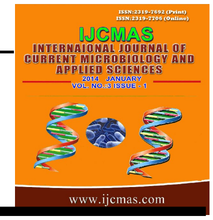

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Original Research Article

Green technology for Glycerol waste from Biodiesel plant

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A B S T R A C T

Keywords

Biodiesel; hydrogen; 1,3-PDO; n-Butanol; Di-hydroxyacetone Propanoic acid;

With the reduction of fossil fuel reservoirs, biodiesel is seen as an alternative future fuel to drive all machines in future. This ecofirendly fuel produces crude glycerol as the waste which is to be addressed before setting up of the biodiesel plants. Biological activity of this glycerol and bioconversion is very less addressed topic from bio-prospecting angle. Even the industrially important compounds used in pharmaceuticals, cosmetics, solvents, ethanol, hydrogen, 1,3-PDO, n-Butanol, Organic acids, Dihydroxyacetone can be produced from crude glycerol waste from biodiesel plants. This paper highlights the microbial strains used to produced various industrial important materials as by-products.

Introduction

Humans are inventing new instruments, machines and substance in the name of development. The energy is required to run these machines which may be natural, biological, chemical, electrochemical or physical. One of them is petroleum and its products viz. petrol, diesel, gasoline etc. The over dependence on these products has now resulted not only in depletion of fossil fuels but also in has created global ecological disturbance.

On an average, human utilizes fossil fuels which results in the release of 29 gigatonnes CO_2 each year. These figures point towards 'M. King Hubbert's peak theory' according to which peak oil is the point in time when the maximum rate of petroleum extraction is reached, after which the rate of production is expected to enter terminal decline (Hubbert, 1956). This critical situation has led to the emergence of an eco-friendly, alternative fuel Biodiesel. According to United States Environmental Protection Agency, the volume requirement of the biomass based diesel in 2013 is 1.28 million gallons which accounts for 1.13% of the total renewable fuels.

The feedstock materials for biodiesel are vegetable oil and animal fats. Biodiesel is basically a long chain of alkyl esters, formed by trans- esterification of triglycerides with alcohol resulting in glycerol as a waste product (Leon et al., 2004). The estimated market of biodiesel by 2016 is expected to reach 37 billion gallons with an annual growth of 42% indirectly producing 4 billion gallons of crude glycerol (United States Environment Protection Agency - Renewable fuel standards; 2013).This would result in increased establishment of biodiesel plant, dramatically increasing the amount of crude glycerol in market.

The massive waste material in form of crude glycerol produced by biodiesel plants are not so easy to convert into useful product by physical or chemical means. The world is expected reach face pollution and environmental problems due to the huge glycerol waste, which will be reflected in the market as increase in cost of production of biodiesel.

The process of purifying crude glycerol is expensive and cumbersome. Increasing biodiesel production has also increased the production of crude glycerol; as a result a considerable fraction is disposed off as waste. This has caused a negative effect on the cost of biodiesel. To counteract this effect and reduce the cost of biodiesel we need efficient methods to convert the crude glycerol into useful products. Converting the surplus crude glycerol into valuable products is a promising approach towards decreasing the cost of biodiesel and also using the products formed from crude glycerol in a meaningful way.

The review of literature has shown that only few literatures available on bioconversion of glycerol into industrially useful products. In order to meet tomorrow's inevitable energy demand for environment friendly technology and green technology this paper is drafted to fill these future lacunae. It highlights production of valuable products using microbes.

Glycerol

Glycerol as it is commonly called glycerin is widely used product in different forms in industries, laboratories and for daily purposes. It is trihydric alcohol and simplest of all alcohols and is also called as propane-1,2,3- triol according to IUPAC. The commercial names are trihydroxypropane, glyceritol or glycidic alcohol. It is an oily liquid, viscous, odorless, colorless and has a syrupy sweet taste (Table no. 1) . A glycerol molecule has three hydrophilic hydroxyl groups responsible for its solubility in water and hygroscopic nature (Koichi et al, 2012).

