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Enhancement of the valorization of renewable glycerol: The effects of the surfactant-enzyme interaction on the biocatalytic synthesis of glycerol carbonate

Madalina Tudorache, Alina Negoi, Vasile I. Parvulescu *

University of Bucharest, Department of Organic Chemistry, Biochemistry and Catalysis, Bd. Regina Elisabeta 4-12, Bucharest 030016, Romania

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ABSTRACT

The effect of the addition of a surfactant during the bio-conversion of a renewable glycerol into glycerol carbonate (GlyC) has been investigated. Lipase enzyme immobilized on magnetic particles assisted the GlyC synthesis via the carbonylation of glycerol originating from different oils with dimethyl carbonate (DMC). Glycerol from food production residues was compared with glycerol produced from the transesterification of soybean, rape, olive, palm, sunflower, and corn oils. Tween 20, Tween 80, Triton X or CTAB was added as surfactant in the biocatalytic system with the scope to enhance the biocatalyst performance via a direct surfactant-lipase interaction. Optimum conditions of the system were set up testing different types and concentrations of surfactant. Indeed, the biocatalyst exhibited a higher catalytic activity in the presence of the surfactant. Thus, TON increased from 13.0×10^5 to 22.6×10^5 using only 0.1% (w/w) CTAB. Beside these, the effect of the lipase-surfactant interaction was investigated in the context of biocatalyst preparation and storage conditions.

1. Introduction

Glycerol is a co-product of the biodiesel manufacture (renewable glycerol), and, as a consequence, it is generated in high quantities (e.g. 10 kg Gly for each 100 kg biodiesel product). For the moment, this production exceeds the market necessity [1]. However, glycerol represents an important raw compound in numerous fine chemistry (e.g. food, drug, cosmetic and tobacco industry) and bulk industrial chemical processes due to its high chemical reactivity [2]. The restriction in the use of the renewable glycerol is a direct consequence of its composition generated by the random presence of several impurities coming from the biodiesel process (e.g. catalyst, residual methanol, water, salts, soap and free fatty acids) [3]. These impurities can strongly affect the transformation of glycerol into the requested products. They can also inhibit the biologic processes with glycerol acting as nutrient for the cell growth [4]. Therefore, the purification of renewable glycerol is generally required in order to yield a commercial grade compound. Filtration, chemical additions, and fractional vacuum distillation are the common alternatives for the glycerol purification, also leading to the increase of the cost of the final product. Such operations are even more required for food, cosmetics or drugs applications requesting additional purifications by bleaching, deodorizing or ion exchange in order to remove all the impurity traces [5]. For these reasons, more attention has to be paid in the future on the identification of new possibilities of valorization of the renewable glycerol without any pretreatment (crude glycerol).

The biotransformation of glycerol into value-added products represents a current alternative for its valorization. Thus, 1,3-propanediol might be produced by anaerobic fermentation in fed-batch cultures of *Klebsiella pneumonia*/*Clostridium butyricum* [6–8], citric acid by fermentation with *Yarrowialipolytica* mutant cell [9], hydrogen and/or ethanol by photo-fermentation with *Rhodopseudomonas palustris* bacterium[10,11], and polyesters and biosurfactants by bacterial digestion [12,13]. Beside these, several strategies were proposed for the conversion of the impure glycerol, but most of the examples refer to the use of pure glycerol "poisoned" with impurities (i.e. artificial mixer of pure glycerol and impurities) [1,14–19]. However, these examples are far away from the real case because they cannot reproduce entirely the complex composition/effects of the matrix impurities.

A valuable alternative for the use of renewable glycerol is the biocatalytic production of glycerol carbonate (GlyC) as one of the value-added products of glycerol. This has been firstly reported by our research group [20]. GlyC is a relative new product for chemical industry holding great "valences" for the chemical synthesis area.

* Corresponding author.
E-mail address: vasile.parvulescu@g.unibuc.ro (V.I. Parvulescu).

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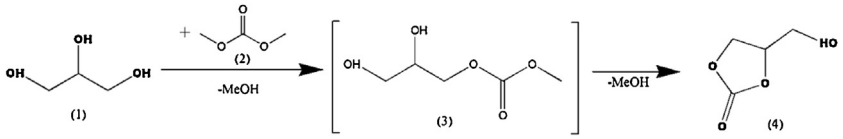

Scheme 1. Reaction of glycerol with DMC for GlyC synthesis. (1) glycerol; (2) DMC; (3) unstable intermediate; (4) glycerol carbonate.

Thus, GlyC is considered to be a "green solvent" due to its properties (e.g. high stability, low toxicity, low evaporation rate and low flammability) and a valuable intermediate for the production of resins and plastics [21–23].

