene glycol are used in the production of flexible or rigid foams. Polybutadiene based polyol are made mainly for use as solid rocket propellant binders [37, 38].

Before 1950, Dai-Ichi Karkaria and Castrol (I) Ltd., were producing urethane grade polyol. Now, there are some more industries which have commenced manufacture of polyol for

polyurethane. These industries are Manali Petrochemicals Ltd, UB Petroproducts Ltd., Shivathene LinopackLtd. NOCIL. Malabar Polyols and Expanded Incorporation. Manali Petrochemicals and UB Petro products have started production of polyether polyol in 1990. There are the only two units in the country manufacturing polyol from the grass root level, using propylene as the raw material. Propylene is converted to propylene oxide and then into polyol, whereas all other units are vegetable oil based or else they start with propylene oxide [39, 40].

There so, in the present work transesterification of cottonseed oil was carried out with methanol to obtained crude glycerol. Then crude glycerol was converted into 1,3-PDO using E. coli, Pseudomonas, Enterobacter aerogen and the resulting 1,3-PDO was utilized for the synthesis of polyol as well as unsaturated polyester resin. 1,3-PDO was characterized by FTIR and gas chromatography (G.C.). Polyester and polyester polyol were characterized by FTIR spectroscopy.

MATERIALS AND METHODS

Materials

Cottonseed oil, sodium hydroxide and nutrient broth medium, tryptone soya broth medium, terephthalic acid, phthalic anhydride, maleic anhydride, zinc acetate, p-toluene sulfonic acid (PTSA), tin, zinc chloride, potassium hydroxide (KOH), hydroquinone and nutrient broth medium were purchased from Sigma Aldrich. Solvents viz. xylene, chloroform, methanol and styrene were purchased from Merck India Private Ltd. Other solvents and chemicals were used of A.R grade.

Transesterification of cottonseed oil

Transesterification of cottonseed oil is carried out by methanol and sodium hydroxide (1% based on cottonseed oil) at 65^0C for 2.5hrs. During the process methyl ester and crude glycerol was produced with 3:1 ratio. The reaction mass was cooled and transfer into separating funnel where it was allowed for layer separation. Two layers viz. lower layer rich in glycerol and upper layer rich in methyl ester are formed after 1hr. Lower layer is separated and neutralized by concentrated HCl to obtain crude glycerol while methyl ester is used for biodiesel application.

Bioconversion of crude glycerol to 1, 3-propanediol

1. **Escherichia coli**

Bioconversion of crude glycerol was carried out using E-coli in the minimal medium. The composition of minimal medium is shown in table: 1. Crude glycerol (60gm) was dissolved

in 1liter distilled water. The prepared medium was sterilized in an autoclave at 121^0C at 15lb/in^2 for 30min. E. coli strain was inoculated in 150ml autoclaved minimal medium at various temperature viz. 30^0C, 35^0C, 40^0C and 45^0C for 5, 10, 15, 20, 25, 30, 35, 40hrs.

Table: 1 Composition of Minimal medium

Sr. No.	Composition	Gms/litre
1.	K_2HPO_4	2.0
2.	KH_2PO_4	2.00
3.	$MgSO_4$	1.0
4.	$CaCl_2$	0.4
5.	$FeCl_3$	1.0
6.	NH_4NO_3	1.0

2. Pseudomonas

Bioconversion of waste glycerol was carried out using Pseudomonas in the nutrient broth medium. The composition of the nutrient broth medium (25gm) is shown in table: 2. Waste glycerol (30, 40, 50 and 60gm) was dissolved in 1liter distilled water. The prepared medium was sterilized in an autoclave at 121^0C at 15lb/in^2 for 30min. Pseudomonas was added in each 250ml flasks containing 150ml medium. The flask was incubated at 30^0C for 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39, 42, 45, 48, 51, 54, 57, 60, 63, 66, 69, 72, 75, 78, 81, 84, 87, 90, 93, 96, 99, 102, 105, 108, 114hrs.

