Bioresource Technology 150 (2013) 1–8

Contents lists available at ScienceDirect

Bioresource Technology

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

Microbial recycling of glycerol to biodiesel

Liu Yang [a,b,1], Zhi Zhu [a,1], Weihua Wang [a], Xuefeng Lu [a,*]

[a] Key Laboratory of Biofuels, Shandong Provincial Key Laboratory of Energy Genetics, Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, No. 189 Songling Road, Qingdao 266101, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China

HIGHLIGHTS

- *De novo* biodiesel production using glycerol as sole carbon source.
- Genetic engineering of fatty acid ethyl ester (FAEE) biosynthetic pathway to improve biodiesel production.
- High production level of FAEEs (813 mg L^{-1}) from glycerol by fermentation optimization.

ARTICLE INFO

Article history:
Received 7 July 2013
Received in revised form 13 September 2013
Accepted 17 September 2013
Available online 2 October 2013

Keywords:
Biodiesel
Glycerol
Escherichia coli
Genetic engineering
Fermentation

ABSTRACT

The sustainable supply of lipids is the bottleneck for current biodiesel production. Here microbial recycling of glycerol, byproduct of biodiesel production to biodiesel in engineered *Escherichia coli* strains was reported. The KC3 strain with capability of producing fatty acid ethyl esters (FAEEs) from glucose was used as a starting strain to optimize fermentation conditions when using glycerol as sole carbon source. The YL15 strain overexpressing double copies of *atfA* gene displayed 1.7-fold increase of FAEE productivity compared to the KC3 strain. The titer of FAEE in YL15 strain reached to 813 mg L^{-1} in minimum medium using glycerol as sole carbon source under optimized fermentation conditions. The titer of glycerol-based FAEE production can be significantly increased by both genetic modifications and fermentation optimization. Microbial recycling of glycerol to biodiesel expands carbon sources for biodiesel production.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Worldwide increasing petroleum consumption induces economical and environmental problems at present time (Tarabet et al., 2012). Considerable attention was focused on the development of alternative fuel sources (Hansen et al., 2005). Biodiesel, fatty acid alkyl ester, is considered to be an alternative for petroleum-based diesel fuel. The most common technique for producing biodiesel is transesterification, which refers to a catalyzed chemical reaction of triacylglycerides (TAGs) with alcohols (Ma and Hanna, 1999; Rottig et al., 2010). Despite of possessing attractive prospects, the traditional biodiesel production still faces some bottlenecks as follows: the limited supply of lipid feedstock which generally relies on geographical and seasonal conditions, and the chemical transesterification which is energy consumption intensive and needs further waste treatment processes (Du and Liu, 2012; Yousuf, 2012). Glycerol as a low-price byproduct is inevitably produced in current biodiesel industry (Hoydonckx et al., 2004; Posada et al., 2012).

The global biodiesel market is estimated to reach 37 billion gallons by 2016 with an average annual growth of 42%, which means about 4 billion gallons of raw glycerol will be generated (Anand and Saxena, 2012; Yang et al., 2012). Glycerol stemming from biodiesel production flooding the market led to a dramatic decrease in prices of glycerol (Anand and Saxena, 2012; Dharmadi et al., 2006; Durnin et al., 2009). Thus, the process converting low value glycerol into high value products via biological or chemical routes currently attracts increasing interest (Chatzifragkou et al., 2011; Gungormusler et al., 2011; Posada and Cardona, 2010; Posada et al., 2012).

In terms of the issues on feedstock supply, waste cooking oils, oils accumulated by the heterotrophic microbes and photosynthetic microalgae have been used as feedstock for biodiesel production (Chisti, 2007; Ratledge and Cohen, 2008; Thanh le et al., 2010). Especially, oil from microalgae has been considered to be one of the most promising TAGs resources which provide a clue to produce biodiesel with environmental benefits and large net

* Corresponding author. Tel.: +86 532 80662712; fax: +86 532 80662629.
E-mail addresses: yangliu@qibebt.ac.cn (L. Yang), zhuzhi@qibebt.ac.cn (Z. Zhu), wangwh@qibebt.ac.cn (W. Wang), lvxf@qibebt.ac.cn (X. Lu).
[1] These authors contributed equally to this work.

