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

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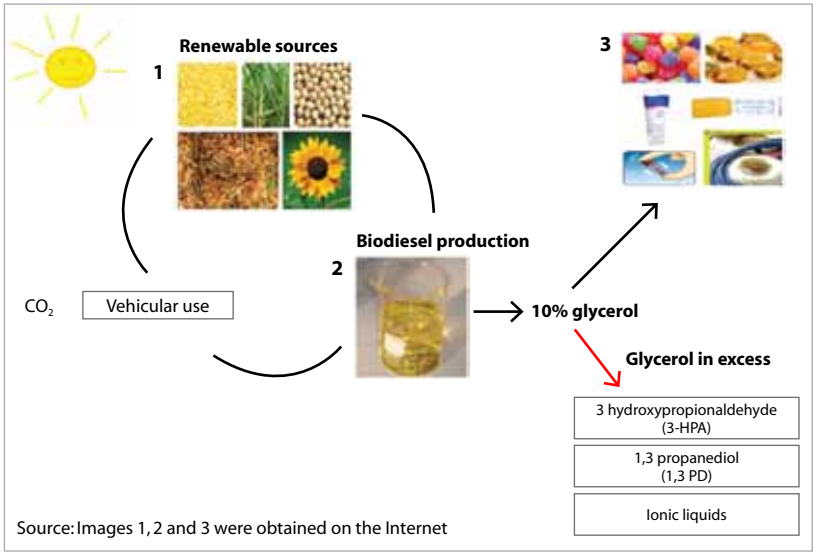
Glycerol has important features that are useful in industry in general. However, as an abundant co-product in biodiesel production, the quantity will certainly exceed industry demand. This will probably cause an economic issue and environmental problems unless new technologies are developed quickly to take advantage of this surplus. A noble purpose and profit can be obtained from glycerol by its conversion to 3-hydroxy-propionaldehyde (3-HPA) and/or 1,3-propanediol (1,3 PD), products with high added market value. These compounds are potential key raw materials for food preservation and for the production of polymers. The conversion of glycerol to these compounds by microbiological routes has been developed by several groups, but still requires new insights in order to be sufficiently productive for commercial application. The identification and characterization of microrganisms with desirable characteristics could be an alternative for achieving this goal.



Source: Images 1, 2 and 3 were obtained on the Internet

In order to obtain a more efficient biological system that is able to tolerate large amounts of 3-HPA, the genes from the pathogenic microorganism *Klebsiella pneumoniae* that encode the proteins involved in the conversion of glycerol to 3-HPA and 1,3-PD will be introduced into *Caulobacter crescentus* by using suitable expression vectors. Although *Caulobacter* has not been used previously in biotechnological processes, *Caulobacter crescentus* is a free-living, readily cultivated bacterium with clear potential for reducing cost in biotechnological processes. These same genes of *Klebsiella* will be cloned in pET vector for production of the enzymes of interest in *E. coli,* which will hopefully allow the conversion of glycerol to 3-HPA and 1,3-PD *in vitro*. High Performance Liquid Chromatography (HPLC) will be used to identify and quantify the products 3-HPA and 1,3-PD in the bacterial cultures and in the *in vitro* transformations of glycerol. When suitable glycerol-transforming bacteria are obtained, operational conditions for glycerol fermentation will be optimized in pilot scale reactors designed for this purpose. A final objective of this project is the chemical transformation of glycerol into a series of novel ionic liquids via a straightforward sequence of reactions. Ionic liquids are non-volatile organic salts that are liquid at or around ambient temperatures. They are excellent "green" solvents for a variety of chemical processes of practical interest and widely used as a reaction medium for catalytic reactions, in particular those that involve dispersed nanoparticles.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Six out of seven genes from *Klebsiella pneumoniae* that encode proteins involved in the conversion of glycerol to 3-HPA and 1,3-PD have been cloned and their integrity confirmed by DNA sequencing. These genes are being transferred to *E. coli* vectors, and the transgenic strains are being tested for their efficiency and activity. We are transferring these genes to *Caulobacter crescentus* by using suitable expression vectors. These cloning experiments will provide transgenic strains with prospective industrial application, which hopefully would reduce cost and allow large-scale production of the enzymes required for the glycerol conversion. Vectors bearing the genes of enzymes involved in glycerol conversion under the control of promoters inducible by specific conditions (oxygen tension, concentration of glucose, or specific carbon source etc.) are being constructed. The conversion of glycerol to 3-HPA and 1,3-PD by these transgenic strains is being tested by cultivating the *E. coli* strains under different conditions. Several synthetic routes for the transformation of glycerol into ionic liquids via chemical reaction (green chemistry) are currently being explored. Several intermediate compounds along these routes have been obtained in good yields and optimization of the reaction conditions for the final step in the preparation of an ionic liquid by one of these routes is currently in progress.

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