Table: 2 Composition of Nutrient broth medium

Sr. No.	Composition	Gms/litre
1.	Peptone	10
2.	Beef extract	10
3.	Sodium chloride	05

3. Enterobacter aerogen

E. aerogen strain was inoculated in previously autoclaved Tryptone soya broth medium for 24hrs. The prepared pre-culture medium was then utilized for bioconversion of crude glycerol. The composition of tryptone soya broth medium (30gm) is shown in table: 3 and crude (50gm) glycerol was dissolved in 1liter distilled water. The prepared medium was sterilized in an autoclave at 121^0C at 15lb/in^2 for 30min. Pre-culture medium was added in each 250ml flasks containing 150ml medium. The flask was incubated at 200rpm at 25^0C, 30^0C, 35^0C and 40^0C for 2, 4, 6, 8, 10, 12days.

Table: 3 Composition of Tryptone soya broth medium

Sr. No.	Composition	Gms/litre
1.	Pancreatic digest of casein	17.0
2.	Papaic digest of soyabean meal	3.0
3.	Sodium chloride	5.0
4.	Dipotassium hydrogen phosphate	2.50
5.	Dextrose (glucose)	2.50

Separation

The biomass obtained using various strain was separated by centrifugation at 5000rpm for 20min. Then 1, 3-PDO was isolated by chloroform as solvent in extraction process. The chloroform was recovered by distillation.

SYNTHESIS OF POLYESTER POLYOL

Polyester polyol was prepared by reacting an excess of the stoichiometric amount of difunctional glycol with dibasic acid. The manufacture was carried out through a batch process.

A mixture of terephthalic acid and 1,3-propanediol in a molar ratio of 1:2 (a typical load: 200 -220gm) and 0.1% tin as catalyst were stirred at 225-230^{0}C in a three-necked flask equipped with a mechanical stirrer, thermometer, and a distillation head.

As the rate of distillation significantly decreased and the mixture became clear, further distillation was continued under reduced pressure (250-300mm Hg) until the lowest acid number was obtained.

SYNTHESIS OF POLYESTER RESIN

1,3-propanediol obtained from bioconversion of glycerol using various strains was utilized for polyester resin synthesis. 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 cool up to160^{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

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.

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

Where, N = Normality of the alcoholic KOH solution.

Gas chromatography

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

FTIR spectroscopy

The 1,3-PDO was characterized by FTIR spectroscopy using Perkin Elmer spectrum GX instrument.

RESULT AND DISCUSSION

Bioconversion of crude glycerol to 1,3-propanediol

1. E. coli

The results of bioconversion of crude glycerol using E-coli in the minimal medium are tabulated in table: 4.

Table: 4 Bioconversion of glycerol using E. coli

Sr. No.	Hours	Yield (gms/litre)			
		30^0C	35^0C	40^0C	45^0C
1.	5	1.4	2.1	7.3	4.4
2.	10	8.3	8.3	15.5	11.2
3.	15	18.2	18.2	25.8	20.7
4.	20	26.4	26.4	32.6	27.5
5.	25	30.9	30.9	35.9	33.7
6.	30	33.5	33.5	39.5	34.1
7.	35	34.7	34.7	39.5	34.2
8.	40	34.7	34.7	39.5	34.1

The presence of small amount of methanol and sodium hydroxide in the crude glycerol do not affect the bioconversion process. Also, the high pH due to presence of NaOH is helpful for the process. There so crude glycerol was used directly without refining process. Bioconversion was carried out with 50gm/L glycerol concentration at 30^0C, 35^0C, 40^0C and 45^0C for 5, 10, 15, 20, 25, 30, 35, 40hrs using E. coli strain. At 40^0C, highest 1, 3-PDO yield was obtained as shown in table: 4. Optimum yield was obtained during 30hrs of bioconversion process, while almost same yield was noted in up to 40hrs. Yield of 1,3-PDO was increases at certain as the bioconversion process time increases. At the end of 30hrs, at 35^0C, the bioconversion of crude glycerol gives optimum results.

