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# Techno-economic assessment of the use of solvents in the scale-up of microbial sesquiterpene production for fuels and fine chemicals

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**Abstract.** Sesquiterpenes are a group of versatile, 15-carbon molecules with applications ranging from fuels to fine chemicals and pharmaceuticals. When produced by microbial fermentation at laboratory scale, solvents are often employed for reducing product evaporation and enhancing recovery. However, it is not clear whether this approach constitutes a favorable techno-economic alternative at production scale. In this study empirical correlations, mass transfer and process flow sheeting models were used to perform a techno-economic assessment of solvent-based processes at scales typical for flavors and fragrances ( $25 \text{ MT year}^{-1}$ ) and the fuel market ( $25\,000 \text{ MT year}^{-1}$ ). Different solvent-based process options were compared to the current state of the art, which employs surfactants for product recovery. The use of solvents did reduce the sesquiterpene evaporation rate during fermentation and improved product recovery but it resulted in costs that were higher than, or similar to, the base case due to the additional equipment cost for solvent-product separation. However, when selecting solvents compatible with the final product formulation (e.g. in a kerosene enrichment process), unit costs as low as  $\$0.7 \text{ kg}^{-1}$  can be achieved while decreasing environmental impact. © 2018 The Authors. *Biofuels, Bioproducts, and Biorefining* published by Society of Chemical Industry and John Wiley & Sons, Ltd.

*Supporting information may be found in the online version of this article.*

**Keywords:** microbial biofuels; fine chemicals; bioprocess integration; fermentation; product recovery; techno-economic performance; emulsion

## Introduction

Sesquiterpenes are 15-carbon isoprenoids with applications in different markets like flavors, fragrances, cosmetics, pharmaceuticals, foams, lubricants,

and biofuels.<sup>1,2</sup> Normally sesquiterpenes are extracted from plants in which they naturally occur. However, this method is costly, presents low yields, and raw materials are usually scarce, resulting in high product prices ranging from ~100 to ~1000 EUR kg<sup>-1</sup>.<sup>3</sup> The use of genetically

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modified microorganisms to produce sesquiterpenes via fermentation is a promising alternative to overcome these problems. Recently developed strains can secrete sesquiterpenes to the extracellular medium, reaching titers in the order of grams per liter.<sup>4,5</sup> Sesquiterpene forms a separate oil phase with lower density than water, which is very attractive from the point of view of product recovery. Several companies like Amyris, Isobionics, Allylix, and Evolva are currently developing processes at commercial scale. For example, Amyris already produces farnesene (a precursor for farnesane, commercialized under the name of Biofene<sup>®</sup>). Moreover, they have successfully developed a microorganism for the production of amorpho-1,4-diene, a precursor for the malaria medicine artemisinin, while Sanofi Aventis is currently working on scaling up and commercializing the process.

Despite these industrial developments, the literature on process technology and quantitative data is limited to a few patents.<sup>6–10</sup> Scientific publications are mainly focused on metabolic improvements and fermentation yields.<sup>4,5,11–16</sup> Those laboratory-scale studies briefly describe the processing of sesquiterpenes for analytic purposes (Fig. 1(A)), and typically employ solvents during fermentation and sample handling.<sup>8,13</sup> However, the reason for applying this method,

the impact of the solvent in the process, or its applicability at industrial scale, are not explicitly stated. In the following section the mechanisms in which solvents play a role in the laboratory scale protocols, and their potential application at large scale, are discussed.

## Roles of solvent in the production of sesquiterpenes

### Lowering evaporation rate of sesquiterpene

Sesquiterpenes are relatively volatile molecules (Table 1), and thus part of the product can be transferred to the gas phase during fermentation. Evaporation rates in the order of  $\text{mg h}^{-1}$  have been reported to occur at laboratory scale fermentations reaching product titers in the order of  $\text{mg L}^{-1}$ .<sup>16</sup> Three percent of product loss has also been reported in a 2 L scale bioreactor having a product titer in the order of g/L.<sup>6</sup> A typical solution is to add an overlay of 10%–20% v/v of a relatively low volatile organic solvent (e.g. decane or dodecane) to the fermentation medium (Fig. 1(A)), capturing the hydrophobic sesquiterpene molecules in the organic phase.<sup>8,11,13,16,24</sup> Although this is a common practice at laboratory scale, the actual impact of the solvent on the product evaporation rate is unknown,

