Enzyme and Microbial Technology 69 (2015) 19–23

Contents lists available at ScienceDirect

Enzyme and Microbial Technology

journal homepage: www.elsevier.com/locate/emt

Enzymatic production of glycerol acetate from glycerol

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ARTICLE INFO

Article history:
Received 4 September 2014
Received in revised form 16 October 2014
Accepted 21 November 2014
Available online 29 November 2014

Keywords:
Glycerol
Glycerol acetate
Novozym 435
Optimization

ABSTRACT

In this study, we report the enzymatic production of glycerol acetate from glycerol and methyl acetate. Lipases are essential for the catalysis of this reaction. To find the optimum conditions for glycerol acetate production, sequential experiments were designed. Type of lipase, lipase concentration, molar ratio of reactants, reaction temperature and solvents were investigated for the optimum conversion of glycerol to glycerol acetate. As the result of lipase screening, Novozym 435 (Immobilized *Candida antarctica* lipase B) was turned out to be the optimal lipase for the reaction. Under the optimal conditions (2.5 g/L of Novozym 435, 1:40 molar ratio of glycerol to methyl acetate, 40 °C and tert-butanol as the solvent), glycerol acetate production was achieved in 95.00% conversion.

1. Introduction

Although the demand for fossil fuels is steadily increasing, their supply is not adequate to cover the increasing requirement. Therefore, the price of fossil fuels has been increasing. The price of petroleum, typical fossil fuel, has huge range of fluctuation, because of its dependence on the situation of oil-producing country. It causes many problems for the petrochemical industry [1]. The increase in fossil fuel price brings problems not only for vehicle gas, but also a great portion of the products from chemical process. Thus, alternative fuel source has been receiving significant attention [2].

Many researchers have focused on bioenergy and value added chemicals based on biomass. It can be produced by the renewable sources and emits less greenhouse gases than the fossil fuels. There are many studies about the sources for production of bioenergy and value added chemicals. Initially, edible plants such as sugar cane were used. Then, the range was extended to lignocellulosic biomass and during the past few years, to microalgae [3]. In recent years, animal oil or vegetable oil are commonly used for biodiesel production by the esterification. Glycerol is produced as a by-product from biodiesel production process, and its amount is 10% of biodiesel production amount (Fig. 1a) [4]. As biodiesel production has been growing, glycerol has been oversupplied. Thus, the value of glycerol has decreased steadily. For economical competitiveness of biodiesel production process, glycerol should be converted to value added chemical [5].

Glycerol has hydroxyl functional groups in the triol form. For adding value, glycerol derivatives are produced by the oxidation, etherification, esterification, and acetalization from glycerol. Glycerol derivatives are used in many fields of industry such as foods, cosmetics, and chemistry. Glycerol acetate, a type of glycerol ester, has got the most interests among the glycerol derivatives because of its extensive applicability. Glycerol acetate exists in three forms, glycerol monoacetate, glycerol diacetate, and glycerol triacetate (Fig. 1b). All of these three forms are able to be used in various industrial fields, such as fuel, food, cosmetic, pharmaceutical, and tobacco industry, etc. [6,7]. The production of biodiesel, glycerol carbonate, and many glycerol derivatives by lipases has been extensively studied. To the best of our knowledge, this is the first successful report on the enzymatic synthesis of glycerol acetate.

Glycerol acetate is industrially produced via chemical methods using bases or acids as a catalyst or using high reaction temperature [8–10]. Enzymatic reaction can synthesize glycerol acetate using lipase as the catalyst for the transesterification at moderate temperature. The reaction needs an acetate donor for the transesterification reaction. Acetate donor should not affect the enzyme stability and be able to react at low temperature. Methyl acetate is a basic form of acetate donor, and it is well known as a stable material for the lipase activity [11]. The solvent and methyl acetate can be reused after removing glycerol acetate and methanol from reaction mixture. For the separation of glycerol acetate and methanol, potential applications are oil-jacketed column and molecular sieve, respectively [12,13].