2. Pseudomonas

The results of bioconversion of crude glycerol using Pseudomonas in the nutrient broth medium are tabulated in table: 5

Table: 5 Bioconversion of glycerol using Pseudomonas

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

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 1,3-PDO yield as shown in table: 5. 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 optimum results.

3. Enterobacter aerogen

The results of bioconversion of crude glycerol using Enterobacter aerogen in the nutrient broth medium are tabulated in table: 6

Table: 6 Bioconversion of glycerol using Enterobacter aerogen

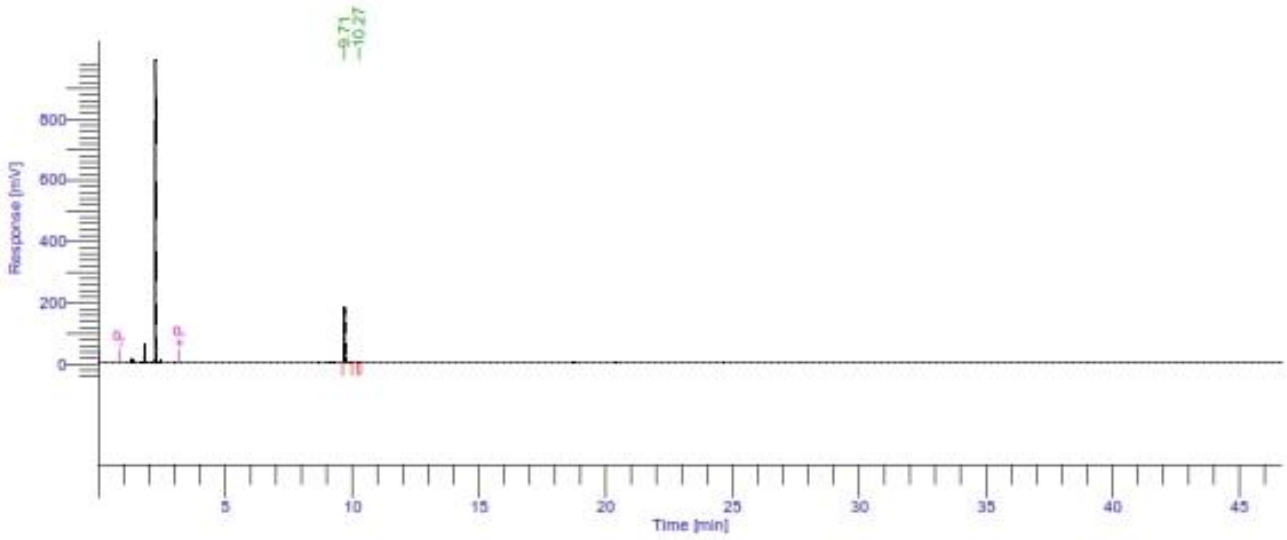
Sr. No.	Days	Yield(mol/mol)			
		25^0C	**30^0C**	**35^0C**	**40^0C**
1.	2	0.062	0.295	0.153	0.086
2.	4	0.112	0.310	0.205	0.133
3.	6	0.198	0.483	0.285	0.156
4.	8	0.278	0.580	0.362	0.202
5.	10	0.361	0.614	0.412	0.220
6.	12	0.361	0.614	0.412	0.221

The crude glycerol obtained during transesterification of cottonseed oil, contains impurities such as unreacted methanol and fatty acids. Generally excess methanol was used to drive the transesterification and if methanol was not recovered properly then it may present in glycerol layer in trace amount along with fatty acid. The crude glycerol contains unreacted methanol and sodium hydroxide in trace amount; do not affect the bioconversion process. So the cost of bioconversion process was decreased as crude glycerol was used directly without refining process. Bioconversion was carried out with 50gm/L glycerol concentration at 25^0C, 30^0C, 35^0C and 40^0C for 2, 4, 6, 8, 10, 12days using E. aerogen strain. Among various temperatures, at 30^0C, highest 1,3-PDO yield was obtained as shown in table:6. Optimum yield was obtained during 10days of bioconversion process, while almost same yield was noted in further days. Glycerol conversion was found maximum at 30^0C temperature while at 35^0C temperature lower yield obtained. Further increase in the bioconversion temperature (40^0C) gives the lowest yield due to

the thermal decomposition of biomass. There so, it is concluded that during 10days process at 30^0C, crude glycerol gives optimum results.

