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Utilization of microbial oil obtained from crude glycerol for the production of polyol and its subsequent conversion to polyurethane foams

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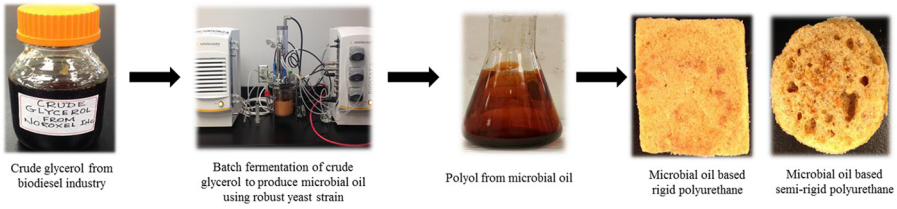
HIGHLIGHTS

- Crude glycerol was converted to microbial oil using a robust oleaginous yeast.
- Microbial oil was chemically converted to polyol.
- Suitability of obtained polyols for polyurethane production demonstrated.
- Carbon present in glycerol waste fixed via polyurethane formation.

GRAPHICAL ABSTRACT



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ABSTRACT

We have demonstrated possible use of microbial oil in biopolymer industries. Microbial oil was produced from biodiesel based crude glycerol and subsequently converted into polyol. Fermentation of crude glycerol in a batch bioreactor using *Rhodosporidium toruloides* ATCC 10788 produced 18.69 g/L of lipid at the end of 7 days. The microbial oil was then chemically converted to polyol and characterized using FT-IR and ^{1}H NMR. For comparison, canola oil and palm oil were also converted into their respective polyols. The hydroxyl numbers of polyols from canola, palm and microbial oil were found to be 266.86, 222.32 and 230.30 (mg KOH/g of sample) respectively. All the polyols were further converted into rigid and semi-rigid polyurethanes (maintaining the molar –NCO/–OH ratio of 1.1) to examine their suitability in polymer applications. Conversion of microbial lipid to polyurethane foam also provides a new route for the production of polymers using biodiesel based crude glycerol.

1. Introduction

Fossil resources are used for various applications including energy generation, running transportations, polymer and plastic synthesis. Dependency on such resources and its derivatives have led to various socio-economic and environmental problems. This includes global warming, deforestation, accumulation of non-biodegradable substances, contamination of natural resources and disputes among the countries (Rittmann, 2008; Uprety et al., 2016). In order to overcome these issues, there is considerable focus on the use of alternative renewable energy sources. Biodiesel is one such renewable fuel obtained via transesterification of vegetable oils. It can be blended in various concentrations with fossil based diesel and be used with conventional diesel engine, without any modification. In the past decade, production of biodiesel has increased throughout the world (Uprety et al., 2017).

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However, with an increase in biodiesel production, there is also a rise in the accumulation of the by-product glycerol. During the transesterification of vegetable oils into biodiesel, approximately 10% wt of glycerol is also produced. The glycerol obtained during the biodiesel production process, also known as crude glycerol, contains many impurities. Crude glycerol from biodiesel plant requires numerous purification steps before it can become suitable for direct commercial applications. Due to the buildup of crude glycerol in the past decade, there has been slump in the prices of pure glycerol (Uprety et al., 2016). Thus, carrying out an expensive downstream processing of crude glycerol to obtain its purer form has become uneconomical, mainly for the small and medium scale biodiesel producers. However, the glycerol needs to be diverted to value added products instead of causing environmental problems. Researchers have thus focussed on producing various value added components from crude glycerol in order to add revenues for the biodiesel industries (Ardi et al., 2015; Garlapati et al., 2016). Among many such production processes that are being evaluated, bioconversion of crude glycerol to microbial oils or single cell oils (SCO) has gained considerable attention (Garlapati et al., 2016). The term "single cell oils" were initially coined to include the lipids (rich in triacylglycerol) obtained from heterotrophs such as yeast and fungus (Ratledge, 2013). Ratledge and Wynn (2002) reported the maximum theoretical lipid yield from glycerol to be 30% (w/w). Use of various types of yeasts and fungi to convert glycerol into lipids or microbial oil was previously reported by various authors (Kitcha and Cheirsilp, 2013; Marchand et al., 2013; Xu et al., 2012). Since the fatty acid characteristics of obtained microbial oils were similar to vegetable oils, all the reported works had stressed its use for the production of biodiesel. However, exploring other applications of obtained microbial oils from such sources will make the overall conversion process more economically feasible and sustainable.

