DEVELOPMENT OF STRATEGIES FOR TRANSFORMATION OF GLYCEROL VIA BIOTECHNOLOGY AND CHEMICAL ROUTES

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1,3-Propanediol (1,3 PDO) is a chemical compound of remarkable value in the synthesis of terephthalate polymers, cosmetics, lubricants, among others; 3-hydroxypropionaldehyde (3-HPA) is used for food preservation, polymer production and as a precursor to many chemicals, such as acrolein, citric acid and also of 1,3 PDO itself. Both compounds can be produced biologically by microrganisms via fermentation of glycerol, which is generated in excessive amounts as a byproduct of biodiesel production. The project goal is to obtain conversion of glycerol to 3-HPA and 1,3 PDO through two microbiological strategies. One is to perform genetic modifications in other organisms using genes from *Klebsiella pneumoniae* bacteria, which is one of the largest producers of 1,3 PDO. The other strategy is to improve the conversion of these products (mainly 1,3 PDO) by *Lactobacillus reuteri*, which is also a natural producer microorganism. Another objective set out in the project is the chemical modification of glycerol to generate ionic liquids, so-called green solvents. These compounds have relevance for the development of new materials replacing organic solvents, which are used in such synthesis reactions.

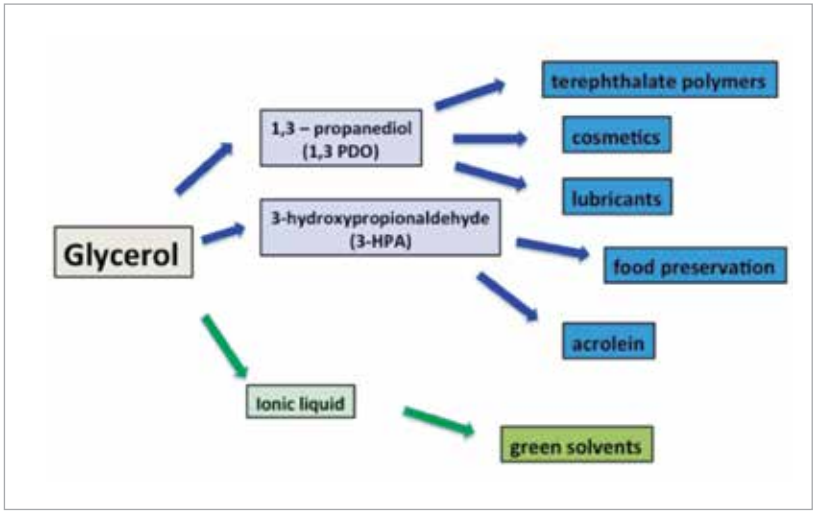

Figure 1. Glycerol from diesel waste may be converted by the microbiological (blue) or chemical (green) pathways to other sustainable products, as indicated

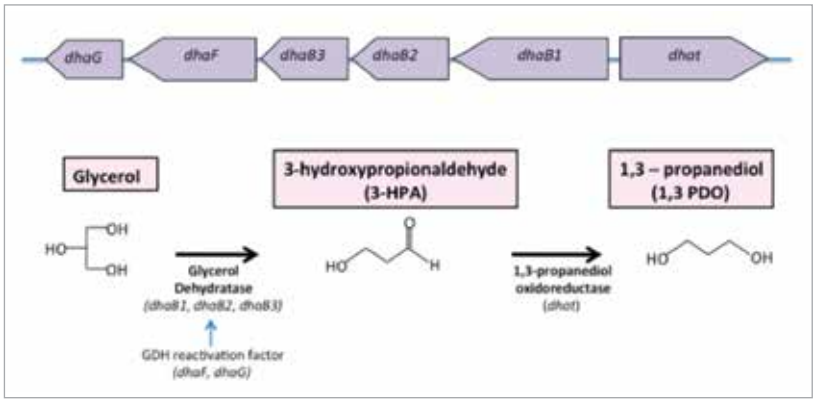

Figure 2. Glycerol assimilation through the fermentative pathway, showing the reductive pathway, producing the 3-HPA and 1,3 PDO. In this pathway, glycerol is reduced to 1,3-PDO by the successive action of glycerol dehydratase (GDH) (encoded by three dhaB genes) and 1,3 propanediol oxidoreductase (encoded by dhaT gene). On top, the genomic arrangement of dha genes present in Klebsiella pneumoniae is depicted; arrows indicate the direction of transcription. The genes refer to those found in K. pneumoniae (modified from Martins-Pinheiro et al, submitted to publication)

