Journal of Biotechnology 162 (2012) 390–397

Contents lists available at SciVerse ScienceDirect

Journal of Biotechnology

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

Tandem transformation of glycerol to esters

Maria V. Sotenko[a], Martin Rebroš[b], Victor S. Sans[a], Konstantin N. Loponov[a], Matthew G. Davidson[c], Gill Stephens[d,**], Alexei A. Lapkin[a,*]

[a] School of Engineering, University of Warwick, Coventry CV4 7AL, UK
[b] Institute of Biotechnology and Food Science, Slovak University of Technology, Bratislava 812 37, Slovakia
[c] Centre for Sustainable Chemical Technology, Department of Chemistry, University of Bath, BA2 7AY, UK
[d] Faculty of Engineering, University of Nottingham, Nottingham NG7 2RD, UK

ARTICLE INFO

Article history:
Received 19 January 2012
Received in revised form 7 May 2012
Accepted 18 May 2012
Available online 13 July 2012

Keywords:
1,3-Propanediol
Rhizomucor miehei lipase
Biphasic catalysis
Esterification
Membrane contactors

ABSTRACT

Tandem transformation of glycerol *via* microbial fermentation and enzymatic esterification is presented. The reaction can be performed with purified waste glycerol from biodiesel production in a continuous mode, combining continuous fermentation with membrane-supported enzymatic esterification. Continuous anaerobic fermentation was optimized resulting in the productivity of 2.4 g L^{-1} h^{-1} of 1,3-propanediol. Biphasic esterification of 1,3-propanediol was optimized to achieve ester yield of up to 75%. A hollow fibre membrane contactor with immobilized *Rhizomucor miehei* lipase was demonstrated for the continuous tandem fermentation-esterification process.

1. Introduction

In the majority of chemical industries there is an increasing focus on security of feedstocks and a desire to develop routes to key intermediates as well as to new products starting from renewable bio-feedstocks. The highly oxygenated nature of bio-feedstocks, together with their low purity and variability, however, make them unsuitable for most conventional chemical processes, traditionally used in the supply chain of petrochemicals. Biocatalysis, on the other hand is adaptive to new and impure substrates, and thus appears to be more suitable for such transformations. Within an integrated biorefinery process (Smith, 2007), one of the roles of biocatalysis is to convert oxygenated feedstocks to a small number of common platform molecules that can be effectively transformed to the desired products using either biocatalytic or chemical methods. Conventional wisdom dictates that the fermentation products must be recovered from the aqueous mixture prior to downstream chemical conversion. However, recovery of products from dilute aqueous fermentation product streams is a rather difficult challenge, and it would be desirable to circumvent the need for energy intensive, costly separations.

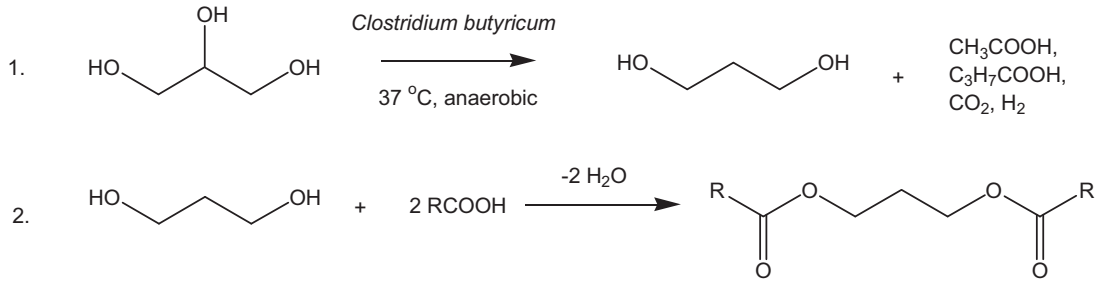
The particular focus of the current study is further development of tandem reactions where the first step is whole cell catalysis, as a means of avoiding separation after the fermentation. There are a number of examples of biocatalytic–chemical tandem reactions in the literature where the biocatalyst is an enzyme, since this is technologically a much simpler process. For example, as early as 1985, a combined bio- and chemocatalytic conversion of D-glucose/D-fructose mixtures into D-mannitol (62–66% yield) was achieved using an isomerase and a copper catalyst immobilized onto silica in an aqueous buffer (Makkee et al., 1985). More recently Walker (2004) showed conversion of codeinone to oxycodone using consecutive biological and chemical catalysis in 1-(3-hydroxypropyl)-3-methylimidazolium glycolate ionic liquid. Furthermore, one-pot tandem biocatalytic–chemical systems are widely used for resolution of racemic mixtures of alcohols and aminoacids (Alexandre et al., 2002; Pàmies and Bäckvall, 2003).

