Appl Biochem Biotechnol (2010) 161:491–501
DOI 10.1007/s12010-009-8839-x

Microbial Fed-batch Production of 1,3-Propanediol Using Raw Glycerol with Suspended and Immobilized *Klebsiella pneumoniae*

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Received: 23 May 2009 / Accepted: 25 October 2009 /
Published online: 18 November 2009

Abstract The production of 1,3-propanediol (1,3-PD) was investigated with *Klebsiella pneumoniae* DSM 4799 using raw glycerol without purification obtained from a biodiesel production process. Fed-batch cultures with suspended cells revealed that 1,3-PD production was more effective when utilizing raw glycerol than pure glycerol (productivity after 47 h of fermentation, 0.84 $gL^{-1}h^{-1}$ versus 1.51 $gL^{-1}h^{-1}$ with pure and raw glycerol, respectively). In addition, more than 80 g/L of 1,3-PD was produced using raw glycerol; this is the highest 1,3-PD concentration reported thus far for *K. pneumoniae* using raw glycerol. Repeated fed-batch fermentation with cell immobilization in a fixed-bed reactor was performed to enhance 1,3-PD production. Production of 1,3-PD increased with the cycle number (1.06 $gL^{-1}h^{-1}$ versus 1.61 $gL^{-1}h^{-1}$ at the first and fourth cycle, respectively) due to successful cell immobilization. During 46 cycles of fed-batch fermentation taking place over 1,460 h, a stable and reproducible 1,3-PD production performance was observed with both pure and raw glycerol. Based on our results, repeated fed batch with immobilized cells is an efficient fermentor configuration, and raw glycerol can be utilized to produce 1,3-PD without inhibitory effects caused by accumulated impurities.

Keywords 1,3-Propanediol · Glycerol · Immobilization · Klebsiella pneumonia · Fed-batch

Introduction

In recent years, biodiesel production has increased considerably because it is an environmentally friendly and renewable alternative diesel fuel. During the production of biodiesel, a large amount of raw glycerol is generated as a by-product in the range of 10% (*w*/*w*) of biodiesel production [1]. The tremendous increase in the glycerol supply has led to a remarkable drop

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in its price, resulting in a market price of raw glycerol of about 2.5 cents/lb in 2006 [2]; therefore, there is a need to develop novel technical methods using glycerol as a raw material.

Glycerol can be converted to 1,3-propanediol (1,3-PD) by either chemical or biochemical methods. Recently, 1,3-PD has attracted much attention because it can be used as an essential monomer to synthesize a new type of polyester, polytrimethylene terephthalate (PTT) and as an ingredient in polymers, cosmetics, foods, lubricants, and medicines [3]. Although glycerol can be converted to 1,3-PD using chemical catalysts, there are several disadvantages such as the high pressures and temperatures required for the chemical reaction, the use of toxic organic solvents, and the especially low yields (5~15% *w*/*w*) for 1,3-PD and various by-products [e.g., 1,2-propanediol; 4, 5]. Unlike the chemical conversion of glycerol to 1,3-PD, the biological conversion of glycerol to 1,3-PD is an environmentally friendly and economical process due to the low-energy requirement with mild reaction conditions in comparison with chemical processes and higher yield of 1,3-PD than that achieved via chemical reaction.

1,3-PD can be produced under anaerobic/microaerobic conditions by various microorganisms such as *Klebsiella pneumoniae*, *Klebsiella oxytoca, Enterobacter agglomerans*, *Citrobacter freundii*, and *Clostridium butyricum* [6]. One efficient way to produce 1,3-PD is the fed-batch process in which the addition of substrate is controlled to achieve a high cell density and high production of 1,3-PD by avoiding substrate inhibition [7–9]. Although there are numerous reports demonstrating a high production of 1,3-PD using fed-batch fermentations, just a few reports have evaluated the feasibility of raw glycerol as a substrate for 1,3-PD production with *Klebsiella* species. Mu et al. [8] used raw glycerol to produce 1,3-PD by *K. pneumoniae* in fed-batch fermentation and obtained a 1,3-PD concentration of 51.3~53 g/L, while 61.9 g/L 1,3-PD was produced with pure glycerol. Mu et al. also examined the combined process of biodiesel production (by lipase) and 1,3-PD production [1] in which glycerol was passed through the membrane for 1,3-PD production. Regarding cell immobilization systems utilized for 1,3-PD production, *K. pneumoniae* encapsulated in sodium cellulose sulfate/poly-dimethyl-diallyl-ammonium chloride microcapsules was studied with a fixed-bed reactor in a fed-batch fermentation mode [10]. The final concentration of the first fed batch using this system was 51.86 g/L for 67 h, but this result was obtained with pure glycerol.