At present, production of varieties of polyols and polymers is mainly dependent on fossil resources (Bradley, 1998; Imre and Pukánszky, 2013; Katiyar et al., 2015). Presently, only 1% of plastic produced are obtained from biological resources mainly vegetable oils. However, the global market of bioplastic is increasing every year and is expected to grow from 4.2 million tonnes in 2016 to 6.1 million tonnes by 2021 (Bioplastic market, 2016). Even though only a very small portion of current plastic market is occupied by bio-based counterpart, the use of renewable sources for polymer production over fossil resources has certainly contributed in reducing the negative effects that fossil based chemicals would cause on environment. Polyurethanes are an important class of thermoplastic and thermoset polymers as it can be used in various fields (Zia et al., 2007). Its production from vegetable oils have been reported by various authors (Hu et al., 2002; Kong et al., 2012; Narine et al., 2007; Pawlik and Prociak, 2012; Saifuddin et al., 2010). Even though polymers (including polyurethanes) from vegetable oils are environmentally friendly, it has some major drawbacks. Firstly, using vegetable oils for polymers production is dependant on many factors including availability of huge arable lands for its cultivation, labor cost, climatic condition of the region and increase in prices of cooking oils and consequently on food security. Secondly, it has longer production lifecycle and are costlier than fossil resources (Liang and Jiang, 2013). Thus, an alternative cheaper renewable feedstock addressing these would be of great interest to the present scientific communities and oleo-chemical industries.

In this study, a novel route to produce polyols from crude glycerol has been reported by using a combination of biological and chemical processes. Firstly, crude glycerol was converted into microbial oil by using an oleaginous yeast *Rhodosporidium toruloides* ATCC 10788. The obtained microbial oil was further converted into its polyol via a chemical reaction. Suitability of obtained polyol for polyurethane (PU) production was also examined by producing two different polyurethane foams (i.e. rigid and semi-rigid). For comparison, polyurethane foams production from canola and palm oil were also carried out under similar reaction conditions. Utilization of such a potential environmental waste (i.e. crude glycerol) to produce polyurethane will help fix the carbon present in crude glycerol. This in turn reduces the release of greenhouse gases to the atmosphere, thus making the whole process a green pathway. To the best of our knowledge, production of polyols from oil obtained from heterotrophic organisms (i.e. yeast or fungal) is not reported so far. Additionally, the uniqueness of our work lies in the fact that a complete conversion of crude glycerol into polyol via the production of microbial oil is demonstrated here.

2. Materials and methods

2.1. Microorganism and chemicals

The oleaginous yeast *R. toruloides* ATCC 10788 used in this work to produce microbial oil was obtained from American Type Culture Collection (ATCC). It was grown in YPG media (10 g/L yeast extract, 20 g/L peptones and 20 g/L glycerol) at 30 °C for 24 h maintaining a shaker speed of 200 rpm. For long term use, the culture was mixed with pure glycerol (1:1 ratio) and stored at −80 °C. Refined, bleached and deodorized (RBD) palm oil was obtained from Saffire Blue Inc. (Canada). Refined pure canola oil was bought at local store located in Thunder Bay, Canada. Crude glycerol containing 44.56% wt glycerol, 13.86% wt methanol, 32.97% wt of soap (as sodium oleate), 0.82% wt of water and 4.38% wt of fatty acid methyl esters (FAME) was obtained from a biodiesel producer in Ontario (Canada). All the chemicals and reagents used in the experiments were analytically pure and obtained from Fisher Scientific, Canada.

2.2. Production of microbial oil from crude glycerol

Microbial oil from crude glycerol was produced using an oleaginous yeast, *R. toruloides* ATCC 10788. Fermentation conditions as described by us earlier was adopted in this study (Uprety et al., 2017). For the preparation of the inoculum, the yeast was grown in a YPG media (10 g/L yeast extract, 20 g/L peptones and 20 g/L glycerol) inside a rotary shaker maintained at 200 rpm, 30 °C for 48 h until viable cell count reached $\sim 1 \times 10^8$ cells/ml. The minimal media was prepared by using distilled water and maintained a pH of 6.0. It contained 1.0 g/L KH_2PO_4, 1.0 g/L $Na_2HPO_4{\cdot}12H_2O$, 1.5 g/L $MgCl_2{\cdot}6H_2O$ and 10 ml/L of trace elements. The trace elements mixture was prepared by mixing 4.0 g $CaCl_2{\cdot}2H_2O$, 0.55 g $FeSO_4{\cdot}7H_2O$, 0.52 g Citric acid$\cdot H_2O$, 0.10 g $ZnSO_4{\cdot}7H_2O$, 0.076 g $MnSO_4{\cdot}H_2O$, and 100 μl 18 M H_2SO_4 in 1 L of distilled water. Crude glycerol and ammonium chloride (NH_4Cl) were added in the media to obtain the overall C:N molar ratio of 100.

Shake flask experiments were carried out in 125 ml Erlenmeyer flask with a working volume of 50 ml. Similarly, larger scale production of microbial oil was carried out in 1 L bioreactor (Sartorius, Biostat A) using 750 ml of minimal media, containing crude glycerol as a carbon source. Fermentation was carried out at 30 °C, 300 rpm stirrer speed and 1.2 vvm aeration. The pH of the media was maintained at 6 by automatic addition of 1 M NaOH or 1 M HCl. Sample analyses were performed in replicates and percentage errors were expressed as mean ± standard deviation.

2.3. Analytical methods

Quantitative determination of methanol and glycerol in crude glycerol were done by using an Agilent 1260 infinity HPLC