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NEW STRATEGIES FOR THE PRODUCTION OF BUTANOL

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KEYWORDS

Biofuels, butanol, *Clostridium pasteurianum*, granular sludge

INTRODUCTION

Biofuels are an attractive alternative to current petroleum-based fuels as they can be utilized as transportation fuels with little change to current technologies and have significant potential to improve sustainability and reduce greenhouse gas emissions. Even though gaseous biofuels such as methane and hydrogen can be produced microbiologically, liquid energy carriers (ethanol, butanol, among others) offer some advantages in the transportation sector (Karakashev et al. 2007). Among liquid biofuels, the biologically derived n-butanol is receiving considerable attention.

Butanol is an aliphatic saturated alcohol having the molecular formula of C_4H_9OH that, in addition to its potential use as fuel or fuel additive, it can be used as an intermediate in chemical synthesis and as a solvent for a wide variety of chemical and textile industry applications.. Recently, increasing demand for the use of renewable resources as feedstock for the production of chemicals combined with advances in biotechnology through omics, systems and synthetic biology, metabolic engineering and innovative process developments is generating a renewed interest in fermentative butanol production (Fischer et al. 2008). 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. In Europe alone, the production of glycerol has tripled within the last 10 years (http://theglycerolchallenge.org/) and, even though high purity glycerol is an important compound in several industries (food, drug, tobacco, among others), crude glycerol has a low price due to impurities. Moreover, purification of crude glycerol is costly and not affordable for most small and medium scale biodiesel producers. (Pachauri and He, 2006).

Fermentation of low grade glycerol to butanol has been proven (Andrade and Vasconcelos, 2003). However, there is still place for process optimizations in order to improve the production yields and reduce the toxicity of butanol to the producing organisms (Brenner et al. 2008), which are the main objectives of this PhD thesis. To accomplish the proposed objectives, three different approaches are considered; pure culture fermentation, defined mixed culture and undefined mixed culture. It is expected the development of energy based models for butanol producing mixed cultures, and synthetic biology tools will be used in a final stage in order to engineer a model microorganism (*Pseudomonas putida*) for the production of butanol with improved yield and solvent tolerance as a proof-of-principle.

MATERIAL AND METHODS

Clostridium pasteurianum DSM 525 and a granular sludge from a brewery were selected to work as pure and undefined mixed culture respectively. Both, *C. pasteurianum* and granular sludge were anaerobically cultured in batch mode (serum bottles) at 37 ºC. Acids, glycerol and 1,3-propanediol were measured through HPLC, and GC was used to determine the alcohols concentration. *C. pasteurianum* was cultured in a mineral defined media supplemented with vitamins and 5 g/l of crude glycerol and in a semi-defined media where vitamins were replaced by yeast extract and the same crude glycerol concentration was used. Mixed culture fermentations using granular sludge were performed using the semi-defined medium above mentioned containing 3,75 g/l of crude glycerol. Heat and contact with BES (bromoethanesulfonate) were assessed as pre-treatments in order to inactivate the methanogenic strains.

RESULTS AND DISCUSSION

In a first stage, several experiences were conducted for both pure and mixed culture in order to gather suitable data for the following tasks, namely the development of the energy-based models and the design/construction of a new organism using synthetic biology tools

Clostridium pasteurianum was capable of consuming crude glycerol regardless vitamins or yeast extract were used, nevertheless significant differences were found. For the experiments conducted with a defined medium, the butanol yields were around 0,3 g/g, and even though fermentation time decreased considerably when yeast

extract was used, the butanol yield was lower and high amounts of 1,3-propanodiol were found (Figs. 1 and 2).

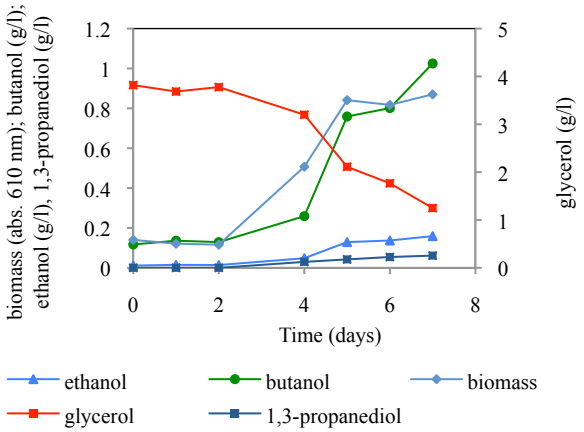

Figure 1. : Biomass, 1,3-propanediol and solvent production by *C. pasteurianum* in defined media with crude glycerol as carbon source

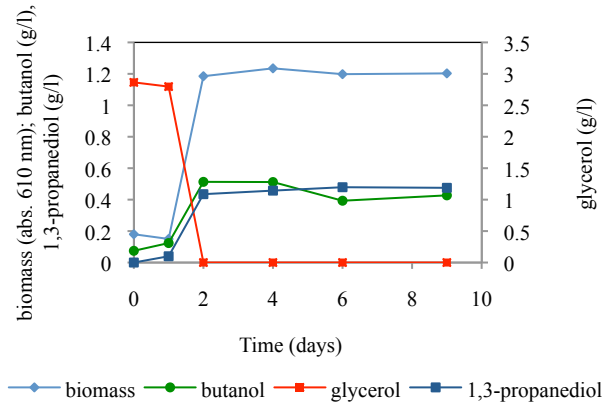

Figure 2. : Biomass, 1,3-propanediol and solvent production by *C. pasteurianum* in semi-defined media with crude glycerol as carbon source

Even though glycerol consumption was found to be faster using granular sludge than in pure culture fermentations, solvents were not present as fermentation products. The main products obtained were acids and 1,3-propanediol.

Finally, *C. pasteurianum* and the granular sludge were transferred to increasing crude glycerol concentrations in order to progressively acclimatize the strain, and eventually obtain an overproducing strain. A rapid growth could be observed up to 25 g/l and 10 g/l, respectively which differs from what has been previously reported in the literature (Taconi et al. 2009)

CONCLUSIONS

It can be concluded that both *C. pasteurianum* and the granular sludge are capable of consuming biodiesel derived crude glycerol showing a fast growth. Thus, they show a great potential for 1,3-propanediol and butanol (*C. pasteurianum*) production from crude glycerol. Nevertheless, optimization is still required since it is essential to increase the yields reducing the quantity of acids produced.

Future work will be focused in the use of selection tools, such as further increases of the crude glycerol concentration for both cultures (pure and mixed) and mutation, in order to obtain butanol-overproducing strains. In parallel, the bioaugmentation potential of *C. pasteurianum* will be assessed by co-culturing this pure strain together with granular sludge in an EGSB (expanded granular sludge bed) reactor.

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AUTHOR BIOGRAPHY

Roberto Gallardo was born in Punta Arenas, Chile and went to the Pontifical University of Valparaíso (Chile) where he obtained the degrees of Biochemical Engineer in 2006 and MSc. In Biochemical Engineering in 2009. His work has been focused in biofuels and since 2009 he is developing his PhD thesis at University of Minho as part of the MIT Portugal program.