In this study, raw glycerol derived from the alkali-catalyzed biodiesel process was utilized without any purification to produce 1,3-PD using *K. pneumoniae* DSM 4799 in batch, fed batch with suspended cells, and repeated fed batch with immobilized cells. In order to investigate any inhibitory effect of raw glycerol, pure glycerol was also tested under the same cultivation conditions. Repeated fed-batch fermentation with immobilized cells was chosen because the immobilized cells can provide sufficient biomass at the beginning of fed-batch fermentation with no need for preculture, leading to accelerated 1,3-PD production without a lag phase. To our knowledge, there has been no other report thus far demonstrating 1,3-PD production using raw glycerol directly in repeated fed-batch fermentation.

Materials and Methods

Microorganism and Medium

K. pneumoniae DSM 4799 was obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Branunschweig, Germany). The preculture medium contained the following (per liter): peptone 5 g and beef extract 3 g. A fermentation medium for 1,3-PD

production contained (per liter): K_2HPO_4 5 g, KH_2PO_4 3 g, $(NH_4)_2SO_4$ 2 g, $MgSO_4 \cdot 7H_2O$ 0.4 g, $CaCl_2 \cdot 2H_2O$ 0.1 g, yeast extract 2 g, peptone 0.5 g, beef extract 0.3 g (Difco Laboratories, Becton, Dickinson & Co., USA), and trace element solutions 1 mL [11]. The carbon sources used were pure glycerol (J. T. Baker, 99.9%) and raw glycerol (Nexenco, Korea), which was a main by-product (purity 80% *w*/*w*) from the methanolysis of soybean oil using an alkali-catalyzed process. The impurities in raw glycerol were methanol (0.27 wt%), water (0.05 wt%), MONG (matter organic non-glycerol) (17.0 wt%), sodium (13,660 mg/kg), potassium (70 mg/kg), and magnesium (1.9 mg/kg).

Batch Fermentation with Serum Bottles

To investigate the effect of initial glycerol concentration on 1,3-PD production, batch experiments were carried out anaerobically in 125 mL serum bottles containing 50 mL fermentation medium. Precultures were cultivated aerobically for 16 h in 250 mL flasks (containing 50 mL medium). The medium in serum bottles was purged with nitrogen gas to remove dissolved oxygen, and then the serum bottles were sealed with septa and aluminum crimp seals. After autoclaving the serum bottles, precultures of *K. pneumoniae* DSM 4799 were inoculated (2.5% [*v*/*v*]) and incubated at 26°C with shaking (150 rpm). Pure and raw glycerol were added to final concentrations of 20, 40, 60, 80, and 100 g/L, and the initial pH was adjusted to 7.

Fed-batch Fermentation

Fed-batch fermentations were conducted in a 3 L fermentor (Fermentec Co. Ltd., Korea) with a working volume of 1.5 L. After autoclaving the reactor and medium and purging it with filtered oxygen-free argon gas, a preculture of *K. pneumoniae* DSM 4799 (grown at the same conditions previously described) was inoculated (5% [*v*/*v*]). The concentration of glycerol was initially at 40 g/L, and it was maintained between 10 g/L and 30 g/L during the course of cultivation by feeding concentrated glycerol solutions (600 g/L). All the fed-batch fermentation experiments were carried out at 30°C and 200 rpm, and pH was controlled automatically at 7.0 with 5 N KOH. During the fed-batch operations, oxidation reduction potential (ORP) was maintained between −150 ~ −450 mV without nitrogen gas supply as long as gas production was observed.