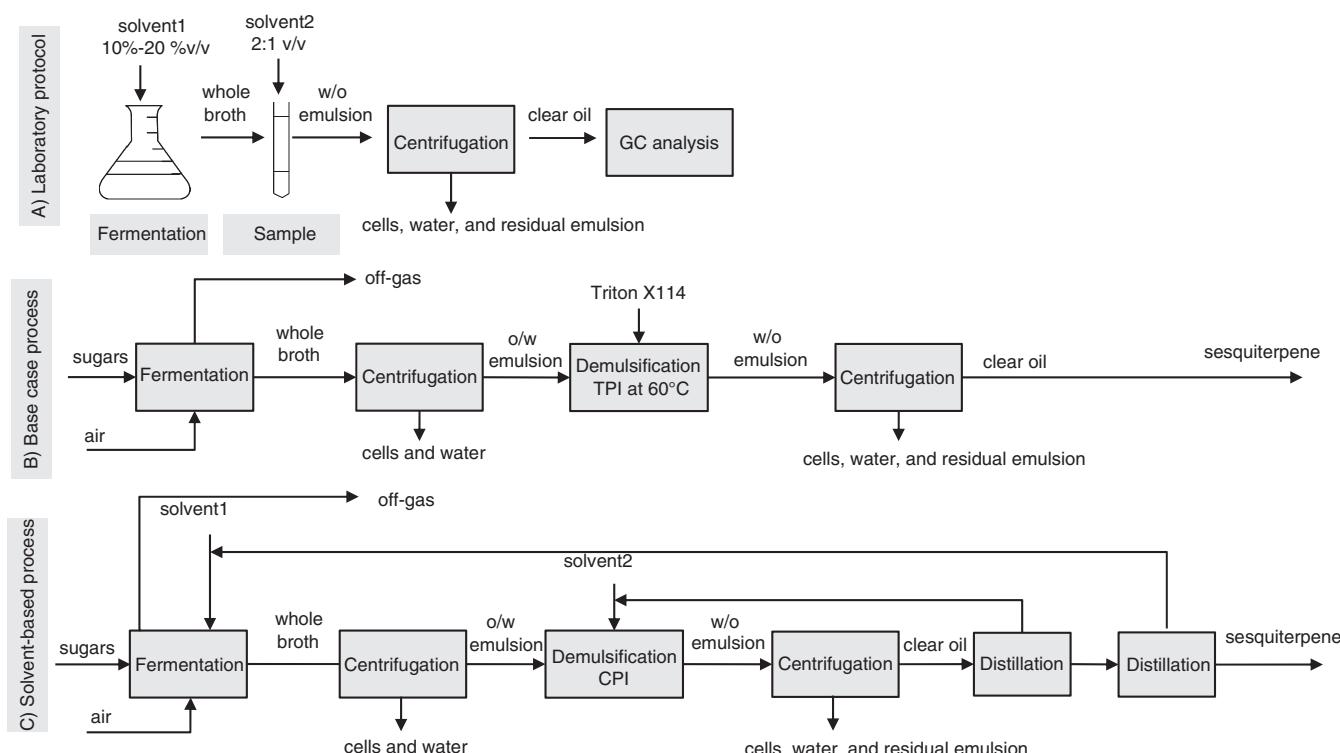


Figure 1. Process options for the production of sesquiterpenes. (A) Lab scale protocol;<sup>8</sup> (B) Base case;<sup>9</sup> (C) Solvent-based process evaluated in this work.

**Table 1. Predicted physical properties sesquiterpenes and solvents typically used in sesquiterpene production. Unless indicated otherwise, source: ChemSpider database (data generated using the US Environmental Protection Agency's EPISuite™) at 25 °C.**

Example	$\rho$ (g mL <sup>-1</sup> )	$T_b$ (°C)	$p^{vap}$ (Pa)	$k_H$ (atm m <sup>3</sup> mol <sup>-1</sup> )	$LogP_{ow}$	$C^w$ (mg L <sup>-1</sup> )	$\sigma_{oa}$ (mN m <sup>-1</sup> )	$\sigma_{ow}$ (mN m <sup>-1</sup> )
Santalene	0.89 <sup>h</sup>	238	7.6	0.39	6.4	0.039	36 <sup>a</sup>	—
Caryophyllene	0.89 <sup>h</sup>	257	4.2	0.69	6.3	0.050	31 <sup>f</sup>	51 <sup>g</sup>
Farnesene	0.86 <sup>h</sup>	261	3.3	0.10	7.1	0.011	26 <sup>a</sup>	—
Amorphadiene	0.90 <sup>a</sup>	258	4.0	0.69	6.3	0.054	26 <sup>a</sup>	—
Dodecane	13	0.75 <sup>h</sup>	206	18	9.35	6.1	0.110	25 <sup>f</sup>
Decane	8	0.74 <sup>h</sup>	165	191	5.30	5.0	1.252	24 <sup>b</sup>
Ethyl acetate	8	0.90 <sup>h</sup>	78	1243	$2.3 \times 10^{-4}$	0.7	$2.99 \times 10^4$	24 <sup>c</sup>
MTBE	17	0.74 <sup>h</sup>	47	33 331	$2.0 \times 10^{-3}$	0.9	$1.98 \times 10^4$	19 <sup>d</sup>
Triton x-114	9	1.06 <sup>i</sup>	—	—	—	Soluble	31 <sup>i</sup>	—