Gas chromatography

The chromatograph of 1,3 propane diol is shown in Figure A.



SICART

SHOPHISTICATED INSTRUMENTATION CENTRE FOR APPLIED RESEARCH AND TESTING

Peak #	Component Name	Time [min]	Area [uV*sec]	Height [uV]	Area [%]
1		9.705	475561.54	181695.39	98.88
2		10.265	5392.59	3177.63	1.12
			480954.13	184873.02	100.00

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Figure: A GC of 1, 3- PDO

The bioconversion product and their quality were determined using Gas Chromatograph using a PE-FFAP column. The sample was run into the GC instrument. The 98.88% purity of 1,3-PDO at 9.706min was observed.

FTIR spectroscopy of 1, 3-PDO

The FTIR spectrum of 1,3-PDO is shown in Figure B. The structure of the 1, 3-PDO contains primary alcohol and alkane substitution. The strong band observed between 2938.52 – 2885.54cm^{-1} due to C—H stretching frequency. The medium vibration observed at

1653.94cm^{-1} which confirms the present of C—C—C stretching. The —CH_2— bending observed at 1474.84cm^{-1}. Due to primary alcohol strong O—H bending vibration and strong C—O stretching vibration observed at 1062.29cm^{-1} and 1233.71cm^{-1}. So above FTIR spectrum confirms the 1,3-PDO.

