

## New Strategies for the Production of Butanol

Bioengineering Systems PhD  
University of Minho

Background: MSc. Biochemical Engineering,  
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### Objectives

Butanol is an aliphatic saturated alcohol that can be used as intermediate in chemical synthesis and as a solvent for a wide variety of chemical and textile industry applications. Moreover, it has been recognized that it has better properties than ethanol for gasoline substitution and can be biologically produced from renewable sources.

In this context, glycerol, generated as a by-product during the production of plant-oil derived biodiesel, arises as a potential substrate candidate for butanol production. Fermentation of low grade glycerol to butanol has been proven. However, there is place for process optimization in order to improve the production yields and reduce the toxicity of butanol to the producing organisms which are the main objectives of this PhD thesis.

### Work plan

Pure and mixed culture: Progressive acclimatization to increasing glycerol concentrations and mutation will be used as selection tools in order to obtain overproducing microorganisms

Modeling: Development of energy based models for butanol producing cultures

Synthetic biology: Engineering of *Pseudomonas putida* strain as a model organism for the production of butanol

from glycerol. Adequate genetic modules will be identified and constructed to facilitate substrate uptake, conduct the required biotransformations and attenuate the toxic effects of butanol, in order to achieve higher butanol yields

### Results

*Clostridium pasteurianum* DMS 525 was assessed for batch fermentation of crude glycerol using a chemically defined medium (Fig. 1 and 2) and a semi-defined medium (Fig. 3 and 4) where pure vitamins were replaced by yeast extract. Main fermentation products were acids, butanol and 1,3-propanediol. For defined medium butanol yields were around 0,3 g/g. Even though fermentation time decreased considerably when yeast extract was used, butanol yield was lower and there was a higher 1,3-propanediol production.

Mixed culture fermentations using granular sludge were performed (results not shown). Heat and contact with BES (bromoethanesulfonate) were assessed as pre-treatments and even though glycerol consumption was faster than in pure culture fermentations, solvents were not present as products. Main products obtained were acids and 1,3-propanediol.

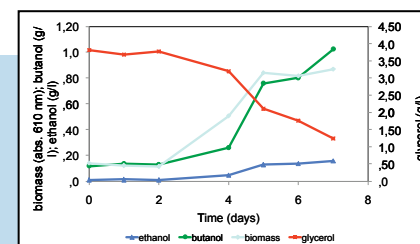


Fig. 1. Biomass and solvent production by *C. pasteurianum* in defined media with crude glycerol as carbon source

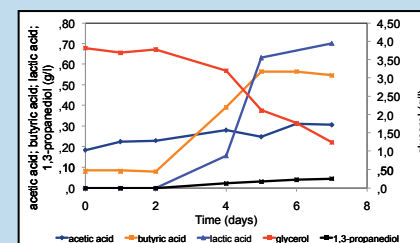


Fig. 2. Acids and 1,3-propanediol production by *C. pasteurianum* in defined media with crude glycerol as carbon source

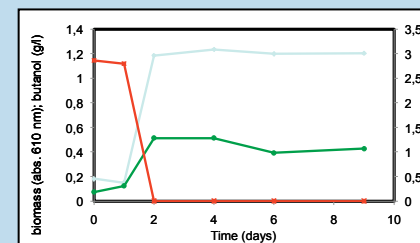


Fig. 3. Biomass and solvent production by *C. pasteurianum* in semidefined media with crude glycerol as carbon source

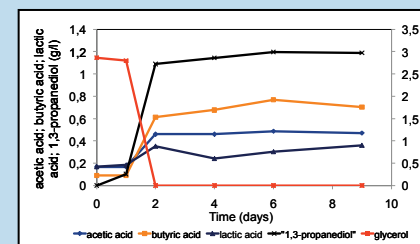


Fig. 4. Acids and 1,3-propanediol production by *C. pasteurianum* in semidefined media with crude glycerol as carbon source

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