Feature Article

Bio-based 1,3-propanediol Production from Crude Glycerol

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ABSTRACT

Biodiesel, an environmental-friendly and renewable fuel has gained market share and popularity as an alternative to fossil fuel. The utilisation of glycerol for the synthesis of value-added chemicals is a topic of great industrial interest because the rapid growth of the biodiesel industry has thus generated an excess of waste glycerol. This occurrence is unavoidable due to its restricted application owing to the presence of impurities. Its natural disposal in the environment is prohibited but at the same time it is costly to store and process this into its pure form. The triglyceride backbone of glycerol can serve as a natural metabolite for biological functions in many cells and has potential to be degraded into high value-added smaller molecules. We found a newly isolated 1,3-propanediol (1,3-PD) producer from palm oil mill effluent (POME) which was capable of fermenting crude glycerol into 1,3-PD (5.28 g litre^{-1}) along with by-products, butanol (0.34 g litre^{-1}) and acetone (0.31 g litre^{-1}) after 48 hr of incubation at 30°C in medium enriched with crude glycerol at 150 rpm. The strain, namely Kluyvera cryocrescens has the potential to be used in the bioprocess of interest in the future.

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INTRODUCTION

The crude glycerol generated thus far from the biodiesel industry has created some environmental and economic challenges as its disposal in the environment is undesirable and its utilisation limited. This surplus by-product produced at an alarming rate of one-tenth of every volume of biodiesel, has received great attention for its potential alternative uses which need to be explored, in the hope to reduce the associated environment impact and improve the economic viability of the biodiesel industry. To date, integration of this waste for high value-added co-production together with biodiesel seems promising.

Owing to its reduced carbon chain length, glycerol can serve as a low-cost carbon source to produce a wide range of value-added products through microbial

fermentation. These include 1,3-propanediol (1,3-PD), citric acid, butanol, ethanol, poly (hydroxyalkanoates), polyglycerol, 1,3-dihydroxyacetone (DHA) and many others. The chemicals and polymers derived have extensive applications as biofuels, fuel additives, detergents, etc. Among the many potential applications, microbial conversion of glycerol into 1,3-PD has recently received much attention (Guo *et al*., 2017; Loh and Stasha, 2016; Da Silva *et al*., 2015; Rossi *et al*., 2013).

The 1,3-PD, a 3-carbon diol, is a specialty platform chemical due to its varying applications in polymers, cosmetics, pharmaceuticals, *etc.* (Kaur *et al*., 2012). It is mainly used as a monomer for the synthesis of polyesters and heterocyclic compounds (Lee *et al*., 2015). Conventionally, 1,3-PD is chemically synthesised via two major routes; reacting 3-hydroxypropionaldehyde with either acrolein or ethylene oxide. However, the chemical routes are costly and require high pressure and temperature. Contrarily, the current interest via biological approach using renewable biomass, including crude glycerol is more promising (Chatzifragkou *et al.,* 2014), as the biological route seems more practical and feasible (more economical, less risky and environmental-friendly). Besides, it is fermentation-based using relatively cheap, replenishable raw materials *i.e.* crude glycerol and naturally occurring microorganisms, thus less energy is required.

The ability of microorganism, *Clostridium pasteurianum* to convert glycerol into 1,3-PD was first discovered in 1881. Later, many other microorganisms have been identified, including *Klebsiella* (Wojtusik *et al.,* 2015), *Clostridium* (Szymanowska-Powalowska, 2014) and *Citrobacter* (Ferreira *et al.,* 2012). Many of these glycerol-catabolising strains are originated from soil and other wastewater resources. Palm oil mill effluent (POME) being cheap and abundant has the potential for such conversion. Hence, the work commenced to identify some potential bacterial strains from POME that can produce 1,3-PD using the crude glycerol derived from biodiesel manufacturing.

Microbial Screening of Palm Oil Mill Effluent

POME samples were collected from a palm oil mill and the potential 1,3-PD producers were isolated by bacterial enrichment in a selective medium. Each isolated bacteria was inoculated into a 250 ml flask with 100 ml enriched medium and incubated at 30°C for 24 hr at 150 rpm. After the incubation period, the samples were subjected to a serial dilution using sterile distilled water and 0.1 ml of each diluted samples were then plated out onto a solid mineral salt medium (MSM) and incubated at 30°C. The culture was then incubated at 30°C for 60 hr in an incubator shaker at 150 rpm. A small portion of the sample was withdrawn at fixed time interval (6 hr, 12 hr, 24 hr, 48 hr and 60 hr) and analysed for 1,3-PD and other by-products production using high performance liquid chromatography (HPLC). The working conditions were as follows: 0.5 mM H_2SO_4 as a mobile phase at 60°C with a flow rate of 1.0 ml min^{-1}. The culture was centrifuged at 4000 rpm for 10 min and the supernatant was filtered through a 0.45-µm membrane filter (Nylon) for analysis.