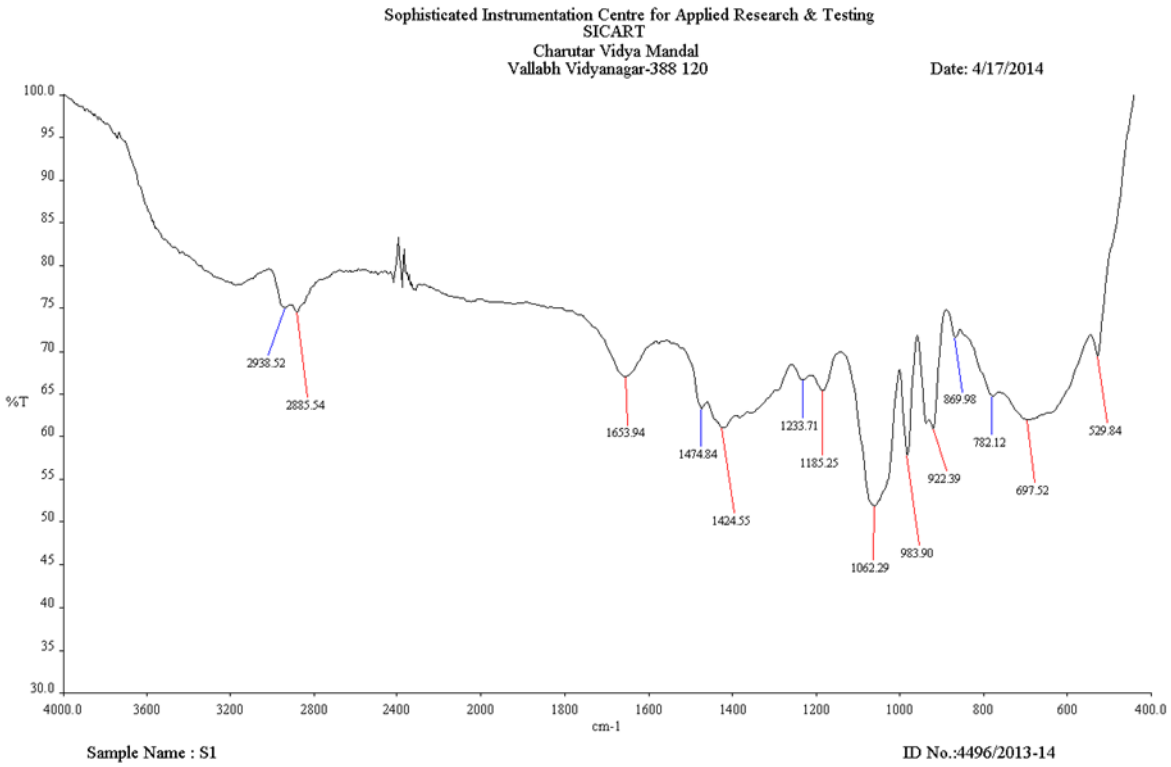

Figure: B FTIR spectra of 1,3 - PDO

1,3 – propane diol obtained after bioconversion of glycerol is used for the synthesis of

1. Polyester polyol
2. Unsaturated polyster

1. POLYESTER POLYOL

The result of optimization of parameters like reaction time, effect of catalyst are tabulated in table: 7

➢ Effect of reaction time

Effect of reaction time on the synthesis of polyester polyol was optimized. As the reaction equilibrium shifted towards the product side, the concentration of acid reactant present in raw material decreased and the acid value of the reaction mass also decreased. There so, the measurement of acid value gives idea regarding the reaction proceeding towards product side. In

another word as the amount of product in the reaction mass increases, the acid value of reaction mass decreases.

The results of effect of reaction time were tabulated in table: 7. High acid value was obtained during 8hrs reaction time. If the reaction time was increased low acid value was obtained using tin, zinc chloride and PTSA as catalyst. There was negligible decrease in the acid value for 20hrs reaction time. There so, 16hrs reaction was economical. In the case of tin catalyst low acid value was obtained compare to other catalyst.

TABLE: 7 Polyester polyol

Sr. No.	Raw Materials	Mole Ratio	Catalyst	Reaction Time (Hours)	Acid Value
1	Terphthalic acid, 1,3-PDO, Xylene, catalyst	1.0:2.0	Zinc chloride	8	89.23
				12	65.21
				16	62.88
				20	63.34
			Tin	8	81.73
				12	50.18
				16	40.76
				20	40.33
			PTSA	8	90.54
				12	62.84
				16	48.02
				20	47.91
2	Terphthalic acid, 1,3-PDO, Xylene, catalyst	1.0:2.0	Tin	16	32.51
3	Terphthalic acid, 1,3-PDO, Xylene, catalyst	1.0:2.0	Tin	16	21.67
4	Terphthalic acid, 1,3-PDO, Xylene, catalyst	1.0:2.0	Tin	16	12.32

- **Effect of catalyst**

Effect of catalyst on the synthesis of polyester polyol was optimized and the results were tabulated in table: 7. Catalyst plays a very important role for any reaction. Tin, PTSA and zinc chloride were used as a catalyst for the process. Low acid value was obtained using tin as catalyst during 16hrs reaction time. While in case of other catalyst comparatively higher acid value were obtained. So, tin proves one of the best as a catalyst for manufacturing polyester polyol.

- **Effect of Temperature**

During the preparation of polyester polyol process, temperature plays a very important role. If the temperature was kept around 250^0C than product may thermally decompose. More specifically at the higher temperature sublimation of materials as well as solvent loss may occur. Ultimately there was shortage of one of the important constituent was taking place. Due to this reason low acid values may not obtained and reactions stop early. The reaction may not forward in the absence of solvent and may obtain high acid value product. If the temperature was maintained around 200^0C properly than reaction proceed in smooth way. So, the minimum chance of solvent loss was there and the product would not decompose.

FTIR spectroscopy for polyol

The FTIR spectrum of polyester polyol is shown in figure C.