Viscosity of glycerol decreases with increase in temperature and with addition of water but increases with addition of electrolytes. Glycerol is miscible in water & ethanol but immiscible in hydrocarbons. The boiling point of glycerol is found to be 290°C at atmospheric pressure which indicates the presence of strong intra-molecular attractive forces between the functional group of molecule as well as with neighboring glycerol molecules (Koichi *et al.,* 2012).

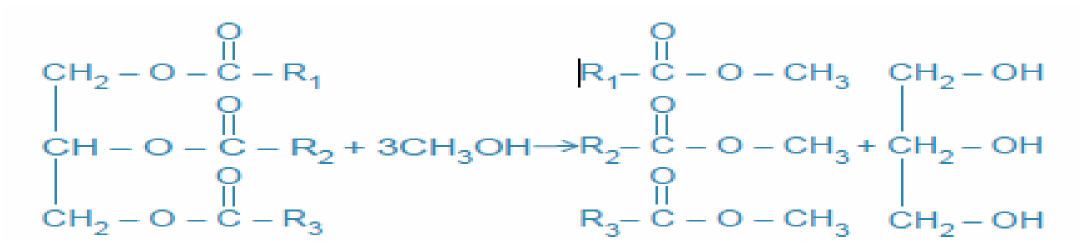
Glycerol formation from biodiesel production

Glycerol is formed as a by-product of the production of biodiesel by transesterification. The triglycerides are treated with an alcohol such as ethanol with catalytic base to give ethyl esters of fatty acids and glycerol(Fig.1).

Table.1 shows the physical and chemical properties of glycerol (Muhammad et al, 2012)

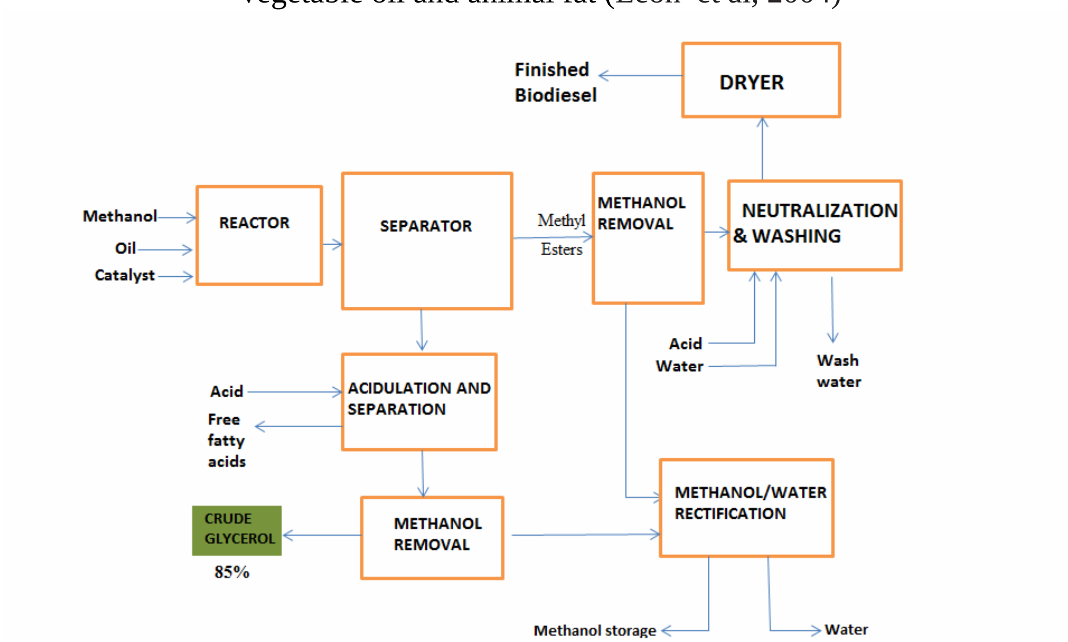
PROPERTIES	**VALUES**
Chemical formula	$CH_2OH–CHOH–CH_2OH$
Formula weight	92.09
Form and color	Colorless and liquid
Specific gravity	1.26050/4
Melting point	17.9 ◦C
Boiling point	290 ◦C
Solubility in 100 parts	
Water	Infinity
Alcohol	Infinity
Ether	Insoluble

