

FAPESP BIOENERGY PROGRAM

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UNDERSTANDING THE EMULSION BEHAVIOUR OF FERMENTATION MIXTURES IN THE MICROBIAL PRODUCTION OF DIESEL AND JETFUEL-LIKE BIOFUELS

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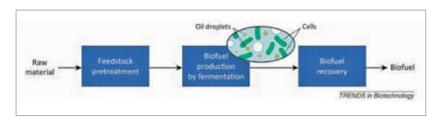


Figure 1. A simplified block diagram of the production of advanced biofuels

Within the many possible renewable energy resources, biofuels dominate the current market. Compared to the well-established alcohol biofuels (e.g. ethanol), the microbial extracellular production of diesel and jetfuel-like biofuels promises advantages in terms of product separation from the fermentation broth. However,

the microbial production of diesel and jetfuel-like biofuels results in a four-phase (S-L-L-G) mixture in the reactor (microbial cells, aqueous medium, lipid product and fermentation gas), which shows emulsion characteristics. Although there have been studies on the emulsion behaviour of similar mixtures, little emphasis has been given to a systematic study of factors relevant for cost-efficient large scale operation. In this context, understanding the mechanisms behind emulsion formation is key for a rationale feedstock (and the pre-treatment associated to it) selection, for defining process (fermentation and product recovery) conditions and for identifying targets for synthetic biology of producing microorganisms (e.g. robustness at low pH, high salt concentration). Thus, this project aims at cooperation between two research groups able to contribute to the consolidation of processes for the large scale production of biofuels. The group of Delft University of Technology is expert in integrated bioprocesses, while the group at the University of Campinas has experience in emulsions and proteinbased emulsifiers. As a result, it is expected to understand the mechanisms that lead to the formation of emulsion in the fermentation process to develop an efficient and cost-competitive integrated fermentation/ recovery process, while at the same time preserving the microbial cell viability.



SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The project was divided into three steps in which the first one was the characterization of the process over time, determining the composition and properties of each phase obtained during the industrial process. Subsequently, a bottom-up study was performed with the building of model emulsions that showed increased complexity in order to understand the mechanisms involved in the emulsification process. Finally, a top-down study was proposed in which demulsification strategies were proposed.

In general, the evaluation of the stability mechanisms of model emulsions allowed understanding the phase behavior of these emulsions, as well as the interface thereof. Such information was used to evaluate the various strategies for demulsification aiming a greater separation of oil, making easier the downstream processes of biofuels production.

The strategies used in the demulsification process were based on the application of mechanical forces, changes in charge density of the droplets and reducing the viscosity of the dispersed phase.

Use of ultracentrifugation was the only process

that caused total separation of the components of the cream phase. Temperature increase (up to 60 °C) could only partially recover the oil since viscosity decreasing was not enough to promote total phase separation. The use of alcohols caused a reduction of phases viscosity and greater affinity for the hydrophobic portion, leading to higher separation rates. However an additional step to recover the solvent would be required after the demulsification process. The use of salts, polymers, acids and bases was not efficient to destabilize the cream phase only by the action of gravitational forces. The combination of the strategies adopted with a later centrifugation step resulted in a more efficient separation.

The destabilization of the cream phase, almost entirely, it was possible through the use of magnetic nanoparticles by applying a magnetic field. This process is interesting because no additional steps of separation are needed.

Thus, it was possible to understand and evaluate the stability mechanism of the model emulsions, allowing study and examine various strategies demulsification aiming greater separation of oil.

MAIN PUBLICATIONS

Heeres AS, Picone CSF, Cunha RL, Wielen LAM, Cuellar MC. 2014. Microbial advanced biofuels production: overcoming emulsification challenges for large-scale operation. *Trends in Biotechnology*. **32(4)**: 221-229.

Furtado GF, Picone CSF, Cuellar MC, Cunha RL. 2015. Breaking oil-in-water emulsions stabilized by yeast. *Colloids and Surfaces Biointerfaces*. **128(1)**: 568-576.

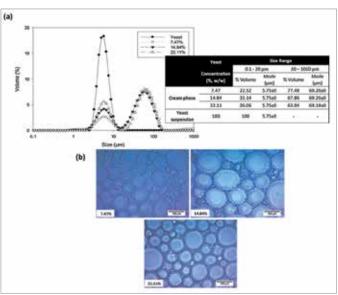


Figure 2. Particle size distribution by volume and particle size ranges (a) and micrographs (b) of the yeast suspension and cream phase of the emulsions prepared at varied yeast concentration (%, w/w)

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