In the case of tandem systems with bacteria as catalysts, the earliest published reports involve complex extraction and purification of intermediates and consecutive chemical reaction under conditions which would be totally incompatible with the upstream bio-process. Thus, consecutive bio- and chemical catalysis for hydroquinone synthesis from D-glucose was reported by Frost's

Abbreviations: AA, acetic acid; BA, butyric acid; CLEA, cross-linked enzyme aggregates; DA, decanoic acid; Gly, glycerol; HF, hollow fibre; LA, linoleic acid; LacA, lactic acid; PD, 1,3-propanediol; PES, polyethersulphone; PP, polypropylene; Tetrad, tetradecane; C^0, initial concentration, g L^{-1}; C^{final}, final concentration, g L^{-1}; *D*, dilution rate, h^{-1}; *F*, flow rate, mL min^{-1}; *N*, number of membranes; *Q*, productivity, g L^{-1} h^{-1}; *r*, reaction rate; *S*, interfacial area, cm^2; *V*, volume, cm^3; *Y*, yield, mol mol^{-1}.

* Corresponding author. Fax: +44 02476 418922.
** Corresponding author. Fax: +44 01159 514115.
E-mail addresses: gill.stephens@nottingham.ac.uk (G. Stephens), a.lapkin@warwick.ac.uk (A.A. Lapkin).

0168-1656/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jbiotec.2012.05.026



Scheme 1. Tandem biocatalytic transformation of glycerol to 1,3-propanediol and esters.

group (Li et al., 2005; Ran et al., 2001). Glucose was converted to quinic acid using genetically modified *Escherichia coli* but then it was necessary to remove the cells and purify the product stream before converting quinic acid to hydroquinone using a chemical catalyst. A similar approach was used to convert glucose to protocatechuate, followed by extraction of the product and thermal decarboxylation to catechol.

More recently, the need to separate the intermediate fermentation product has been eliminated. Thus, Liu et al. (2009) demonstrated fermentation of glycerol to 1,3-propanediol using *Clostridium butyricum*, followed by direct use of the fermentation broth to produce secondary amines using hydrogen transfer catalysis. The key advance was to use a biphasic system containing the unpurified fermentation broth with the catalyst dissolved in an ionic liquid, so that a reactive separation could be achieved (Liu et al., 2009). This avoids the significant technological challenge involved in separating 1,3-propanediol from fermentation broths by distillation (Saxena et al., 2009). However, it was impossible to operate the fermentation and the catalytic step together, because of mutual incompatibility of the bio- and chemocatalytic processes (Liu et al., 2009). The incompatibility was caused by: (i) toxicity of chemicals (substrates, products, by-products, solvents or chemical catalysts) for the bio-system, (ii) low activity of chemical catalysts due to low temperature and pressure, and the presence of water, (iii) low rates of downstream chemical reactions due to diluted reagents, and (iv) general problems of stability.

The problem of compatibility of bio- and chemical catalysts can potentially be resolved by improved spatial and/or temporal separation of both systems within a single reaction space. Potential approaches include the use of supported catalysts, immobilized cells, biphasic media or membranes.