Fig.1 Shows the formation of the crude Glycerol during the formation of the biodiesel (Monoalkyl Ester)



Triglyceride + Methanol ⟶ Monoalkyl Ester + Crude Glycerol

Fig.2 The figure shows the process of biodiesel production from vegetable oil and animal fat (Leon et al, 2004)



Industrial production of biodiesel

The initial reaction occurs between methanol and vegetable oil in the presence of catalyst like potassium hydroxide. The reaction is carried out in reactor which is attached to a separator. The separator separates the products into methyl esters and free fatty acids and acids. The excess methanol is removed from the methyl esters and free fatty acids. This methanol is reused for reaction. The neutralization and washing of the methyl esters is done by acids and water, which is further dried in a dryer to get finished biodiesel. The methanol rectification of the waste yields crude glycerol which is used for industrial purposes (Fig. 2).

The crude glycerol produced henceforth is environmentally unfriendly in large amounts, as well as posses threat for the future generation. This crude glycerol can be converted into the industrially useful byproducts by green technology using environment friendly bacteria as shown in Fig. 3.

Industrially useful products and the organisms used

1, 3- Propanediol $[CH_2(CH_2OH)_2]$

It is an three carbon diol $[CH_2(CH_2OH)_2]$ which is used in building block in the production of polymers such as polytrimethylene terephthalate (PTT). It is used to produce composites, adhesives, laminates, coatings, moldings, aliphatic polyesters, copolyesters. Wood paints, antifreeze and solvent are also prepared 1,3 propanediol.

Bacteria *Citrobacter freundii (DSM15979)* and *Pantoea agglomerans (DSM30077)* are reported to convert crude glycerol into 1,3-Propanediol (1,3-PDO). The conversion yield was found to be 43% by *C. freundii* whereas it was 68% by *P. agglomerans* (Silvia et al,2012).

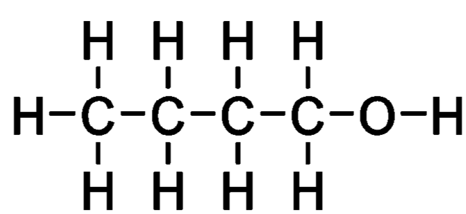
Klebsiella oxytoca converts biodiesel derived crude glycerol into 1,3-PDO under batch and fed-batch fermentation conditions. In batch fermentation with an initial concentration of 120g/L glycerol, ethanol was obtained as 47% (w/w) yield. In fed-batch fermentation with an initial concentration of 126g/L of glycerol, 1,3-PDO is formed along with ethanol which was 20% w/w (Maria et al, 2012).

N-Butanol $[C_4H_9OH]$

The n-butanol acts as an excellent solvent for antibiotics, vitamins, hormones and paints. Also it can be used as alternative transportation fuel. It also acts as feedstock material for formation of various polymers (Swati et al., 2013).

It is an important compound in pharmaceutical industry, polymer production, pyroxylin plastics, weedicide esters, urea formaldehyde resins, melamine formaldehyde resins and printing industry.

Clostridium pasteurianum when immobilized on Amberlite, converts crude glycerol into n-butanol by anaerobic fermentation. The bacteria yielded maximum n-butanol at 25g/L of initial glycerol concentration (Swati et al, 2013).



Hydrogen [H_2]

Hydrogen is seen as an ideal renewable fuel for future as it is an eco-friendly fuel and does not produce green house gases. Hydrogen, the only fuel to produce water as a byproduct and has highest energy content amongst other gaseous fuel. It can be given to internal combustion engines.

Hydrogen is also used in consumption process in petroleum and chemical industries, like upgrading of fossil fuel and ammonia production. It is used as reducing agent of metallic ores during extraction. It is used as coolant in turbo generators. Dangling bonds of amorphous silicon and carbon are stabilized by hydrogen in semiconductor industry.