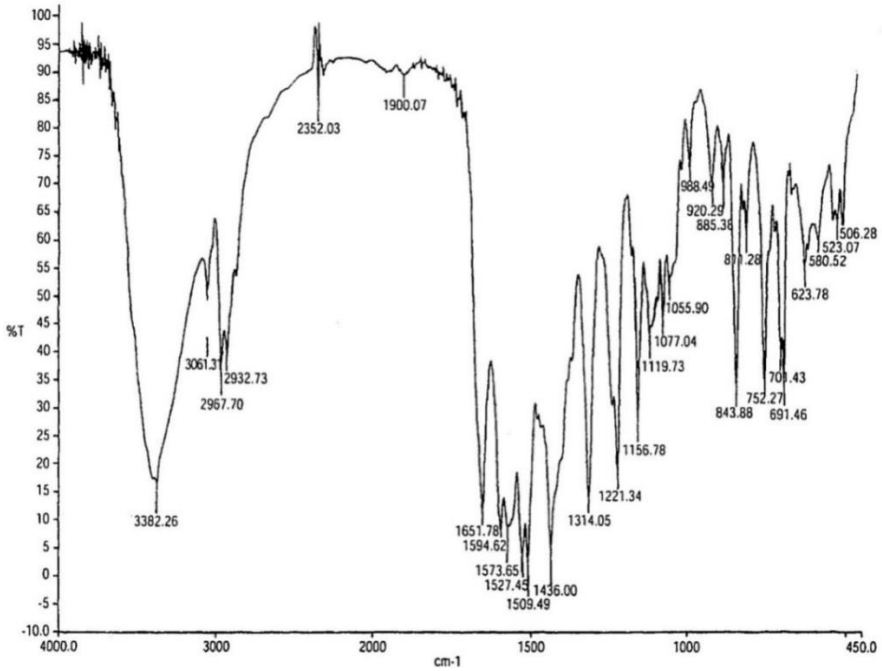

Figure: C FTIR spectra of polyol

The main structure of the polyester polyol sample which contain alcohol, ester, aromatic ring and anhydride. The C=C group confirmed by the peak observed at 704.43cm^{-1}. The C=O stretching observed at 1651.78cm^{-1} which confirms the carbonyl group. Vibration observed at 704.43cm^{-1}& 752.27cm^{-1}due to meta substituted benzene deformation. While incorporation of excessive 1,3-PDO into polyester polyol confirms by strong absorption band at 1077.04cm^{-1}.

2. UNSATURATED POLYESTER

The effect of reaction parameters like mole ratio, reaction time, effect of solvent and catalyst were optimized. The results were tabulated in table: 8

TABLE: 8 Unsaturated Polyester Resin

Sr. 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
				16	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 solvent**

40ml of xylene was used as solvent in the esterification reaction. Toluene and benzene can also be used but the higher boiling point of xylene compare to other is advantageous for the reaction. Solvent helps for proceeding of the reaction in forward direction. By using xylene as solvent, reaction mixture becomes homogeneous and excessive xylene was separated out using distillation

- **Effect of reaction time**

Effect of reaction time on the synthesis of polyester was optimized. The results were tabulated in table: 8. 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 16hrs reaction time so 12hrs reaction was economical. In the case of zinc acetate catalyst low acid value was obtained compare to other catalyst.

- **Effect of catalyst**

Effect of catalyst on the synthesis of polyester was optimized and the results were tabulated in table: 8. 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 one of the best catalyst for manufacturing unsaturated polyester resin.

FTIR spectroscopy of polyester resin

The FTIR spectrum of polyester resin is shown in figure D. 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 987.81cm^{-1} which confirms the present of benzene group. The C=O streching observed at 1724.13cm^{-1} which confirms the carbonyl group. The bending observed at 731.24cm^{-1}which confirms the C=C group. Due to meta substituted benzene deformation, vibration observed at 731.24cm^{-1}& 780.80cm^{-1}. While incorporation of 1,3-PDO into polyester confirms by strong absorption band at 1074.80cm^{-1}.

