Enhanced 2,3-butanediol production by *Enterobacter ludwigii* in fed-batch and continuous culture: Substrate and product inhibition from sugarcane

Vasileios Sdrallis[1], Sofia Maina[1], Anestis Vlysidis[2], Dimitrios Ladakis[1,3], Seraphim Papanikolaou[1], Aline Machado de Castro[4], Apostolos Koutinas[1*]

[1] Department of Food Science and Human Nutrition, Agricultural University of Athens, Iera Odos 75, 11855, Greece

[2] School of Chemical Engineering, National Technical University of Athens, Iroon Polytechneiou 9, 15772, Greece

[3] Department of Chemical Engineering, University of Patras, Rio 26504, Greece.

[4] Biotechnology Division, Research and Development Center, PETROBRAS, Av. Horácio Macedo, 950. Ilha do Fundão, Rio de Janeiro 21941-915, Brazil

Presenting author email: sofiamaina@aua.gr

ABSTRACT

2,3-butanediol (BDO) is an important building block for the chemical industry with potential applications in agrochemical, pharmaceutical and fine chemical production processes. It can be produced by bacterial strains under anaerobic or oxygen limiting conditions in order to prevent intracellular acidification, as a carbon and energy reserve and for NADH co-factor recycling. Any pathway leading to NADH recycling is also associated to the production of metabolites (e.g. lactate, ethanol) that are antagonistic to BDO synthesis (Celinska and Grejek, 2009). Organic acids along with substrate could strongly inhibit BDO production. Improving BDO production, yield and productivity by optimizing the fermentation conditions are desirable to increase the economic feasibility of industrial BDO production. Different fermentation strategies have also been evaluated for enhanced BDO production (Zeng and Sabra, 2011). Batch fermentation result in low productivity and substrate inhibition usually occurs. To overcome this problem, fed-batch operations should be carried out. However, metabolic by-product formation could gradually inhibit bacterial growth and BDO production (Wong et al, 2014). Continuous cultures could be employed in order to increase the productivity of BDO production.

In this study, raw sugar cane was initially used as carbon source for BDO production by a newly isolated bacterial strain, namely *Enterobacter ludwigii*. The effect of substrate and metabolic products on bacterial growth was studied in microplate and shake flask fermentations. A model was proposed to describe the multiproduct inhibited growth of *E. ludwigii*. Succinic acid, acetic acid and lactic acid concentrations higher than 20 g/L resulted in a linear reduction of specific growth rate. Initial formic acid concentration higher than 5 g/L resulted in decreased specific growth rate by 62%. The presence of organic acids along with BDO hindered microbial proliferation and BDO production.

Batch cultures carried out with increasing sugarcane concentration led to BDO production up to 55.5 g/L when the initial sugarcane concentration was 120 g/L. Nonetheless, the increment in sugarcane concentration entailed an increase in by-product formation resulting in lower sugar to BDO conversion yield. Maximum yield (0.45 g/g) and productivity (1.02 g/L/h) were achieved at sugarcane concentration of 75 g/L. Two fed-batch fermentations were carried out with initial bacterial mass concentration of 1 and 4 g/L. Although the productivity reached 3.14 g/L/h until 8 h when 4 g/L of initial bacterial mass concentration was used, the final BDO concentration (87 g/L), productivity (1.23 g/L/h) and yield (0.25 g/g) attained similar values in both fed-batch cultures. Continuous culture was finally investigated using different dilution rates. BDO along with by-product formation were determined. Although higher BDO productivities than fed-batch cultures were achieved, high dilution rates resulted in sugar accumulation leading to lower BDO yield.

References

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