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Breaking oil-in-water emulsions stabilized by yeast

Guilherme F. Furtado[a], Carolina S.F. Picone[a,b], Maria C. Cuellar[c], Rosiane L. Cunha[a,*]

[a] Department of Food Engineering, School of Food Engineering, University of Campinas, Campinas 13083-970, SP, Brazil
[b] School of Technology, University of Campinas, Limeira 13484-332, SP, Brazil
[c] Department of Biotechnology, Delft University of Technology, Julianalaan 67 Delft, 2628BC, The Netherlands

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ABSTRACT

Several biotechnological processes can show an undesirable formation of emulsions making difficult phase separation and product recovery. The breakup of oil-in-water emulsions stabilized by yeast was studied using different physical and chemical methods. These emulsions were composed by deionized water, hexadecane and commercial yeast (*Saccharomyces cerevisiae*). The stability of the emulsions was evaluated varying the yeast concentration from 7.47 to 22.11% (w/w) and the phases obtained after gravity separation were evaluated on chemical composition, droplet size distribution, rheological behavior and optical microscopy. The cream phase showed kinetic stability attributed to mechanisms as electrostatic repulsion between the droplets, a possible Pickering-type stabilization and the viscoelastic properties of the concentrated emulsion. Oil recovery from cream phase was performed using gravity separation, centrifugation, heating and addition of demulsifier agents (alcohols and magnetic nanoparticles). Long centrifugation time and high centrifugal forces (2 h/150,000 × *g*) were necessary to obtain a complete oil recovery. The heat treatment (60 °C) was not enough to promote a satisfactory oil separation. Addition of alcohols followed by centrifugation enhanced oil recovery: butanol addition allowed almost complete phase separation of the emulsion while ethanol addition resulted in 84% of oil recovery. Implementation of this method, however, would require additional steps for solvent separation. Addition of charged magnetic nanoparticles was effective by interacting electrostatically with the interface, resulting in emulsion destabilization under a magnetic field. This method reached almost 96% of oil recovery and it was potentially advantageous since no additional steps might be necessary for further purifying the recovered oil.

1. Introduction

Several kinds of processes such as bioremediation, two-phase fermentation, aqueous extraction of edible oil from oilseeds and microbial production of diesel and jetfuel replacements can result in an undesirable formation of oil-in-water emulsions. The presence of microbial cells can make the oil separation quite complex since these particles can act as stabilizers, emulsifiers or even producing biosurfactants by the own cells [1–9]. Thus, in these processes the emulsion formation can bring difficulties associated with process yield, quality and efficiency which could lead to a high-cost process and a non-competitive product [4,5]. A good knowledge of the physical and physicochemical properties of emulsions can help to design process conditions leading to a maximum oil separation and recovery. The attractive or repulsive interactions between droplets depend on the electrostatic and steric forces as well as on interfacial tension between the immiscible phases. Therefore, droplets coalescence depends on the emulsion composition and process conditions to obtain this emulsion [10].

Oil separation could require previous flocculation and coalescence steps, which can be achieved by physical or chemical methods, or by a combination of both [11]. The more simple method to promote the creaming of droplets is gravitational separation. However large tanks are necessary to avoid a high separation rate that could promote shear between the droplets and favor re-emulsification [12]. On the other hand, a long residence time would be necessary if the creaming rate is low or when the emulsion is kinetically stable. The application of centrifugal forces is a technique widely used in the petroleum industry, allowing separation of small droplets, but with high investment costs and maintenance [13]. Demulsification can also be promoted by increasing the temperature of the emulsion effectively changing the viscosity of the phases [10,14]. Chemical methods affect the interfacial

* Corresponding author. Tel.: +55 19 35214047; fax: +55 19 35214027..
E-mail address: rosiane@fea.unicamp.br (R.L. Cunha).

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properties improving coalescence by adding a constituent which effectively changes the viscosity of the phases and/or the surface charge [15]. The combination of physical and chemical methods has been used to obtain a more effective emulsion breakup. Alcohols have been successfully used for breaking up microbial stabilized emulsions [16,17] and recent studies have shown that the use of magnetic nanoparticles coated with amphiphilic material is effective in the stabilization and destabilization of emulsions. These particles act as emulsifying agents stabilizing the interface but in the presence of a magnetic field these particles migrate rapidly towards to the applied field, destabilizing the droplets interface and thereby inducing coalescence of the droplets [18,19]. This method has shown growing interest due to the rapid and easy separation of complex multiphase systems [20]. In order to provide information leading to the maximum oil separation the use of model systems, with components having known properties, allows a better evaluation of the physical and physicochemical phases and interface properties of the emulsions.

In this study, we have studied model systems of emulsions, using hexadecane as the oil phase and baker's yeast as model microorganism. The emulsions have been evaluated to allow understanding the mechanisms involved in their stabilization, and to further develop the demulsification protocols. Finally, different chemical and physical demulsification methods were performed in order to evaluate oil recovery.