Likewise, fed-batch operation with immobilized cells was carried out using 5×5×5 mm porous hydrophobic polyurethane media [12]. A schematic description of the fermentor and fixed-bed reactor is shown in Fig. 1. A fixed-bed reactor (FBR), 29 cm in length, with a 7-cm inside diameter, and a total volume of 1.1 L was made of a glass column packed with the immobilization media. After autoclaving the fermentor containing 2.5 L of medium and FBR, the medium was purged with filtered argon gas and circulated through the FBR to create anaerobic conditions. After circulating medium through the FBR for 30 min, the volume in the fermentor vessel was 1.5 L, and consequently, 1 L of medium was retained in the FBR. The fermentor was inoculated (2.5% [*v*/*v*]) and operated at 30°C, 200 rpm, and pH7.0 using 5 N KOH, unless otherwise stated. Cell immobilization was achieved by circulating the fermentation broth through the FBR at a pumping rate of 43 or 86 mL/min. Recirculation of the fermentation broth also helped establish well-mixed conditions in the FBR, reducing the difference in glycerol, 1,3-PD, and pH between the fermentor and FBR. For repeated fed-batch operation, when 1,3-PD concentration reached 30 ~ 60 g/L, the fermentation broth was removed from the fermentor, and then a fresh medium (1.5 L) was added to start a new fed batch. The initial working volume for each fed batch was 2.5 L, as

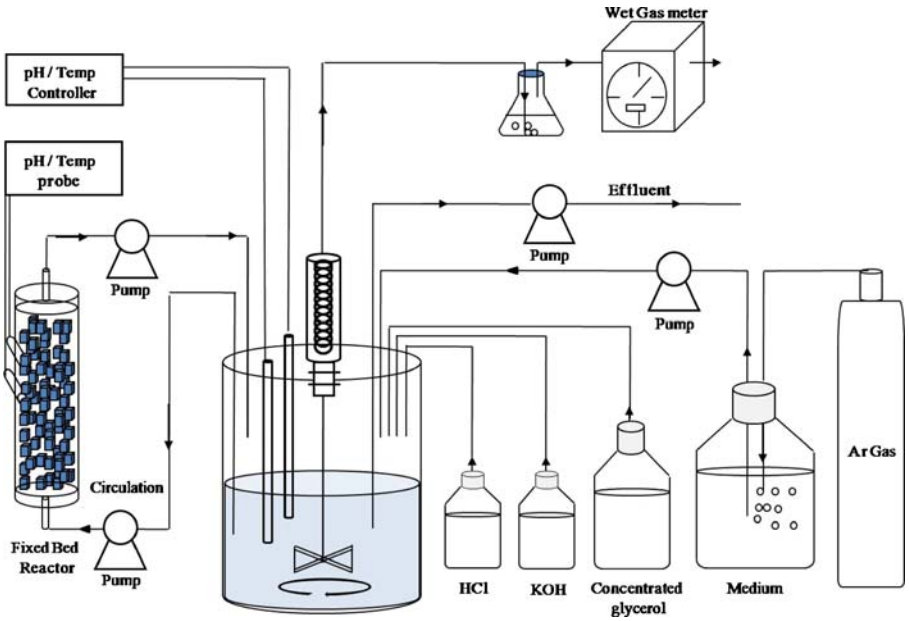

Fig. 1 Schematic diagram of the fixed-bed reactor and fermentor

1 L of fermentation broth was retained in the FBR. The ORP was maintained at −150~ −450 mV without nitrogen gas supply. A concentrated complex nitrogen source was added along with glycerol during the fed-batch fermentation to balance carbon and nitrogen nutrients.

Analytical Methods

The cell concentration was determined by measuring optical density at 600 nm with a UV spectrophotometer (UVmini-1240, Shimadzu, Japan). Glycerol was measured by an enzyme reaction with free glycerol reagent (Sigma, USA). 1,3-PD, 2,3-butanediol (2,3-BD), acetic acid, and ethanol were measured by gas chromatography (Shimadzu GC-1200, FID, Agilent HP-INNOWax column [30 m×0.32 mm×0.25 μm]) under the following conditions: oven temperature 50~240°C at a rate of 30°C/min; injector temperature, 200°C; detector temperature, 240°C; and carrier gas (N_2) flow rate, 28 mL/min.

