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Engineering *Saccharomyces cerevisiae* for efficient utilization of crude glycerol from biodiesel industry

Candida Lucas

University of Minho, Portugal

Glycerol is a well-known product with a large variety of applications in processes and products. Bio-diesel industry generates amounts of glycerol that largely exceed their potential applications. Nevertheless, the crude glycerol does not substitute for glycerin from petrochemical origin since the refining process requires large-scale complex technology and therefore prices do not compete. In consequence, crude glycerol, in opposition to glycerin, is poorly utilized charging the biodiesel industry with a high waste-disposal cost. In this context large amounts/low cost crude glycerol became an interesting carbon source for the biotechnology industry, but this depends on the use of amenable microorganisms with recognized industrial applications. *Saccharomyces cerevisiae* was genetically engineered aiming to obtain strains that consumed crude glycerol efficiently. For this purpose, several proteins were overexpressed and freed of glucose repression and other tight regulation mechanisms combining several construction strategies. These included proteins from the glycerol consumption pathway, the glycerol plasma membrane high affinity transporter (Stl1p) and kinase (Gut1p) and other proteins indirectly influencing ethanol production flux, the thiamine plasma membrane permease (Thi10p) and pyrophosphokinase (Thi80p) and Pdc2, a transcription factor for thiamine-regulated genes required for expression of the two pyruvate decarboxylase isoforms. Engineered strains were subsequently challenged with biodiesel industry crude glycerol-based culture conditions for proof of concept.

clucas@bio.uminho.pt

Biobased products from agricultural waste

Cristina Ferreira Silva, Mariana Dias Moreira, Kelly Cristina dos Reis and Rosane F Schwan

Federal University of Lavras, Brazil

Agricultural waste is seen as a new alternative substrate to the fermentation process. Integrated production and re-use of waste is an opportunity to make the process self-suitable in bio-refinery concept. Brazil is known to produce both spirit beverage such as cachaca and coffee generating thousands of dollars per year. However, these products are responsible for tons of liquid and solid waste with high carbon content that represent harmful deposition in the environment. The purpose of the biotechnological process is to use that waste to produce enzymes, pigments, flavor compounds and polyalcohols. Pulp, husk and vinasse are waste known as suitable for the microbial growth, producing add-value products. In this context, coffee waste and vinasse were used to produce carotenoids and flavor compounds, respectively. Both processes are aerobic and show simultaneous decrease of power pollution (evaluated by BOD parameter) and high concentration of add-value products. Productivity of carotenoids reached 21.35 mg/L and they had antimicrobial and anti-oxidant activity. In addition, these carotenoids could be used in cosmetic and pharmaceutical industries. Attainment of biomass from non-*Saccharomyces* strains was possible from vinasse with productivity reaching up to 92% in 70% of vinasse concentration. Nutritional and anti-nutritional parameters were evaluated and their concentration showed that the microbial biomass could be used to supplement food for animals.

cristinafsb@dbi.ufla.br