Bacterial strains are found to convert crude glycerol into Hydrogen under various fermentation conditions. *Rhodopseudomonas palustris* carries out photo fermentation to convert crude glycerol into hydrogen. It produces 6.1 mol H□ per mol of glycerol at 20mM concentration (Dipankar et al, 2012) which is 75% of theoretical value (Guillaume et al, 2009). Theoretical maximum conversion efficiency of 90% was reported by Robert et al., (2013).

Clostridium species under batch fermentation forms hydrogen. At this condition, it uses 36.7% glycerol to give 12.5% hydrogen in gas phase (Rahul et al, 2012). *Klebsiella pneumoniae* TR17 forms 20g/L of Hydrogen for initial glycerol concentration under thermo tolerant fermentation. *Clostridium*and *Klebsiella* species also contribute in hydrogen formation by batch and continuous fermentation (Yung et al, 2013). *Enterobacter aerogens (NRRLB-407)* by response surface methodology forms hydrogen. It is well documented that the production of hydrogen was affected by impurities like soap, NaCl and methanol. The hydrogen production may drop due to impurities from 95.31% to 21.56% (Saurabh et al, 2013).

Ethanol [CH_3CH_2OH]

Ethanol is in great demand as biofuel known as Bioethanol. It is the highest produced alcohol in large scale fermentation. Along with biodiesel, bioethanol is also considered as a future fuel. It is used as alcoholic beverages, in thermometers, as a solvent, and as a fuel. It is used as feedstock for base chemicals like ethyl halides, ethyl esters, diethyl ether, acetic acid, ethyl amines, and butadiene. It is also a wonderful antiseptic

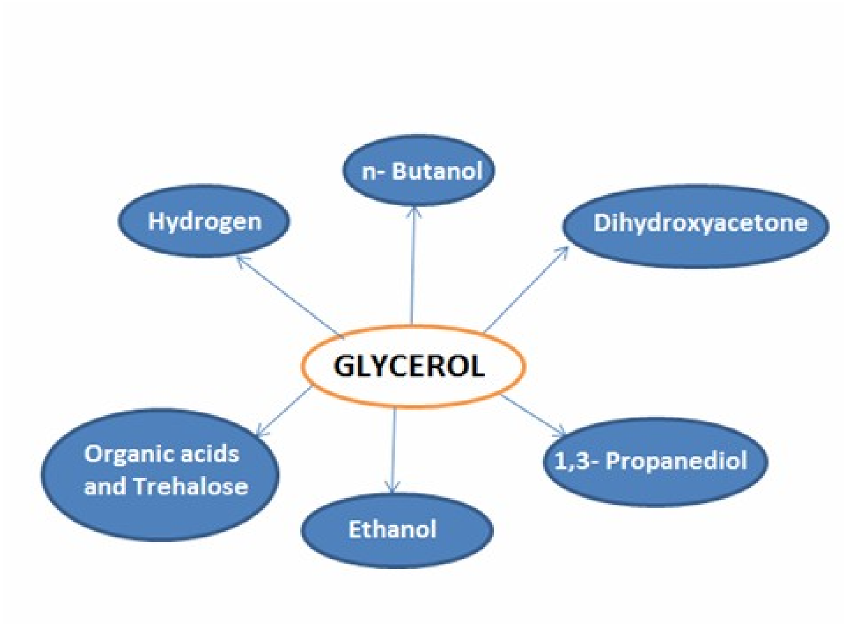
Pachysolen tannophilus (CBS4044) produces ethanol by submerged batch fermentation from crude glycerol. The efficiency of the bacteria to use glycerol was found to be 5%v/v which contradicted with the 56% of theoretical yield suggested (Xiaoying et al,2012).

Klebsiella pneumoniae (GEM167) produces ethanol in batch and fed-batch fermentation conditions equivalent to 21.5g/L with a production rate of 0.93g/L/h. Over expression of spdc and adhII genes encoding Pyruvate dehydrogenase (PDH) and Aldehyde dehydrogenase (ADH) in mutant *Zymomonas mobile* (Baek et al, 2011) have shown the production upto 25g/L.

