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Challenges in Biochemical Engineering and Biotechnology for Sustainable Environment

Bioconversion of Crude Glycerol Into Glyceric Acid : A Value Added Product

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Abstract : Biodiesel acts as a promising alternative to fossil fuels. Increasing demand for alternative fuel leads to the improvement in the production technology day by day. Though it is renewable, the huge amount of waste glycerol generated as a byproduct from these industries poses problem in its disposal. In this research, efforts have been made to the effective utilization of waste glycerol to produce value added products like citric acid, lactic acid, 1,3-dihydroxyacetone (DHA), 1,3-propanediol (1,3-PD), dichloro-2- propanol (DCP), hydrogen and ethanol *etc.* The versatile new applications of glycerol in the everyday life and chemical industry will improve the economic viability of the biodiesel industry. Glyceric acid (GA), an unfamiliar biotechnological product, is currently produced as a small by-product of dihydroxyacetone production from glycerol by ***Glucanobacter Sp***. Here, we have developed a method for the efficient production of Glyceric acid from glycerol. The microorganism used in this work was isolated from the rotten apple. The amount of glyceric acid produced has been optimized using various volumes of $CaCl_2$. The trace elements present in the waste glycerol have also been characterized. Thus, we propose that Glyceric acid is potentially mass producible from waste glycerol.
Keywords: Biodiesel, Glycerol, Glyceric acid, Glucanobacter Sp ,$CaCl_2$.

Introduction

Increasing price and decreasing availability of fossil fuel leads the way to find new and renewable sources of energy. One among them is fatty acid methyl esters (FAME) or otherwise termed as biodiesel[1]. The potential use of biodiesel will greatly benefit the environment, as the emission levels are lower than conventional fossil fuel. They are said to be clean burning alternative fuel composed of mono-alkyl esters of long fatty acid derived from renewable resource such as animal fats and vegetable oils[2].

$$\text{Triglycerides + Methonal} \xrightarrow{\text{Catalyst}} \text{Mixture of fatty acid + Glycerine.}$$

The production of biodiesel leaves a considerable amount of by products like glycerol. 10 kg of glycerol (crude) will be produced for 100 kg of biodiesel [3]. The global biodiesel market is estimated to reach 37 billion gallon by 2016 which means with an annual growth of 42% and about 4 billion gallons of crude glycerol will be produced[4].

Glycerol is colourless, sweet tasting, sticky, odourless and viscous substance[5]. Glycerol has a much higher density so it is easily removed by settling or centrifuge. The glycerol free methyl esters were then reacted with alkali to form soap. The crude glycerine by-product is not utilised by the biodiesel producers.As an alternative to the conventional sources of carbohydrate in fermentation processes, the surplus amount of glycerol generated as a by-product from biodiesel industries can act as a promising carbohydrate source. On the other hand the usage of waste glycerol to produce value added productscan sort out the disposal problemsthereby reducing the waste generation.

Therefore it is imperative to find the alternative uses of glycerol. Glycerol has many uses in different industry such asfood, pharmaceuticals, paint, cosmetics, soap, toothpasteetc[6]. The research has been completed involved the pure food grade glycerol, not a crude glycerol. Therefore further research is required if crude glycerine shows to be feasible food stuff as it contains lot of impurities like methanol, sodium hydroxide, potassium hydroxide and ash.

Many microbes are able to metabolise glycerol aerobically and few are able to do anaerobically and even none of them are used in the industrial scale. Microbes such as *E.coli, Klebsiella, Enterobacter,Glucanobacter, Clostridium, Candida, Aspergillus* are able to convert crude glycerol into value added products[7,8].

Among the recent developments in the conversion of glycerol into commercially value added chemicals, Epicholorohydrin (ECH) and 1,2 propendiol (Propyelene glycol) are commercially synthesised from glycerol by chemical process and Dihydroxy acetone (DHA) and 1,3 propendiol (1,3 PDO) are produced from glycerol by biotechnological process.

Recently, we have focussed our thoughts on the production of a glycerol derivative,an unfamiliar biotechnological word glyceric acid (GA) using a bioprocess technique. Before we began our work, little was known about glyceric acid as a biotechnology product, expect for one Japanese patent on 1987 reported on its by production during DHA production by *Glucanobacter oxydans*[9].

Glucanobacter oxydans is a gram negative rod or oval shaped non motile bacterium belonging to the family Acetobacter[10]. Glucanobacter strains flourishes in sugary substance like ripe grapes, apple, dates, garden soil, and honeybees. Glucanobacter strains are capable of growing in high concentration of sugar solution and low pH values. (Optimal pH for growth is 5.5 – 6.0).These microbes are able to produce many products and we have planned to produce GA from crude glycerol through fermentation process[11].

Materials and Methods

Isolation of Glucanobacter

The microorganism were isolated from rotten apple and serially diluted and later spread plate was done on the selective media comprises glucose-5g/100ml, yeast extract- 3g/100ml, calcium carbonate- 2.5g/100ml, agar- 2g/100ml and incubated for 48 hours $37^{o}c$[12].Those microorganism were confirmed as Glucanobacter by i) motility test by hanging drop method ii) gram staining iii) oxidase test iv) catalase test.

Fermentation of Glycerol to Glyceric Acid

Four flasks which contain crude glycerol and nutrients out of which two was sterilized and another two is unsterilized with the pH of 6.0 are maintained. The sample of Glucanobacter obtained from apple was inoculated into the flask of one sterilised and another unsterilized respectively. The fermentation was carried out for 90hours and the growth of microbes was checked for every 4 hour by colony count and optical density[13].