Results and Discussion

Effect of Raw Glycerol Concentration on 1,3-PD Production

The batch cultures of *K. pneumoniae* were tested to investigate whether the impurities of the raw glycerol would affect 1,3-PD production. Various initial concentrations of pure and raw glycerol were used to study the inhibitory effect of high glycerol concentration on 1,3-PD production. As shown in Table 1, after 24 h of incubation, the 1,3-PD concentrations with raw glycerol were lower than those with pure glycerol by 1.6~6.2 g/L regardless of initial glycerol concentrations. After 48 h of incubation, however, the 1,3-PD concentrations with

Table 1 Effect of pure and raw glycerol concentration on 1,3-PD production and yield under anaerobic conditions after 24 and 48 h of batch cultivation.

Glycerol	Initial glycerol concentration (g/L)	1,3-PD concentration (g/L)		Yield (mol/mol)	
		24 h	48 h	24 h	48 h
Pure	20	8.5	9.7	0.51	0.59
	40	14.2	13.8	0.43	0.42
	60	11.2	16.3	0.42	0.50
	80	7.6	10.6	0.52	0.53
	100	4.2	12.7	0.49	0.61
Raw	20	6.9	10.3	0.42	0.63
	40	8.0	17.1	0.36	0.51
	60	8.7	17.1	0.30	0.43
	80	2.2	11.8	0.11	0.26
	100	0.0	9.4	0.00	0.37

raw glycerol were higher than those with pure glycerol by 0.6~3.3 g/L at the initial glycerol concentration of 20~80 g/L, implying that raw glycerol was successfully converted to 1,3-PD after *K. pneumoniae* DSM 4799 adapted to impurities in raw glycerol. Likely, yields with raw glycerol increased with cultivation time (Table 1). For 60 g/L or higher initial glycerol concentration, the added glycerol was not utilized completely after 48 h of incubation. There was noticeable substrate inhibition of 1,3-PD production when the glycerol concentration exceeded 100 and 80 g/L with pure and raw glycerol, respectively, based on much lower 1,3-PD production than those with 20 g/L of glycerol after 24 h. Mu et al. [8] reported that 1,3-PD concentrations with two different raw glycerol solutions in batch cultures were 11~22% less than those with pure glycerol, which is similar to our results with 20 g/L raw glycerol. Unlike *K. pneumoniae* DSM 4799, several 1,3-PD-producing *Clostridium* species have been reported to be significantly inhibited by raw glycerol [13–15]. Based on our result with batch cultures, the raw glycerol used in this study seems to be able to be used as the sole carbon source for 1,3-PD production by *K. pneumoniae* DSM 4799.

Fed-batch Fermentation with Suspended Cells

Fed-batch fermentation is known to be an effective fermentation mode for producing a high concentration of 1,3-PD without substrate inhibition [6, 9, 16]. Although the batch culture results indicated that raw glycerol could be used for 1,3-PD production, the question of whether a high 1,3-PD production with raw glycerol could be achieved using fed-batch fermentation in which the accumulation of impurities occurs because of raw glycerol feeding needed to be answered. The results of fed-batch fermentation are shown in Fig. 2. With an initial glycerol concentration of 35~40 g/L, 1,3-PD was produced after a lag phase without inhibitory effect of pure and raw glycerol. After 13 h of fermentation, a higher 1,3-PD concentration was achieved with raw glycerol than with pure glycerol (13 and 16 g/L 1,3-PD with pure and raw glycerol, respectively), which is different from the result obtained with batch cultures (Table 1). The 1,3-PD yield with raw glycerol was also higher than that with pure glycerol (0.67 and 0.60 mol/mol, respectively). Interestingly, an improved performance in 1,3-PD production with raw glycerol was observed throughout the fed-batch fermentation with suspended cells, as shown in Fig. 2. After 47 h of fed-batch fermentation, the 1,3-PD