In the present study we aimed to develop a tandem process of fermentation of glycerol to 1,3-propanediol followed by esterification, Scheme 1, using raw glycerol from bio-diesel production. As in our previous study (Liu et al., 2009), we used *C. butyricum* to produce 1,3-propanediol, since this biocatalyst produces high concentrations of the product, contains a vitamin B_{12}-independent glycerol dehydratase (which is not deactivated by glycerol), and the toxic intermediate 3-hydroxypropionaldehyde does not accumulate (Barbirato et al., 1998). Furthermore, the product stream is relatively clean, since the only side products are H_2, CO_2 and small acids, namely acetic, butyric and lactic. In the present study we optimized the continuous fermentation process, which is more amenable for integration into an overall tandem process scheme.

This process resembles the concept of extractive fermentation. For example, in an earlier paper, fermentation of glucose was facilitated by a simultaneous esterification of the product, ethanol, by *Rhizomucor miehei* lipase in a biphasic broth/oleic acid system (Oliveira et al., 1998). Better productivity was achieved because of the simultaneous extraction of the inhibitory ethanol from the fermentation medium, but the process depends on addition of a co-substrate (oleic acid). Mu et al. (2008) developed enzymatic transesterification of oils for biodiesel production, where the resulting glycerol was converted by fermentation to 1,3-propanediol by *Klebsiella pneumoniae*. Both processes raised the problem of mutual toxicity and catalysts stability in complex multiphasic environment, when either emulsified biphasic reactor (Oliveira et al., 1998) or a selective separation membrane (Mu et al., 2008) is used to combine the processes together.

The approach developed in this study eliminates the disadvantages of stability and incompatibility by incorporating a catalytic membrane, which allows reduction of the mutual inhibition and enhances selectivity towards the desired products.

2. Experimental

2.1. Chemicals and materials

Linoleic acid (≥99%), 1,3-propanediol (≥99.6%), tetradecane (≥99%), methanol (≥99.6%), acetone (≥99.5%), potassium dihydrogen phosphate, disodium hydrogen phosphate dodecahydrate, acetic acid (≥99.7%), butyric acid (≥99%), glycerol (anhydrous, >99.5%), and *p*-nitrophenyl decanoate were purchased from Sigma–Aldrich, UK and used without further purification.

The free *R. miehei* lipase (20,000 TBU g^{-1}, 1 TBU catalyses the release of 1 μmol butyric acid per min from tributyrin) was acquired from Aldrich, UK. CLEA lipases (Table 1) were purchased from CLEA Technology (The Netherlands). Novacarb membrane (522 m^2 g^{-1} BET area, 17 nm mesopore diameter, 0.78 μm mean macropore diameter) was obtained from MAST Carbon Ltd. in resin form and carbonized in house. Polyethersulphone (PES) membrane was kindly provided by Veolia International, Paris. Polypropylene (PP) hollow fibres (40 μm i.d., 50 μm o.d., 0.005 cm wall thickness), manufactured by Celgard, Celanese, were kindly provided by Professor Kang Li, Imperial College London. It is known that the pores, 30 nm in diameter, cover about 33% of the of the polypropylene hollow fibre surface.

2.2. Fermentation experiments

C. butyricum strain DSMZ 10703 was maintained on 20% glycerol medium (0.2 mL of glycerol in 0.8 mL of distilled water). The

Table 1
Types and activities of the CLEA enzymes tested.

CLEA name	Activity (TBU g^{-1})
Rhizomucor miehei	81,300
Candida antarctica lipase A	11,400
Candida antarctica lipase B	14,000
Thermomicea lanuginosus lipase	410,000

1 TBU catalyses the release of 1 μmol butyric acid per min from tributyrin (10%, v/v) in 25 mM phosphate buffer, pH 7.5 and 40 °C.