Separation of Glyceric Acid from Glycerol

After fermentation of about 90hour, 20 ml of both sterilized and unsterilized glycerol sample were taken and centrifuged at 2000rpm for 5 min. The supernatant were collected and the pellet was washed away. To the supernatant of Glyceric acid solution 40% calcium salt were added to precipitate. The Glyceric acid calcium salt solution was washed with ethanol repeatedly and filtered, dried then Glyceric acid was collected[14,15].

Confirmatory Test for Glyceric Acid

1 ml of 1% NaoH was added to the Glyceric acid and heated to 80^oc for few minutes. When phenolphthalein indicator added to the heated Glyceric acid which slowly turns to pink colour which confirms the presence of Glyceric acid (tricarboxylic acid) obtained from the fermentation process.

Optimization of Glyceric Acid

50 ml fermented mixture was centrifuged at 2000 rpm for 5 min. The supernatant were collected and mixed with various percentage of calcium chloride 30%, 40%, 50%, 60%, and 70% respectively to precipitate the Glyceric acid salt solution and checked for the maximum precipitation.

The maximum precipitated Glyceric acid calcium salt solution were washed with ethanol twice and filtered, dried to get the free Glyceric acid.

Results

Isolation of Glucanobacter

The microorganism glucanobacteria were isolated from the rotten apples are serially diluted and 10^{-4} were grown on the selective media (fig. 1) and checked by the basic conformation results shows the positive results like gram negative (fig.2), rod shaped and non- motile in gram staining and motility tests.

Fig.1 Serial dilution

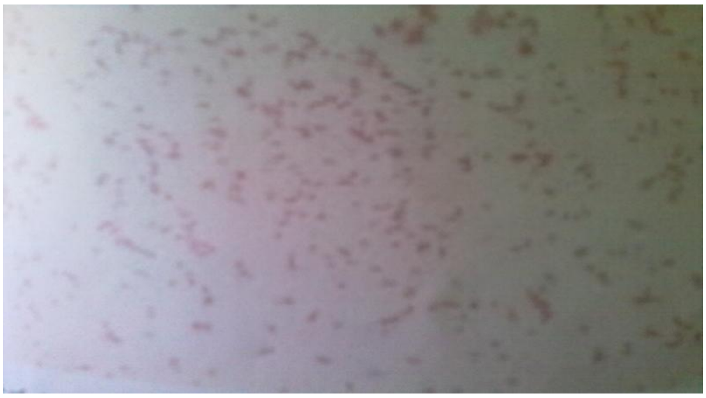

Fig.2 Gram staining

Fermentation of Glycerol to Glyceric Acid

The microorganism was grown well on sterilized crude glycerol with nutrients when compared to the unsterilized crude glycerol with nutrients. This was confirmed by the spread plate technique which has taken for every 4 hours during the fermentation. The initial pH of about 6.0 favours the growth of microbes and as the conversion proceeds the pH is dropped to 2.5 which tend to increase the glyceric acid production.

Separation and Conformation of Glyceric Acid

The supernatant collected after centrifuge were treated with the calcium chloride for the precipitation and later washed with ethanol to obtain the pure glyceric acid. To the glyceric acid 1 ml of 1% NaoH added and heated to which when phenolthalein indicator was added it turns to pink colour (fig. 3). This confirms the presence of tricarboxylic acid (Glyceric acid) as checked with the standard glycerol (fig.4).

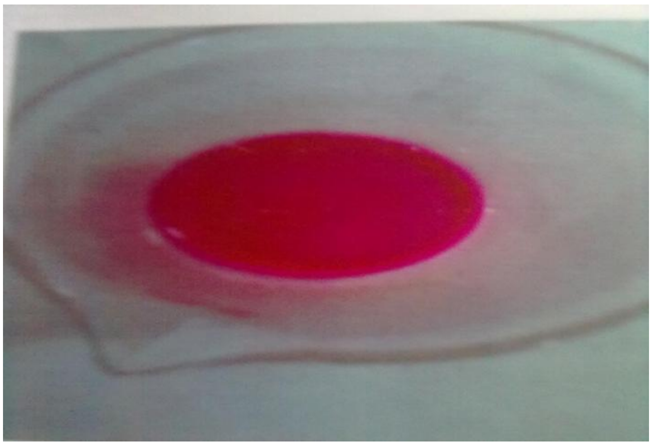

Fig.3 Qualitative test for crudeglycerol

Fig.4 Qualitative test for standard glycerol

Optimisation of Glyceric Acid

When the supernatant treated with the various concentrations of calcium chloride ranges from 30 – 70%, 50% shows the maximum precipitation of glyceric acid calcium salt solution. The glyceric acid calcium salt solution was washed with ethanol repeatedly to remove the salt and filtered, dried and purified to obtain the glyceric acid.

Discussion

An efficient biotechnological production of glyceric acid as a target compound using waste glycerol obtained as by product from biodiesel industry was carried out. *Gluconobacter* species isolated from rotten apple were used for the fermentative production of glyceric acid. Qualitative tests were carried out for the confirmation of the glyceric acid. Thus obtained glyceric acid was optimized using various amount of $CaCl_2$ precipitation.This study can be further extended for the production of Poly Lactic Acid which can be a source in the bio plastic industry for bioplastic production.

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