Dihydroxyacetone [$C_3H_6O_3$]

The Dihydroxyacetone (DHA) is an oral drug for childrens with glycogen storage disease and patients suffering from vitiligo. Its ability to bind with SO2 makes

Fig.3 Depicts the probable discovered industrially useful byproducts from Crude glycerol.



helps it to regulate wine production industry as anti microbial agent in wine industry. It is used in X-ray processing and cosmetic industry to produce sun tanning (Eschenbruch and Dittricha, 1986). Dihydroxyacetone is a value added product as it used in cosmetic industry especially for producing suntans.

Biodiesel derived crude glycerol can converted into DHA using *Gluconobacter frateurii* (CGMCC5397). In shake-flask method this bacteria produces 73.1g/L DHA in 48 hrs where as in fed-batch fermentation 125.8 g/L of crude glycerol was converted to DHA at the rate of 2.6g/L/h with the calculated a yield of 90.8% at 48 hrs (Yu-Peng et al, 2013).

Propanoic acid and Trehalose

Propanoic acid [CH_3CH_2COOH] is a universal preservative for animal and human foods (E280 i.e. INS number 280). It is used as food additives. Cellulose-acetate-propionate, vinyl propionate are used to produce thermoplastics. It it is also used for the manufacture of pesticides, solvents, artificial flavoring and pharmaceuticals products (Bertleff et al 2005)

Trehalose is a reducing sugar having nutraceutical value. Trehalose, is a disaccharide of glucose which has ability to preserve a wide spectrum of biological molecules. It is used as stabilizer in therapeutic products like Herceptin®, Avastin®, Lucentis®, and Advate® (Ohtake and Wang, 2011). It is used in several food and cosmetic products. It is used as cryopreservative

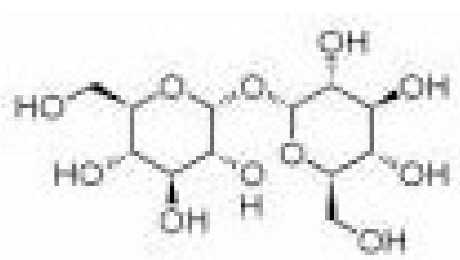
Waste glycerol from biodiesel pant is used by *Propionibacterium freudenreichii* subspecies *shermanii* is and produces propanoic acid, lactic acid and trehalose (Rohit et al, 2012).

Table.2 Depicts the microorganisms which are capable of converting waste glycerol into array of value added products and the fermentation technology employed