0960-8524/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2013.09.073

energy gains (Schubert, 2006; Wijffels and Barbosa, 2010). Nevertheless, the high costs for feedstock and high energy consuming in the downstream processes render the production uneconomical.

Distinct from approaches utilizing the oily fraction of biomass, novel strategies for biodiesel production from sugars hydrolyzed from abundant lignocellulosic biomass were developed through constructing non-native biosynthetic pathways of biodiesel molecules in microbial hosts. This promising concept was initially put into practice in genetically engineered *Escherichia coli* strains co-expressing genes encoding enzymes for ethanol production from *Zymomonas mobilis* and the *atfA* gene encoding acyl-coenzyme A: diacylglycerol acyltransferase (WS/DGAT) from *Acinetobacter baylyi* strain ADP1 (Kalscheuer et al., 2006). Additionally, a thioesterase for releasing feedback inhibition of fatty acid biosynthesis pathway as well as an endoxylanase and a xylanase for utilization of hemicellulose were introduced, which lead to the direct biosynthesis of FAEE with a yield of 11.6 mg L^{-1} from the mixture of glucose (0.2%) and xylan (2%) (Steen et al., 2010).

Compared with traditional biodiesel production, the novel strategy for *de novo* FAEE biosynthesis from hemicellulose possesses obvious advantages due to the abundant feedstock together with simple and shortened downstream processing. Although *de novo* FAEE biosynthesis from hemicellulose could achieve the direct conversion to biodiesel, the yield is considerably low. Lignocellulosic biomass as feedstock for biodiesel production is not available for commercial application currently because of the high costs resulting from pretreatment and enzymatic hydrolysis. So the supply of feedstock is the dominant bottleneck existing in the current biodiesel production. As mentioned previously, the glycerol has recently become cheap and abundant coproduct in traditional biodiesel industry (Almeida et al., 2012). It is a suitable carbon source for *E. coli* in the *de novo* biodiesel production.

In this work, *de novo* biodiesel production with glycerol as sole carbon source was investigated (Fig. 1). Different fed-batch feeding strategies using glycerol as a carbon source for the improvement of FAEE production were compared. In addition, the influence of minimal media on the production of FAEE was examined. Metabolic engineering strategies were also applied to modify *E. coli* for the efficient production of FAEE.

2. Methods

2.1. Enzymes, DNA kits and strains

Taq, *Pfu* DNA polymerase and T4 DNA ligase were purchased from Fermentas (Burlington, Canada) and all restriction enzymes were from Takara (Kyoto, Japan). Plasmid mini kits, PCR purification kits and gel extraction kits were ordered from Omega (Norcross, USA). *E. coli* strain BL21 (DE3) and DH5α were obtained from Takara (Kyoto, Japan).

2.2. Construction of strains and plasmids

Detailed information of plasmids was shown in Table S1. The *E. coli fadD* gene with P_{BAD} promoter was excised from pMD18-T-P_{BAD}-*fadD* by *Nco*I, and inserted into pMSD15 at *Hind*III site, creating plasmid pKC14, so was the formation of pKC15 with *atfA* gene from *A. baylyi* ADP1. The plasmid pMD18-T-P_{BAD}-*atfA* was digested by *Nco*I and *atfA* gene with P_{BAD} promoter was ligated into pMSD15 at HindIII site blunt ended. *FAA2* gene was amplified from *Saccharomyces cerevisiae* INVSc1 with primers faa2-S1-CATATG GCCGCTCCAGATTATGCAC and faa2-A1-GCATGCCTAAAGCTTTTC TGTCTTGACTAC. To construct 5 kb cassette P_{BAD}-rbs-*Nde*I, the fragment excised from pXL49 by *Xba*I and *Spe*I was inserted into pBAD33 at *Xba*I site and digested by enzymes *Nde*I and *Sal*I. Restriction enzymes *Nde*I and *Xho*I were used to insert FAA2 into the cassette P_{BAD}-rbs-*Nde*I by *Nde*I and *Sal*I to generate pKC20. The *Kpn*I–*Sph*I double-digested 2 kb fragment of pKC20 was cloned into pMSD15 by *Hind*III blunt-ended to generate pKC16.