2. Materials and methods

2.1. Materials

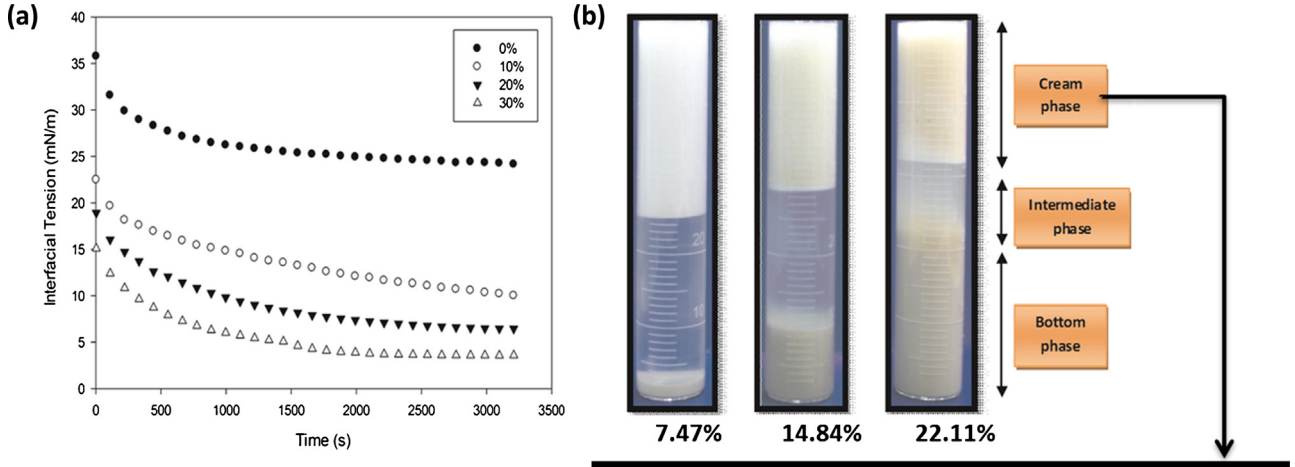
Ultrapure water from a Millipore Milli-Q system (resistivity 18.2 MΩ/cm) was used. Commercial yeast (*Saccharomyces cerevisiae*, Itaiquara, Ltd., Tapiratiba, BRAZIL) was purchased in the local market. Hexadecane was supplied by Sigma–Aldrich Co. (St. Louis, USA) and magnetic nanoparticles (EMG 607) by Ferrotec Corp. (Santa Clara, USA). Iso-butanol and ethanol were of analytical grade.

2.2. Emulsion preparation

The emulsions were prepared by mixing 30% (v/v) of hexadecane to 70% (v/v) of aqueous yeast suspension (10, 20 and 30% w/w) using a mechanical impeller RW20 (IKA, Campinas, Brazil) at 900 rpm (150 s^{-1}) during 30 min. These yeast concentrations were chosen to ensure emulsions stability and they were also based on biotechnological processes. The final yeast concentration in emulsions was 7.47, 14.84 and 22.11% (w/w). Such a wide concentration range allowed understanding the yeast role on the emulsion stability. Hexadecane was used because it presents physical properties (as density and viscosity) similar to some oils obtained from fermentative processes as farnesene [21] (a renewable diesel precursor). All emulsions were prepared in triplicate. In addition, an emulsion prepared at a concentration of 7.47% (w/w) was centrifuged at 10,000 rpm (15,317 × *g*) until complete phase separation (without cream phase). From the hexadecane and aqueous phases recovered after centrifugation, new emulsions were prepared in the same ratios of aqueous and oil phase but with no addition of yeast.

2.3. Yeast characterization

Surface hydrophobicity of yeast was determined by spectrophotometric method based on the microbial cells adhesion to hydrocarbons (as hexadecane) [22,23]. First, cells were suspended in phosphate buffer (0.1 M). Next, three milliliters of this cell suspension was added in a glass tube containing 1 ml of hexadecane. The mixture was shaken for 25 s in a vortex mixer and after 30 min the absorbance of the aqueous phase was measured. Absorbance values of samples was performed using a spectrophotometer Spectro Quest 2800 (UNICO, Dayton, USA) in 600 nm wavelength. Results were expressed as percentage of cells adhered to hexadecane in relation to pure hexadecane. The charge density of yeast cells suspended in Milli-Q water (0.005% w/w) was determined in a Nano-ZS Zetasizer equipment (Malvern Instruments, Worcestershire, UK). The interfacial tension between the aqueous yeast suspension (10, 20 and 30% w/w) and oil phase was measured by the pendant drop method using a TrackerS tensiometer (Teclis,



Yeast Concentration (%, w/w)	CI (%)	OC (%, w/w)	ρ (kg/m³)
7.47	42.4±3.72[a]	60.1±0.41[a]	807.85±0.17[a]
14.84	40.4±2.12[a]	60.5±0.48[a]	811.43±0.06[b]
22.11	37.2±0.02[a]	61.0±0.53[a]	824.23±0.41[c]

Different letters in the same column indicate significant difference ($p < 0.05$).

Fig. 1. Kinetics of interfacial tension between water and hexadecane in the presence of different concentrations of yeast (%, w/w) (a), visual appearance of the emulsions after 24 h, creaming index (CI), oil content (OC) and density (ρ) of the cream phase (b).