



BUTANOL PRODUCTION BY *CLOSTRIDIUM PASTEURIANUM* USING BIODIESEL-DERIVED CRUDE GLYCEROL



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INTRODUCTION

Butanol (C₄H₉OH) is an aliphatic saturated alcohol with potential as fuel/fuel additive, that can also be used in chemical synthesis and for a wide variety of industrial applications. The increasing demand for using renewable resources as feedstock for the production of chemicals combined with advances in biotechnology is generating a renewed interest in fermentative butanol production. In this context, glycerol (by-product from the biodiesel production) arises as a potential substrate for butanol production. In Europe alone, the production of glycerol has tripled within the last 10 years and its price has been considerably reduced.

Although fermentation of low-grade glycerol to butanol has been proven [1], there is still place for process optimization. The main goals of the current thesis are to improve the yield of butanol production from glycerol by *Clostridium pasteurianum* and to reduce the butanol toxicity towards this microorganism.

MATERIALS AND METHODS

Clostridium pasteurianum DSM 525 was anaerobically cultured in 500 ml serum bottles using 200 ml working volume. Ten percent volume was repeatedly transferred to increasing crude glycerol concentrations using a semi-defined medium (crude glycerol-salts-yeast extract). The initial pH was set at $7 \pm 0,2$ and cells were incubated at 37°C. Acids, glycerol and 1,3-propanediol (1,3-PDO) were measured through HPLC (Aminex cation-exchange HPX-87H column) coupled to an UV and RI detector. Butanol and ethanol were determined by GC (TR-WAX column) equipped with a flame ionization detector.

RESULTS

C. pasteurianum was serially transferred from stock cultures to media containing 5, 10, 15, 20, 25, 35 and 50 g/l crude glycerol. The strain was able to consume up to 31,3 g/l of glycerol which is slightly higher than the maximum glycerol consumption obtained by Dabrock et al. [2] (27,6 g/l) but lower to the values reported by Biebl [3] (50 g/l) using pure glycerol. Besides acids (acetic, n-butyric, lactic, formic), the main products found were butanol, ethanol, and 1,3-PDO. Butanol yield increases with increasing glycerol concentrations, while 1,3-PDO yield decreases (Figure 1), thus revealing the competitive nature of these pathways and that glycerol itself has an effect on the relative quantities of these compounds being produced.

For 50 g/l crude glycerol a considerable amount of substrate remained in the culture medium. To overcome this problem, the concentration of different nutrients was evaluated to assess possible limitations. NH₄Cl, CaCO₃, FeCl₂, microelements, salts, and yeast extract were increased independently; nevertheless the glycerol consumption could not be increased. Nonetheless, a simultaneous increase in NH₄Cl (from 1 to 5 g/l) and FeCl₂ (from 1 to 11 mg/l) had a positive effect in the butanol yield (from 0,19 to 0,27 g/g) whereas 1,3-PDO yield decreased (from 0,15 to 0,06 g/g), which is in agreement with [2]. These authors found that an iron limitation somehow inhibit butanol production. A 9 g/l concentration of butanol was obtained (Figure 2) which is in accordance with the values reported for solventogenic clostridia.

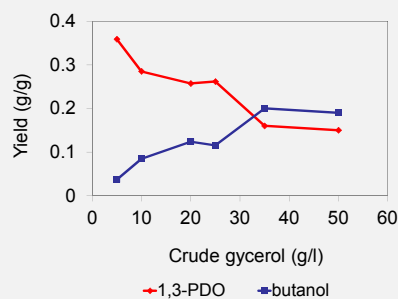


Figure 1: Butanol and 1,3-PDO yield versus crude glycerol concentration in batch fermentation

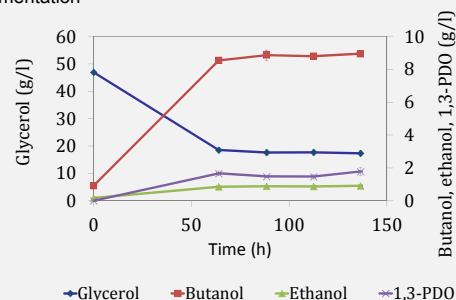


Figure 2: Butanol, ethanol and 1,3-PDO production by *C. pasteurianum* in batch fermentation using a 50 g/l crude glycerol medium.

Based on these results, it is likely that the glycerol consumption is not being affected by nutrient limitation but by some butanol inhibition. It is well known that butanol is very toxic to cells and maximum tolerance between 7 and 13 g/l has been reported for non-manipulated *Clostridium* strains.

In general, the addition of 36 mM sodium butyrate resulted in higher butanol titers, nevertheless, this difference was less pronounced for increasing glycerol concentrations. A slight difference could be observed for the experiments run with 50 g/l crude glycerol (10,11 g/l of butanol). It is important to stress that a higher butanol titer obtained as a result of butyrate addition does not necessarily imply a higher butanol on glycerol yield since butyrate can be directly converted into butanol via butyryl-CoA – butyraldehyde – butanol. Interestingly, as more butanol can be produced at the expenses of butyrate with the same glycerol consumption, it can be suggested that butanol does not inhibit the enzymes involved in its production from butyrate but it exerts a negative effect in the metabolic pathway involved in the glycerol uptake.

CONCLUSION

C. pasteurianum is capable of consuming biodiesel-derived crude glycerol showing a great potential for butanol production from crude glycerol. Nevertheless, butanol toxicity seriously limits butanol titers and therefore, it is important to find ways to overcome this problem. Future work will be focused on this issue considering that the plasmatic membrane is the main target for the negative effect exerted by butanol. It would be desirable to increase the butanol yield, for example, by shutting down genes involved in lactic acid production and/or over expressing enzymes involved in the butanol production. The complete genome of the strain has been sequenced and annotated and will be used in further work.

REFERENCES

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