Identification of 1,3-PD-Producing Microorganisms

There were many bacteria colonies grown on the separation plates packed with MSM. We managed to isolate five different types of colonies and purify them further on Luria Bertani (LB) agar plates. Of these, one potential 1,3-PD producer, showed ability in utilising glycerol as carbon source. This strain performed as a gram-negative rod-shaped bacterium (*Figure 1a*) with its morphology as shown by the SEM micrograph (*Figure 1b*). This newly isolated 1,3-PD producer was

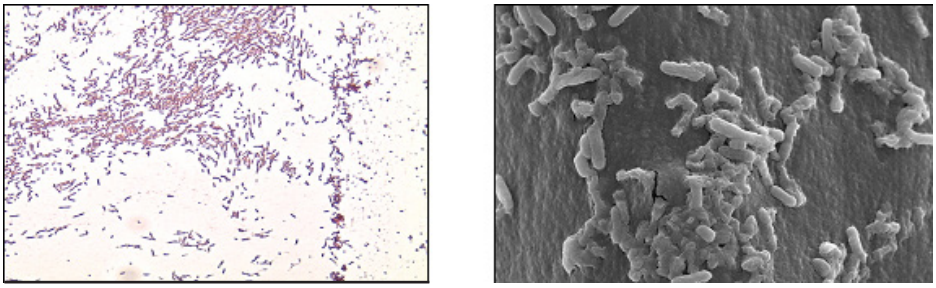

Figure 1. (a) Gram stain test micrograph of Kluyvera cryocrescens *under 40 X magnification. (b) Scanning electron micrograph of* Kluyvera cryocrescens *under 10 000 X magnification.*

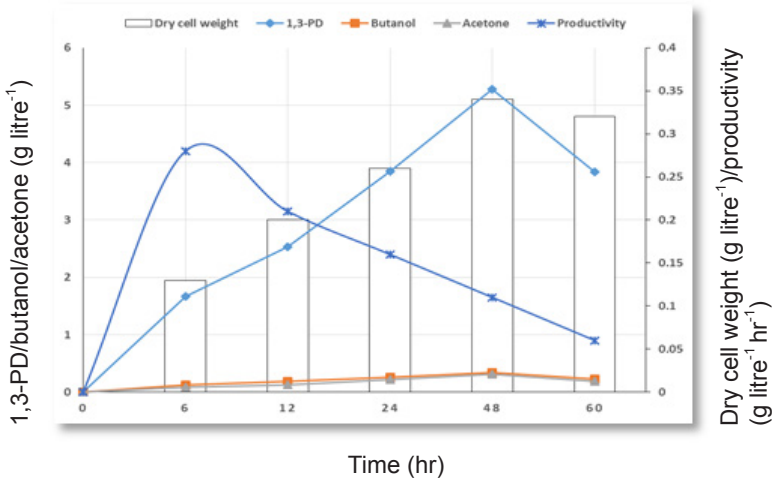

Figure 2. Fermentation of Kluyvera cryocrescens *in crude glycerol for 1,3-PD and other by-products production.*

taxonomically identified to be the species of *Kluyvera cryocrescens* NBRC 102 467 (with 99% similarity via NCBI BLAST).

Production of 1,3-PD by *Kluyvera cryocrescens*

The shake flask fermentation showed that *Kluyvera cryocrescens* could consume the crude glycerol and transform it into 1,3-PD. Although there were impurities in crude glycerol, the strain could withstand it and was able to produce 1,3-PD up to a maximum concentration of 5.28 g litre^{-1} after 48 hr with productivity of 0.11 g litre^{-1} hr^{-1} (*Figure 2*). At the same time, very low concentrations of acetone and butanol were also obtained, 0.34 g litre^{-1} and 0.31 g litre^{-1} (max), respectively. These two by-products can be used as a solvent or a fuel. The production of 1,3-PD and the by-products dropped drastically after that, probably due to nutrient depletion, causing cells die-off with a declined dry cell weight and productivity too.

Commercial Potential

The findings showed that ~5 g of 1,3-PD can be produced from 20 g of crude glycerol. Assuming the biodiesel production in Malaysia is ~500 000 t and the crude glycerol production is ~50 000 t, thus potentially, ~12 500 t of 1,3-PD per year can be produced. The estimated total value of 1,3-PD in a year is RM 19.375 million based on market price of RM 1550 kg^{-1} of 1,3-PD.

CONCLUSION

A newly isolated *Kluyvera cryocrescens* from POME could assimilate crude glycerol derived from the biodiesel industry to produce 1,3-PD as the main product, along with butanol and acetone as by-products. This strain showed high durability even in a contaminated substrate like crude glycerol.

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