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The heterologous expression of genes from *K. pneumoniae* did not lead to the expected high production of 3-HPA or 1,3-PDO in *Escherichia coli*. Therefore, we focused our efforts on two alternative strategies to overcome the difficulties and develop a specific process, with several elements of originality, for the production of 1,3-PDO from glycerol. One of the strategies was to identify functional homologs from the synthetic route in prokaryote genomes through *in silico* analyses. These analyses revealed the evolution of the genes related to the glycerol metabolic route, as this was found to be present in several bacterial species and even in one Archaea species. They also converge with the project goals, identifying new potential genes that can be used for obtaining 1,3-PDO from glycerol. The results were submitted for publication. In the second strategy, the use of the bacteria *Lactobacillus reuteri* was employed with success by increasing the glycerol conversion factor and productivity of 1,3-PDO. We investigated the behavior of Lactobacillus reuteri ATCC23272 when cultivated in batch, repeated batch and continuous modes (chemostat process) during the conversion of glycerol into 1,3-PDO. The production of this product in repeated batch, with cell decantation, was reported for the first time; the results in the chemostat indicated a high productivity of 1,3-PDO (4.92 $gL^{-1}h^{-1}$), with a yield based on glycerol near the theoretical maximum. Moreover, 1,3-PDO productivity was observed to be directly related to the rate of glucose consumption and, because the chemostat process favors glucose uptake rate, it favored production. Thus, a novel process for 1,3-PDO production from glycerol was developed, yielding a patent claim.

Concerning the chemical route to produce ionic liquids, the first trials have shown the feasibility of synthesis using glycerol and 1-methylimidazole as starting materials. The step of preparing the precursor glycerol trimesilate was well established, but nucleophilic substitution with 1-methylimidazole resulted in a mixture of products derived from tri- and di-substitution in a ratio of 3: 2. Optimization of the reaction conditions produced the tri-substituted ionic liquid in higher purity and larger quantities (grams). The presence of a recurrent impurity indicated a possible thermal instability of the tri-substituted ionic liquid, the pyrolysis of which under controlled conditions indeed resulted in the production of another novel ionic liquid, formed by the elimination of the central methylimidazolium group. This thermal instability, not anticipated in advance, greatly limits the use of the tri-substituted ionic liquid in practical applications. Nonetheless, the new disubstituted propenyl ionic liquid, formed as the predominant pyrolysis product, could have interesting and unique properties. The polymerization of compound 4 could potentially form a much more stable polymeric ionic liquid derivative of polypropylene, a possibility not explored further due to the conclusion of the project.

MAIN PUBLICATIONS

Vieira PB, Kilikian BV, Bastos RV, Perpetuo EA, Nascimento C.A.O. 2015. Process strategies for enhanced production of 1,3-propanediol by *Lactobacillus reuteri* using glycerol as a co-substrate . *Biochemical Engineering Journal*. **94**: 30-38.

Martins-Pinheiro M, Lima WC. Asif H, Nascimento CAO, Menck CFM. 2015. Evolutionary and functional relationships of the dha regulon by genomic context analysis. *Plos One*. **11(3)**: e0150772.

Vieira PB, Kilikian BV, Perpetuo EA, Nascimento CAO. A chemostat study of production of 1,3-propanediol from glycerol in *Lactobacillus reuteri*. *In Preparation*.

Patent Claim:

Vieira PB, Kilikian BV, Perpetuo EA, Menck CFM, Nascimento CAO. Process strategies for enhanced production of 1,3 propanediol by *Lactobacillus reuteri* using glycerol as a co-substrate. 2014, Brazil. Patent: Model of Utility. Number of Claim: 61945556, date: February 27th 2014, title: "Process strategies for enhanced production of 1,3 propanediol by *Lactobacillus reuteri* using glycerol as a co-substrate", Claim Institution: United States Patent and Trademark Office.

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