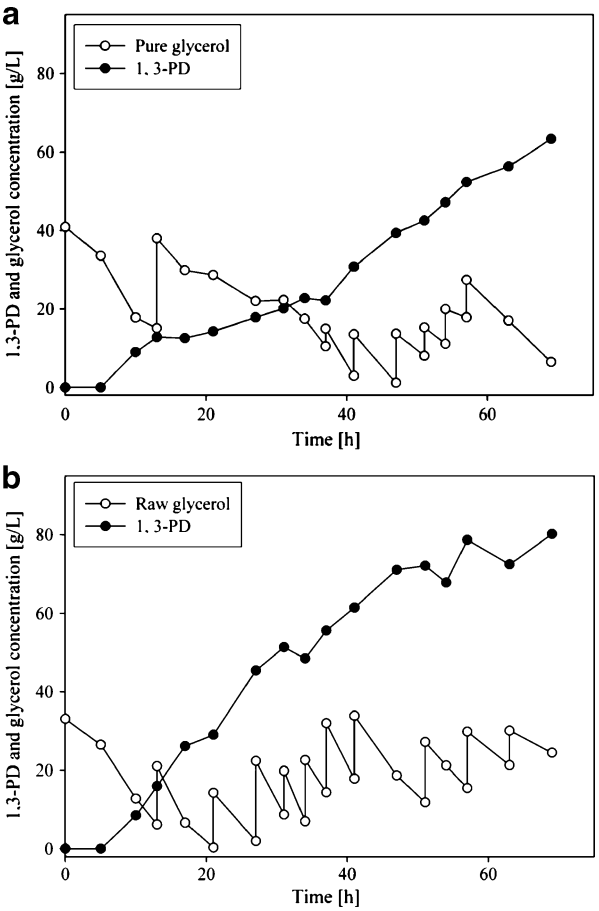

Fig. 2 Fed-batch fermentation with suspended cells of *K. pneumoniae* DSM 4799 using **a** pure glycerol and **b** raw glycerol

concentration with raw glycerol was 1.8-fold higher than that with pure glycerol (39.4 and 71.1 g/L with pure and raw glycerol, respectively). Productivity with raw glycerol was 1.51 g $L^{-1}h^{-1}$, whereas it was 0.84 g$L^{-1}h^{-1}$ with pure glycerol. Because 1,3-PD was successfully produced with 40 g/L of glycerol as shown in Table 1 and Fig. 2 (time=0∼13 h), glycerol concentration profiles shown in Fig. 2a and b are not likely to affect 1,3-PD production significantly. In contrast to our finding of a beneficial effect of raw glycerol on 1,3-PD production, a slight inhibitory effect of raw glycerol on 1,3-PD production was reported with *K. pneumoniae* DSM 2026 [2.0 and 1.7 g$L^{-1}h^{-1}$ with pure and raw glycerol; 8]. It is of interest that our results demonstrating more efficient 1,3-PD production with raw glycerol are different from other reports [1, 8, 13–15], which found reduced or similar 1,3-PD production with raw glycerol when compared to that with pure glycerol. It is not clear what caused an enhanced 1,3-PD production with raw glycerol in our results. A possible reason could be different characteristics of each raw glycerol sample depending on factors such as oil types, catalysts, alcohols, and specialized biodiesel technologies for producers, thus, resulting in different 1,3-PD production performance with various raw glycerol samples. Jerzykiewicz et al. [17] pointed out that even raw glycerol samples from the same producer exhibited diverse characteristics in soap and fatty acid content and other natural compounds originated in the vegetable oils (e.g., phenolic antioxidants).

Table 2 shows a comparison of fed-batch fermentation results with pure and raw glycerol after 47 and 69 h of operation. The 1,3-PD concentration after 69 h with raw glycerol was 80.2 g/L, which was 27% higher than that with pure glycerol. There was a decrease in 1,3-PD productivity and yield with raw glycerol after 69 h compared to that after 47 h of fermentation, likely due to product inhibition. In contrast to the results with raw glycerol after 69 h, 1,3-PD productivity and yield using pure glycerol increased when compared to those after 47 h (Table 2). Mu et al. [8] reported a final 1,3-PD concentration of 51~53 g/L using two different raw glycerol samples and 63 g/L using pure glycerol by fed-batch fermentation. There are numerous reports demonstrating high production of 1,3-PD from glycerol [7–9], but most of them were done using pure glycerol, not with untreated raw glycerol. To the best of our knowledge, this is the highest 1,3-PD concentration achieved using raw glycerol derived from biodiesel processes. On the basis of the fed-batch results with pure and raw glycerol, it could be concluded that the accumulation of impurities in raw glycerol was not likely to interfere with 1,3-PD production.