In this study, enzymatic synthesis of glycerol acetate was achieved by the reaction of glycerol and methyl acetate using lipase. The effects of various reaction parameters such as type of enzyme, enzyme concentration, molar ratio of reactants, reaction

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http://dx.doi.org/10.1016/j.enzmictec.2014.11.004
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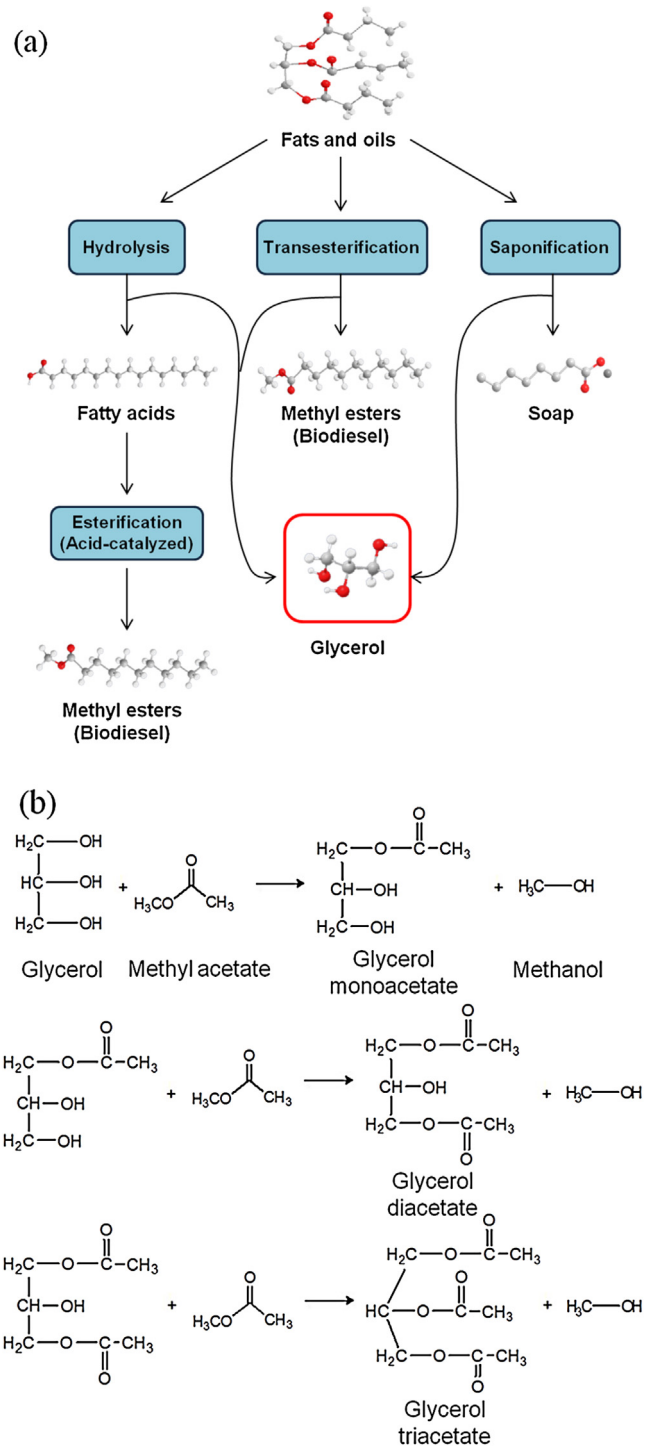

Fig. 1. Scheme of glycerol production from fats and oils and reaction formula of glycerol acetate.

temperature, and solvent were investigated, and the optimum reaction condition was defined. In this study, we synthesized glycerol acetate from glycerol using enzymatic method to make glycerol with added value.

2. Materials and methods

2.1. Materials

Glycerol was purchased from Sigma–Aldrich (St. Louis, MO, USA). Methyl acetate (Daejung Chemicals and Metals Co., Ltd., Gyunggido, Korea) was used as the acetate donor. Novozym 435 (*Candida antarctica* lipase B immobilized on a macroporous acrylic resin) and lipozyme RM IM (*Rhizomucor miehei* lipase immobilized on an anionic resin) were purchased from Novo Nordisk Bioindustry (Bagsværd, Denmark). Amano AK (*Pseudomonas fluorescens* lipase) was purchased from Amano International Enzyme (Nagoya, Japan). Dimethyl sulfoxide (DMSO), acetonitrile, tetrahydrofuran (THF), tert-butanol, 1,2-dichloroethane were used for solvent test. DMSO and 1,2-dichloroethane were purchased from Daejung Chemical and Metals Co., Ltd. (Gyunggido, Korea). Acetonitrile and THF were purchased from Junsei Chemical (Tokyo, Japan). tert-Butanol was purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Transesterification reaction