The biocatalytic route for the GlyC synthesis involves the reaction of glycerol with dimethyl carbonate (DMC) assisted by a lipase (Scheme 1). The use of DMC in excess ensures the proper environment of the reaction and the process is performed under solvent-free conditions. Several alternatives derived from the presented process have been already reported providing valuable solutions to the critical aspects, such as the biocatalyst design, experimental conditions, etc. [24–28]. For renewable glycerol, we reported a biocatalytic system consisting in a lipase covalently immobilized on magnetic particles [20]. With this system renewable glycerol was converted to GlyC in a yield as high as 40%. Experimental evidences on the influences of the impurities present in the renewable glycerol were also provided. More precisely, it was demonstrated how the complex matrix of the renewable glycerol affected the catalytic activity and also the robustness of the biocatalyst [20]. However, in the optimal conditions, the biocatalyst was successfully recycled for tenth cycles. Noteworthy, an activation of the biocatalyst during the first two-three cycles was identified when the value of the GlyC yield increased with 20–50%. The investigation of this aspect is reported herein.

In this study we report an enhancement of the biocatalytic efficiency for the conversion of the renewable glycerol to GlyC using a surfactant (e.g. Tween 20, Tween 80, Triton X and CTAB) as an additive of the reaction mixture. It is well known that the surfactants (detergents) are mostly used in the biological mixtures for the disruption of the cell membrane (cell lysis) and release of the intracellular content in a soluble form. This occurs by breaking down the protein-protein/protein-lipid/lipid-lipid associations, denaturation of proteins and other macromolecules, and avoiding the unspecific binding of proteins [29–31]. Also, the surfactants are used for the preparation of stable biocatalysts in the organic mediums. Enzyme coating with surfactants occurs through a non-covalent interaction. In this way, the enzyme is protected against the denaturation and many synthetic reactions can be performed in organic solvents. However, a disadvantage of this method is the relative low yield of the preparation of the surfactant-enzyme biocomposite [32,33].

Another objective of this study was to establish the way through which the surfactant stabilizes the biocatalyst and enhances the biocatalytic interaction lipase-glycerol/DMC. Screening of glycerol separated from different biodiesel processes was performed in order to choose the most representative glycerol sample (crude/renewable glycerol). Working with crude (renewable) glycerol, which means glycerol directly separated from the biodiesel mixture without any additional purification, is less favorable due to the fast deactivation of the catalysts by either the acid/bases or other impurities. In the present example, glycerol was collected only by a simple decantation. So that, in addition to water and methanol, it contained different other impurities like metals, fatty acids, soap, etc. In our previous studies, we investigated the individual effect of these impurities [20]. However, to clarify this complex problem, in this study we enlarged our research looking for the influence of the entire matrix including all these impurities on the biocatalytic behavior. The effect of the operation conditions on the biocatalytic conversion of the renewable glycerol to GlyC have been also investigated in details.

2. Experimental

2.1. Chemicals and solutions

Aspargillus niger lipase and dimethyl carbonate (DMC) were purchased from Sigma-Aldrich (USA). Magnetic nano-particles with 50 nm external diameter and shell structure of Fe_3O_4 magnetic core covered by polyethylenamine (PEA) (Chemicell, Rostock, Germany) were used for the lipase-biocatalyst preparation. 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) was used as immobilization reagent. A solution of 0.1 M 2-(N-morpholino)ethanesulfonic acid (MES), pH 4.7/7.4 was used as a coupling buffer for the enzyme attachment. Phosphate buffer saline (10 mM PBS) was prepared by the dilution of the stock PBS solution (100 mM PBS containing 8 g NaCl, 0.2 g KCl, 1.43 g $Na_2HPO_4 \times 2H_2O$ and 0.34 g KH_2PO_4 in 1 L distilled water) and checking/adjusting the pH value to 7.4. All the synthesis reagents were purchased from Sigma-Aldrich (USA). Tween 20 (polysorbate 20), Tween 80 (polysorbate 80), Triton X (polyethylene oxide) and CTAB (cetyltrimethylammonium bromide) were purchased from Sigma-Aldrich (USA), as well. Derivatization reagents (BSTFA - N,O-bis(trimethylsilyl) trifluoroacetamide, TMCS - trimethylchlorosilane (BSTFA:TMCS = 99:1) and pyridine) were purchased from Macherey-Nagel Corp. (Duren, Germany) and Fluka (Switzerland). The organic solvents used in all the experiments were of the analytic purity.

2.2. Production of renewable glycerol

Renewable glycerol was prepared from different oil sources (e.g. residual, soybean, rape, olive, palm, sunflower, and corn oils) using a reported biodiesel procedure [34]. The produced glycerol was separated from the reaction mixture by simple decantation. Rape and corn oils were of industrial purity (unrefined oils), while the others were refined oils (e.g. soybean, sunflower, olive, and palm oils). Glycerol from residual sun-flower oil recovered from the cooking process was used as well.

2.3. Preparation of the biocatalyst

Aspergillus niger lipase was covalently immobilized on the magnetic nano-particles based on a protocol reported previously [28]. The particles were added in a solution containing 10 mg/mL EDC and 43 μg/mL of lipase dissolved in 0.1 M MES (pH 4.7). The suspension was stirred gently for 2 h. The resulted lipase-beads biocatalyst was washed with a PBS solution (0.1 M, pH 7.4) for 3 times. Then, the biocomposites were re-suspended in the blocking solution in order to remove the enzyme excess and to avoid the unspecific binding on the particle surface. BSA solution (0.1% BSA and 0.05% sodium azide in 0.1 M PBS) were tested for the blocking step. After preparation, the biocatalyst was stocked in PBS solution (0.1 M PBS, pH = 7.4) before use.