Repeated Fed-batch Fermentation with Immobilized Cells

To improve the productivity of 1,3-PD, the *K. pneumoniae* DSM 4799 cells were immobilized using hydrophobic polyurethane media in the FBR. In a previous study from our lab, the same immobilization media were found to provide a successful immobilization matrix through a long-term fermentation [12]. As the fermentation broth was circulated through the FBR, and the fed-batch process was repeated using a fill-and-draw process, visible biofilms were developed on the surface of the immobilization media. The fed batch was started up and repeated using pure glycerol to avoid any inhibition effect of impurities in raw glycerol. Because there was a certain amount of product retained in the FBR after removing fermentation broth at the end of each cycle, the concentrations of 1,3-PD, 2,3-BD, and ethanol were considerable at time=0 for a new fed batch. To minimize overestimation of product concentrations, the net production was determined using the difference of concentrations between at time=0 and at a specific fermentation time for each cycle. The net produced concentration profile of 1,3-PD, 2,3-BD, and ethanol is shown in Fig. 3. Over a time period of 1,460 h (61 days), 46 fed-batch cycles were carried out mostly every 20~48 h, revealing a stable and reproducible 1,3-PD fermentation behavior.

The first five cycles were run for 48 h or longer to examine whether a high concentration of 1,3-PD could be consistently achieved batch by batch with immobilized cells in the FBR. During the first fed-batch cycle, there was a lag phase that lasted about 6 h, and 64 g/L of

Table 2 Comparison of product concentrations, 1,3-PD yield and 1,3-PD productivity with pure and raw glycerol by suspended cells of *K. pneumoniae* DSM 4799 after 47 and 69 h of fed-batch fermentation.

	Pure glycerol		Raw glycerol	
	47 h	69 h	47 h	69 h
1,3-PD (g/L)	39.4	63.3	71.1	80.2
2,3-BD (g/L)	5.7	15.8	13.5	14.2
Ethanol (g/L)	2.2	6.5	4.1	8.2
Acetic acid (g/L)	3.6	3.9	3.6	3.0
1,3-PD yield (mol/mol)	0.50	0.64	0.67	0.55
1,3-PD productivity (g $L^{-1}h^{-1}$)	0.84	0.92	1.51	1.16

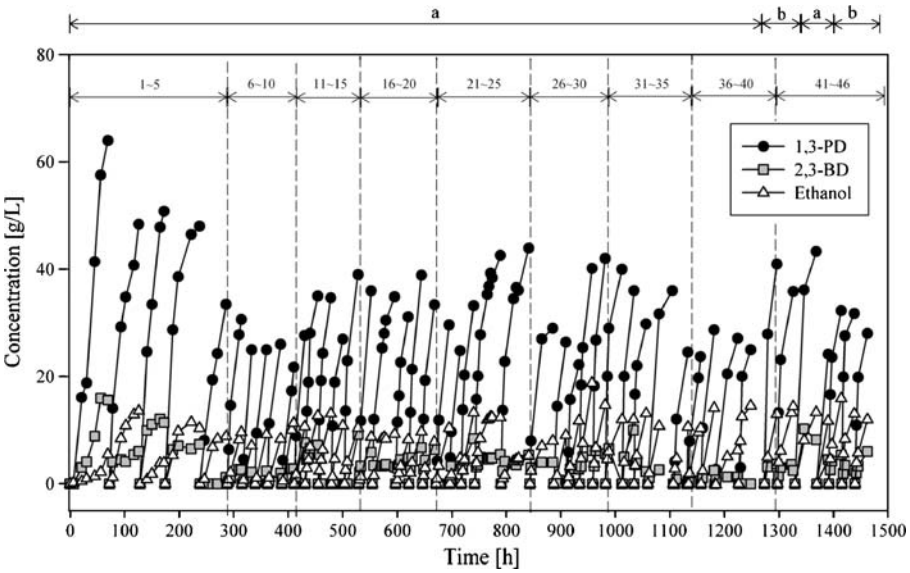

Fig. 3 Concentration profiles of 1,3-PD, 2,3-BD, and ethanol from repeated fed-batch fermentation with immobilized cells using pure and raw glycerol. Pure glycerol and raw glycerol were used during periods *a* and *b*, respectively. The concentration on the *y* axis is the net production during time=0 to t for each fed batch. The *dotted lines* are placed every five fed-batch cycles and *numbers* indicate the corresponding cycle numbers