Glycerol acetate was produced by the transesterification reaction of glycerol and methyl acetate using lipase. The reaction requires acetate group as the acetate donor. 1 mol of glycerol was reacted with 1 mol of methyl acetate, affording 1 mol of glycerol acetate and 1 mol of methanol. In this study, sequential experiments were performed to find the optimum conditions for the production of glycerol acetate. A 50 mL screw-cap flask was used, and the working volume was 10 mL. For maintaining the temperature and mixing velocity, a shaking incubator was used (Jeio-Tech, Korea). Reactions occurred for 12 h at 180 rpm.

2.3. Condition optimization

To investigate the optimum condition, sequential reaction procedure was designed. At first, the effect of regioselectivity and immobilization of lipase on the conversion of glycerol acetate was investigated. Novozym 435 and Lipozyme RM IM were loaded at 5.0 g/L as immobilized lipases and the non-immobilized lipase, Amano AK was loaded at 0.5 g/L. The molar ratio of glycerol to methyl acetate, the reaction temperature and the solvent were 1:5, 30 °C, and THF, respectively.

Various enzyme concentrations were investigated: 1.0, 2.5, 5.0, 10.0, and 15.0 g/L based on total reaction volume. Novozym 435 was used as the enzyme. The molar ratio of glycerol to methyl acetate was 1:5 and reactions were performed at 30 °C. THF was used as the solvent.

To analyze the effects of molar ratio of glycerol to methyl acetate on the conversion, various molar ratios such as 1:5, 1:10, 1:20, 1:40, and 1:60 were investigated. The concentration of Novozym 435, reaction temperature and solvent were 2.5 g/L, 30 °C, and THF, respectively.

The effects of reaction temperature on the conversion of glycerol acetate were investigated from 20 to 50 °C at 10 °C intervals. 2.5 g/L of Novozym 435 was used as the enzyme and the molar ratio of glycerol to methyl acetate was 1:40. THF was used as the solvent.

DMSO, acetonitrile, THF, tert-butanol, and 1,2-dichloroethane were selected based on their hydrophilicity. Although there is small difference in the hydrophilicity between THF and tert-butanol, both of them were selected. Because THF is generally used as an organic solvent for various industrial fields, and tert-butanol is known as an appropriate solvent for the enzymatic reaction [14]. 2.5 g/L of Novozym 435 was loaded in each sample. The molar ratio of glycerol to methyl acetate was 1:40. Reactions were performed at 40 °C in five different solvents.

2.4. Application of crude glycerol

To confirm the applicability of the enzymatic reaction in real process, pure and crude glycerol were used. Reactions were performed at optimum condition (2.5 g/L of Novozym 435, 1:40 molar ratio, 40 °C, and tert-butanol).

2.5. Analytical methods

1.0 mL of sample was taken from the reaction mixture and placed in 1.5 mL tube using a syringe and centrifuged at 12,000 rpm for 10 min. Then supernatant was filtered to remove the enzyme. Hydrophobic filter (Advantec DISMIC-13JP PTFE 0.20 μm, Japan) was used. The prepared samples were analyzed using an Agilent 7890A gas chromatograph, equipped with a DB-5MS capillary column (5% phenyl methyl polysiloxane capillary, 30.0 m × 250.0 μm × 250.0 μm) and a flame ionization detector. 1 μL of the sample was injected in the gas chromatograph and nitrogen was used as the carrier gas. Oven temperature was programmed: initial temperature was 140 °C for 2 min, and then oven was heated at a rate of 20 °C/min to 240 °C. The oven temperature was maintained at 240 °C for 2 min. The inlet and detector temperatures were set at 210 and 250 °C, respectively. The conversions of glycerol acetate were calculated by the following Eq. (1).

$$\text{conversion of glycerol acetate}\,(\%) = \frac{\text{mol of glycerol (mono + di + tri) acetate}}{\text{mol of glycerol in the sample}} \times 100\% \quad (1)$$