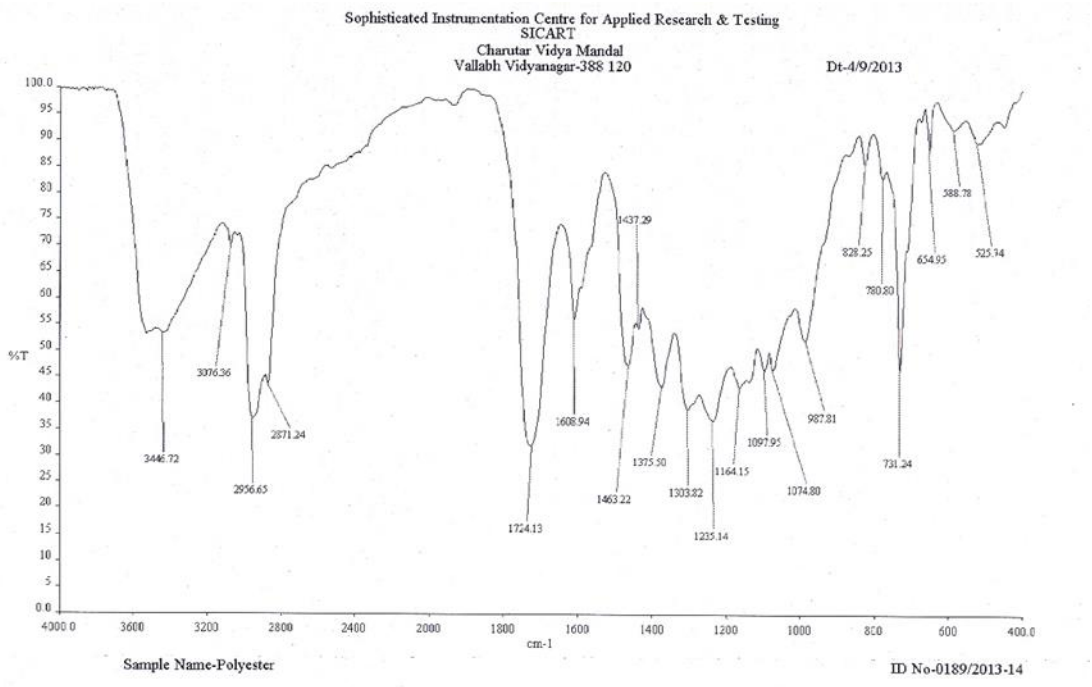

Figure: D FTIR spectra of unsaturated polyester

CONCLUSION

The trans-esterification of cotton seed oil was successfully carried out which produced biodiesel and crude glycerol. The bio conversion of crude glycerol which is separated from trans-esterification production mass was carried out by various stains viz. E. coli, E. aerogen and Pseudomonas. The resulted 1, 3-PDO was separated from biomass. The polyol and unsaturated polyester were synthesized from the resulted 1,3-PDO. The reaction parameters like temperature, mole ratio, solvent, catalyst and reaction time were optimized. The best parameters for manufacturing polyester polyol were, 1:2 molar ratios of terephthalic acid, 1,3-propanediol in the presence of tin catalyst for 16 hours at 200^0 C, where the 12.32 acid value was obtained. The best parameter for unsaturated polyester resin were obtained using zinc acetate catalyst after 12hrs using xylene as solvent. The products obtained during the present work were characterized by gas chromatography and Fourier transforms infrared spectroscopy.

PUBLICATIONS

The following two research papers were published as the deliverables of the work.

1. Bioconversion of waste glycerol to 1,3-propanediol and its application, Accounts of Biotechnology Research, 2014, 1(1), 29-33.

2. A novel biological route for 1,3-propanediol synthesis through transesterification of cottonseed oil, Accounts of Biotechnology Research, 2015, 2(1), 32-36

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