2.3. Cell transformation

BL21 (Δ*fadE*) competent cells were transformed with pMSD8 and pXT11 plasmid, and the BL21 (Δ*fadE*) (pMSD8/pXT11) mutant was screened on solid LB plates containing carbenicillin (25 μg ml^{-1}) and kanamycin (25 μg ml^{-1}). Plasmid pMSD15 was transformed into competent cells of BL21 (Δ*fadE*) (pMSD8 pXT11) to generate KC3 mutant. The plasmid pKC14 harboring *fadD* gene, pKC15 overexpressing two-copy of *atfA* gene and pKC16 harboring *FAA2* gene was co-transformed into KC2 strain together with pMSD8 and pXT11 to generate YL14, YL15 and YL16 strain respectively, which were screened on solid LB plates containing carbenicillin (25 μg ml^{-1}), kanamycin (25 μg ml^{-1}) and chloramphenicol (17 μg ml^{-1}).

2.4. Culture medium

For shake flask seed cultivation, LB media supplemented with 25 mg L^{-1} ampicillin, 25 mg L^{-1} kanamycin, and 17 mg L^{-1} chloramphenicol were used. LB media consisted of 1% w/v tryptone, 0.5% w/v yeast extract, 1% w/v NaCl. The detailed information of the fermentation media used here was shown in Table S2 of the additional files. Complex medium and chemically defined medium were evaluated as the initial medium, complex medium contained 2% w/v tryptone, 1% w/v yeast extract and 1% w/v sodium chloride, and chemically defined medium contained (%, w/v): Na_2SO_4 0.2, $(NH_4)_2SO_4$ 0.268, NH_4Cl 0.05, K_2HPO_4 1.46, $Na_2HPO_4 \cdot 12H_2O$ 0.802, $(NH_4)_2$H-citrate 0.1, $MgSO_4 \cdot 7H_2O$ 0.05, VB_1 0.001, glycerol 2 and 3 ml trace element solution ($CaCl_2$ 0.05, $ZnSO_4 \cdot 7H_2O$ 0.018, $MnSO_4 \cdot H_2O$ 0.01, Na_2-EDTA 1, $FeCl_3$ 0.8, $CuSO_4 \cdot 5H_2O$ 0.016, $CoCl_2 \cdot 6H_2O$ 0.018). Appropriate antibiotics were added (see above). The chemically defined medium used as feed solution contained 7% glycerol, 0.2% $MgSO_4 \cdot 7H_2O$ and 0.1% $(NH_4)_2SO_4$ with the exception of the complex medium for the feed solution with 7% yeast extract.

2.5. Shake flask cultures and seed cultures

Recombinant strains of *E. coli* were streaked onto LB agar plates with antibiotics (see above) and incubated at 30 °C overnight. Single colonies were picked and inoculated into 10 ml of LB media in 50 ml flasks, and the flasks were incubated at 30 °C in a rotary shaker at 200 rpm for 12 h. For seed cultures, the cells were collected by centrifugation at 5000 rpm for 1 min, resuspended into 150 ml of sterilized LB in 500 ml flasks and were incubated at 30 °C and 200 rpm for 12 h. For shake flask fermentation cultures, the cells were collected by centrifugation at 5000 rpm for 1 min, resuspended into 50 ml of sterilized chemically defined medium as mentioned above with exception for 5 g L^{-1} glycerol instead of 20 g L^{-1} in 250 ml flasks, and shaken until an OD_{600} of 1.5–2 was reached. Arabinose was subsequently added into the culture to a final concentration of 0.4% for induction of the *araBAD* promoter. One hour later, IPTG was added to a final concentration of 0.5 mol L^{-1} for induction of the T7 promoter. At about 20 h after induction, the culture was extracted for GC–MS analysis.

2.6. Fed-batch fermentation

Fed-batch fermentation was performed in a 5 L fermentor (Biostat Bplus, Sartorius) with a working volume of 3 L. The pH

Download English Version:

https://daneshyari.com/en/article/7079216

Download Persian Version:

https://daneshyari.com/article/7079216

Daneshyari.com