S. No	BACTERIA	SUBSTRATE	SOLID SUBSTRATE FERMENTATION	PRODUCT	EFFICIENCY	REFRENCES
1.	*Citrobacter freundii* (DSM15979)	Crude glycerol	1. shake flask batch 2. packed bed reactor (continuous)	1,3-propanediol	At 20g/l of initial glycerol concentration, *C. freundii* produces 1,3-PDO with 43% conversion yield.	Silvia et al (2012)
2.	*Pantoea aglomeranse* (DSM30077)	Crude glycerol	-shaken flask batch -packed bed reactor (continuous)	1,3-propanediol	At 20g/l of initial glycerol concentration, *Pantoeaaglomeranse* produces 1,3-PDO with 68% conversion yield.	Silvia et al (2012)
3.	*Clostridium pasteurianum*	Crude glycerol	Anaerobic fermentation Immobilization and amberlite	n-Butanol	25g/l initial glycerol concentration. Maximum n-butanol whereas at 150g/l it shows inhibitory effect.	Swati et al (2011)
4.	*Rhodopseudomona spalustris*	Crude glycerol	Photofermentation	Hydrogen	6.1 mol H_2/mol crude glycerol at 20mM concentration.	Dipankar et al (2011)
5.	*Clostridium*	Crude glycerol	Batch fermentation	Hydrogen	At pH 6.5 and 40°C uses organism converted 36.7% glycerol and produced 12.5% H_2 in gas phase= 0.7 mol/H_2/mol of glycerol.	Rahul et al (2012)
6.	*Klebsiella pneumoniae* TR17	Crude glycerol	Thermotolerant fermentation	Maximum Hydrogen; 1,3 PDO; 2,3 Butandiol ethanol	At 40°C and pH 8 uses 20g/l crude glycerol= 27.7 mmol H_2/L.	Teera et al (2012)
7.	*Clostridium butyricum Clostridium pasteurianum Klebsiella*	Crude glycerol	Batch and Continuous fermentation	Hydrogen		Yung-chunglo et al (2013)
8.	*Enterobacteraerogenes* (NRRLB-407)	Crude glycerol	Response surface methodology	Hydrogen	Impurities like soap, NaCl, methanol reduce H_2 production by 21.56 - 95.31%.	Saurabhjyoti Sharma et al (2013)
9.	*Rhodopseudomona spalustris*	Crude glycerol	Photofermentation	Hydrogen	6 moles H_2/mole of glycerol which is 75% of theoretical value.	Guillaume et al (2009)
10.	*Pachysolentannophilus* (CBS4044)	Crude glycerol	Submerged batch fermentation	Ethanol	17.5g/l on 5%(v/v) crude glycerol corresponding to 56% of theoretical yield.	Xiaoying et al (2011)
11.	*Rhodopseudomona spalustris*	Crude glycerol	Photofermentation	Hydrogen	Gives 97 mol% H_2 at a conversion efficiency nearing 90% of the 7 mol H_2 theoretical maximum.	Robert et al (2012)
12.	*Klebsiellaoxytoca* (FMCC-197)	Biodiesel derived glycerol	Batch and fed batch bioreactor fermentation	1,3-PDO 2,3-BDO Ethanol	-At 120 g/l glycerol, yields 47% (w/w) - at 150-170 g/l = 1,3-PDO decreases (batch fermentation)	Maria et al (2012)

					-126 g/l glycerol- 50.1 g/l of 1,3-PDO also 25.2 g/l ethanol (20% w/w).	
13.	*Gluconobacterfrateurii* (CGMCC5397)	Crude glycerol	-Shake flask fermentation - Fed batch fermentation	Dihydroxyacetone	73.1 g/l DHA in 48 hrs (shake-flask) At concentration 125.8 g/l productivity is 2.69/l/h and yield is 90.5% at 48 hrs	Y.P. Liu et al (2013)
14.	*Klebsiella pneumonia* (GEM167)	Crude glycerol	Batch fermentation Fed batch fermentation	Ethanol	Maximum= 21.5 g/l with productivity of 0.93 g/l/h production increases to 25 g/l when a mutant strain of the bacteria is used.	B.R. Oh et al (2011)
15.	*Propionibacterium freudenreichii* subspecies *shermanii*	Crude glycerol	Batch fermentation	Organic acids trehalose	Mutant strain= 391 mg/g of biomass, 90 mg/g of substrate consumed= 3-4 times higher than present strain. Also 0.42 g/g substrate consumed (P.A) and 0.3 g/g substrate consumed (L.A).	R. Ruhal, B. Choudhury (2012)
16.	*Citrobacter freundii*	Solvent assisted crude pretreated glycerol biodiesel desired	Anaerobic fermentation	1,3-PDO		Pinki et al (2012)

Biodiesel, an alternative fuel, is a part of Green Technology making the environment safer and free of green house gases. The residual glycerol derived from this biodiesel production can be good source of other economically valuable products. This conversion can be useful to reduce the amount of glycerol which is disposed off as waste. As far as market concerned, this idea of converting glycerol will reduce the saturation of glycerol, in turn reducing the high cost of biodiesel making it more efficient fuel.



Waste glycerol produced from biodiesel plants are not waste if treated biologically using appropriate microorganism in a specific abiotic conditions.

It is multipurpose compound which has broad industrial applications using green refinery processes favoring environmental health and energy efficient.

Rhodopseudomonas palustris. strains of bacteria can produce hydrogen by photo fermentation; *Citrobacter freundii* can produce 1,3-PDO and *Klebsiella pneumonia* and *Klebsiella oxytoca* can produce ethanol as future fuel.

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