<sup>a</sup>ACD/Labs Percepta Platform - PhysChem Module; <sup>b</sup> Dataphysics<sup>18</sup> @20 °C; <sup>c</sup> Demond and Lindner<sup>19</sup> (Temperature not reported);

<sup>d</sup> CAMEOdatabase<sup>20</sup> @20 °C; <sup>e</sup> Montaño et al.<sup>21</sup> @20 °C; <sup>f</sup> Hickel et al.<sup>22</sup> (Temperature not reported); <sup>g</sup> Experimentally determined in this work at room temperature; <sup>h</sup> estimated from experimental results using<sup>23</sup>; <sup>i</sup> Chem Src Safety Data sheets; <sup>j</sup> Dow Safety Data Sheets

and studies on sesquiterpene evaporation, and VLE physical properties of sesquiterpenes are scarce; see for example Schuhfried *et al.*<sup>25</sup>

Solvent selection criteria for sesquiterpene fermentations are, among others, low volatility, low tendency to form emulsions, and an octanol/water partition coefficient higher than 10<sup>5</sup> ( $\log P_{ow} > 5$ ), which, in principle, excludes toxicity problems in microorganisms like *Saccharomyces cerevisiae*.<sup>26</sup> When using solvents at production scale, additional solvent selection criteria like high relative volatility should be considered for a cost-effective solvent-product separation. Alternatively, this separation step could be bypassed by choosing solvents compatible with the final product formulation. Examples include the use of methyl oleate or isopropyl myristate in the production of amorphadiene,<sup>5</sup> canola oil in the production of the sesquiterpene alcohol bisabolol,<sup>27</sup> and farnesene as solvent in the production of the monoterpene limonene.<sup>28</sup>

## Enhancing oxygen transfer during fermentation

In aerobic fermentation, oxygen is generally supplied by sparging air bubbles into the bioreactor. The oxygen is transferred from the bubbles into the aqueous phase, and once there, it is available to be consumed by the microorganisms. The oxygen transfer rate (OTR) depends on the overall mass transfer coefficient ( $k_L a$ ), and the difference in oxygen concentration between the gas/liquid interface and the bulk liquid phase ( $\Delta C_{O_2}$ ) (Eqn (1)):

$$OTR = k_L a \cdot \Delta C_{O_2} \cdot V \quad (1)$$

The  $k_L a$  depends on physical properties of the system, bioreactor geometry, and hydrodynamic conditions.<sup>29</sup> The OTR is typically one of the limiting factors in the scale-up of aerobic fermentations.<sup>29</sup> Due to the low solubility of oxygen in water, oxygen limitation may occur, affecting the fermentation performance. Oxygen limitation can be avoided by increasing the power input of the system but this is highly energy demanding (e.g., due to aeration and agitation), especially at large scale. As oxygen presents ten times higher solubility in hydrocarbons than in water,<sup>30</sup> a possible alternative is using solvents as oxygen vector to enhance OTR. This concept has been claimed in a patent application by Isobionics for the production of the sesquiterpene valencene.<sup>31</sup> However, the net effect of the solvent on the OTR is controversial because it depends on the oil fraction used, among other factors.<sup>30</sup>

## Product recovery: enhancing coalescence and creaming of the oil phase

Sesquiterpenes are hydrophobic liquids, with a lower density than water (Table 1). During fermentation, microorganisms synthesize and secrete sesquiterpene to the extracellular medium, where it forms a separated phase (the oil phase) dispersed as droplets due to the mixing in the reactor. Dispersed oil droplets can and coalesce into larger ones. These large droplets can rise due to their lower density than the aqueous medium. This mechanism is called creaming, and its velocity ( $v_d$ ) depends on the size ( $d_{oil}$ ) and the density of the oil droplets ( $\rho_{oil}$ ) (Eqn (2)):

$$v_d = \sqrt{4 \cdot g \cdot (\rho_l - \rho_{oil}) \cdot d_{oil} / (3 \cdot C_D \cdot \rho_{aq})} \quad (2)$$