1,3-PD was produced at 69 h, which is similar to that observed in fed-batch fermentation without the FBR (Fig. 2a). From the second to fifth fed-batch cycle, however, 1,3-PD production seemed to be inhibited when 1,3-PD concentration in the fermentor (i.e., not the net produced concentration) reached 45~50 g/L (i.e., net production of 35~40 g/L) after about 24 h of each fed batch, resulting in less net 1,3-PD production (48~51 g/L) than that of the first cycle. In contrast to the final concentration and productivity, the initial productivity of the first five cycles increased with the cycle number; productivities of 1.06, 1.39, 1.52, and 1.61 $gL^{-1}h^{-1}$ were achieved for the first to fourth cycles of fed-batch fermentation, respectively, after 20~24 h of fermentation. Based on the improvement in initial productivity, it seems that a high-cell density was established by the successful immobilization of *K. pneumoniae* DSM 4799 cells in the FBR through the repeated fed batch. Even with the high initial 1,3-PD productivity, a reduction in final 1,3-PD concentration from the first to fourth cycle occurred (Fig. 3), possibly due to a mixing and mass transfer limitation in the FBR, leading to product inhibition and a localized glycerol depletion and pH drop [10].

After the fifth fed-batch cycle, the fermentation broth was replaced with fresh medium every 20~24 h to further increase the amount of immobilized biomass by reducing the product inhibition effect. The overall final concentration of 1,3-PD was relatively stable at 30~40 g/L and the productivity around 20~24 h of fermentation remained stable in the range of 1.3~1.7 $gL^{-1}h^{-1}$. Despite circulation of fermentation broth through the FBR at 43 mL/min, the pH was lower in the FBR than in the fermentor by 0.2~0.5. This pH drop in the FBR was noticeably observed during 0~10 h of fermentation for each cycle after a fresh medium was added and circulated through the FBR. A pH decrease in 1,3-PD fermentation has been reported in other studies, and pH-controlled 1,3-PD fermentation has

been employed for high production of 1,3-PD [7, 9, 18]. To reduce a pH drop in the FBR, the pH value in the fermentor was regulated at 7.2 (the 21st~23rd cycles for 670~740 h in Fig. 3) or 7.5 (the 33rd~34th cycles for 1,035~1,080 h in Fig. 3) and the circulation rate of the fermentation broth through the FBR increased to 86 mL/min (the 35th~39th cycles for 1,100~1,250 h in Fig. 3) for a better mixing state; however, there was no beneficial effect of high pH values and circulation rate on 1,3-PD production, implying that the mixing problem needs to be solved using a different reactor configuration, using different immobilization media or removing products in situ to reduce inhibitory effects. Additionally, because vitamin B_{12} is known to enhance 1,3-PD production by *K. pneumoniae* [19], the fed-batch fermentation was performed with 0, 5, and 10 mg/mL vitamin B_{12} in an effort to improve 1,3-PD production. In contrast to a result from Huang et al. [19], the addition of vitamin B_{12} also did not exhibit a beneficial effect on 1,3-PD production (the 37th~39th cycles), presumably because enough vitamin B_{12} was already supplied by yeast extract in the medium; the excess vitamin B_{12} appeared not to improve 1,3-PD production further. When a 48-h fermentation was attempted for the 24th and 25th fed-batch cycles (740~840 h in Fig. 3), 1,3-PD concentration increased by 21% and 27% (42.6 and 43.9 g/L) compared to the 23 h fermentation in the 24th and 25th cycles (35.3 and 34.5 g/L), respectively; but, the

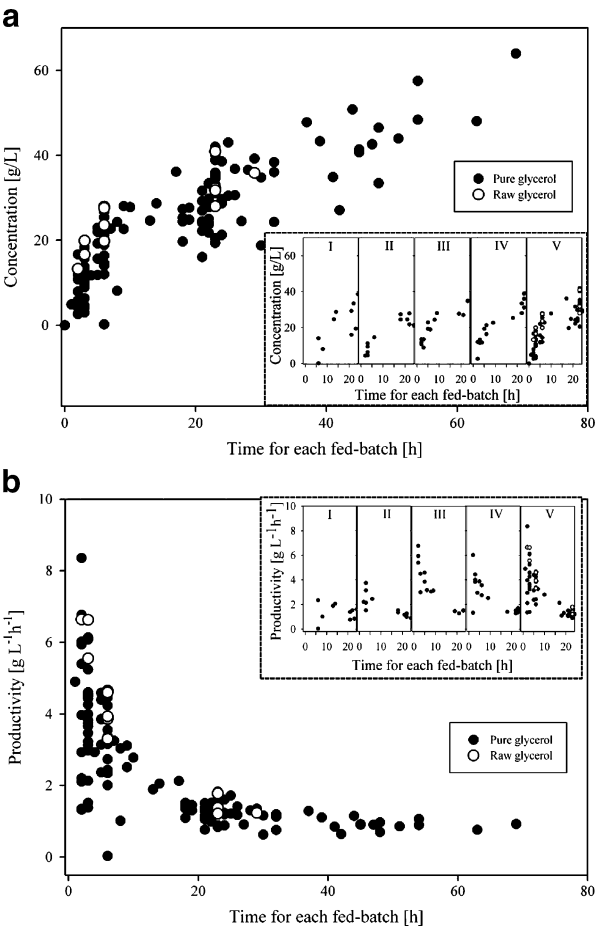

Fig. 4 Profile of **a** 1,3-PD concentration and **b** productivity with fermentation time for each fed batch with immobilized cells using pure (*filled circles*) and raw glycerol (*circles*). The inset graphs show 1,3-PD concentration and productivity during 0~24 h for each fed batch in the 1st~5th (*I*), 6th~10th (*II*), 11th~15th (*III*), 16th~20th (*IV*), and 21st~46th cycles (*V*)

productivities decreased more than 40% (1.54 $gL^{-1}h^{-1}$versus 0.91 $gL^{-1}h^{-1}$ in the 24th cycle and 1.50 $gL^{-1}h^{-1}$ versus 0.86 $gL^{-1}h^{-1}$ in the 25th cycle).

The profiles of 1,3-PD productivity and concentration during the whole repeated fed-batch process are shown in Fig. 4 with respect to the fermentation time for each fed-batch cycle. As shown in Fig. 4a, a rapid production of 1,3-PD was observed during 0~10 h of each fed-batch fermentation after filling with fresh medium, indicating a successful cell immobilization. The productivity during 0~10 h of each fed-batch fermentation tended to increase from the 1st to 15th cycles due to the increase in immobilized cells (Fig. 4b). No significant increase in productivity was observed from the 16th to 46th cycles. The productivity appeared to decrease significantly with increasing fermentation time for each fed batch, especially for fed batches with higher cycle number (Fig. 4b) in which the mixing and mass transfer problems became more severe due to the increase in immobilized cells in the FBR.

To investigate the feasibility of using raw glycerol in repeated fed-batch fermentation, raw glycerol was added in the 40th~41st cycles (1,275~1,325 h in Fig. 4) and the 44th~46th cycles (1,390~1,462 h in Fig. 4). As shown in Figs. 3 and 4, the 1,3-PD productivity and concentration using raw glycerol were similar compared to those with pure glycerol, demonstrating the feasibility of using raw glycerol as the carbon source in repeated fed-batch fermentation without an inhibition effect.

Conclusion

Fed-batch fermentation with suspended cells and repeated fed-batch fermentation with immobilized cells were conducted with pure and raw glycerol derived from the alkali-catalyzed biodiesel process using *K. pneumoniae* DSM 4799 under anaerobic conditions. In fed-batch fermentations, cultures utilizing raw glycerol showed more effective 1,3-PD production than those with pure glycerol. During repeated fed-batch fermentations with immobilization media, 1,3-PD productivity increased as cells were successfully immobilized, and a stable and high production of 1,3-PD was achieved with either pure or raw glycerol as the sole carbon source, demonstrating the feasibility of repeated fed batch with cell immobilization for 1,3-PD production. Based on the results presented above, it can be concluded that raw glycerol is an efficient carbon source for 1,3-PD production by *K. pneumoniae* DSM 4799 in batch, fed batch, and repeated fed batch with immobilized cells configurations.

Acknowledgments The authors wish to acknowledge their financial support from the Korea Energy Management Cooperation (KEMCO). This work has been performed as part of the Energy Technology Innovation Project (ETI) under the Energy Resources Technology Development Program.

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