Coalescence and creaming contribute to the production of phase separation and are therefore desirable mechanisms for reducing product recovery costs. When fermentation is performed in the presence of solvent there is a larger total oil fraction in the bioreactor. For example, a 15% v/v oil phase was described in amorphadiene fermentation, of which 10% v/v accounted for solvent.<sup>5</sup> Higher oil fractions increase droplet collision, leading to a larger average droplet size.<sup>32,33</sup> Moreover, depending on the density of the selected solvent (Table 1), the overall density of the oil phase can be reduced, contributing to its creaming. Implementing solvents for reducing product evaporation could therefore also contribute to improving product recovery.

### Demulsification of the oil phase by phase inversion

Several components of the fermentation broth (e.g., salts, glycolipids, proteins, cells and cells debris) can hinder coalescence by lowering the oil/water (o/w) interfacial tension and/or stabilizing the o/w interface.<sup>34</sup> As a result, the product is not a homogeneous continuous phase but a stable emulsion. Although the formation of sesquiterpene emulsions is usually not mentioned in laboratory-scale studies,<sup>27,35</sup> this problem has been reported at larger scales.<sup>9</sup> Reported recovery methods include inducing phase inversion and obtaining an emulsion of water in a continuous oil phase (w/o), which is separated afterwards by centrifugation. Phase inversion can be (i) transitional (TPI), (ii) catastrophic (CPI), or (iii) induced by partial crystallization of the solvent.

Transitional phase inversion involves adding a nonionic surfactant and increasing the emulsion temperature until the surfactant becomes more soluble in the oil phase.<sup>36</sup> Tabur and Dorin<sup>9</sup> report the use of Triton-X114 and temperatures of 60 °C for TPI of sesquiterpene emulsions in a large-scale (300 L) fermentation. Catastrophic phase inversion is induced by adding oil phase until a critical concentration is reached.<sup>36–38</sup> The applicability of CPI as a recovery step in a production process involving microbial emulsions has been reported by Glonke *et al.*<sup>39</sup> There is no experimental data on critical o/w ratios for inversion of sesquiterpene emulsions; however, laboratory-scale protocols report the addition of two volumes of solvent per volume of broth<sup>8,13</sup> (Fig. 1(A)). Finally, phase inversion by partial crystallization of the solvent can be induced by first lowering the temperature to form a crystal network of solvent across the droplets' walls, and then heating the emulsion above the solvent's melting

temperature. To the best of our knowledge there are no data regarding the applicability of this method to sesquiterpene emulsions.

Using CPI instead of TPI has the advantage of avoiding the use of costly surfactants and changes of temperature. The main disadvantage of CPI is that it requires an extra step for solvent-product separation. However, TPI might also require additional purification steps like distillation to meet the purity specifications of some applications (e.g., 92–94% purity for cosmetics).<sup>2</sup> Furthermore, solvent-product separation costs can be reduced by selecting solvents with high vapor pressure; for example methyl-*tert*-butylether (MTBE), ethyl-acetate, or heptane are typically used at laboratory scale.<sup>8,13</sup> On the other hand, these solvents can be toxic for cells due to their higher solubility in water, compromising the possibility of cell recycling. Hence, interesting alternatives for reducing solvent-product separation steps include using the same solvent as in the bioreactor to reduce evaporation, using a solvent compatible with the final product formulation (e.g., diesel for sesquiterpene-based biofuels), or increasing the oil fraction by recycling sesquiterpene.

### Aim of this work

This work studies the effect of solvent on the evaporation rate, droplet size, and oil-phase recovery in sesquiterpene fermentations by using empirical correlations and transfer models based on predicted VLE properties. It also evaluates the techno-economic impact of using solvents in a microbial sesquiterpene production process by means of flow sheeting at two scales, namely 25 MT year<sup>−1</sup> (flavors and fragrances market) and 25 000 MT year<sup>−1</sup> (aviation fuel market).

## Materials and methods

### Experiments

#### Preparation of o/w dispersions

Oil in water dispersions was prepared in a 2 L jacketed vessel (Applikon, The Netherlands) containing 1.275 L of demineralized water, and 0–10% v/v of sesquiterpene and dodecane (Sigma Aldrich, > 99% purity). Experiments were performed using the sesquiterpene caryophyllene (kindly provided by Firmenich, > 95% purity), as it provided higher stability than commercially available synthetic farnesene, and their physical properties are expected to be similar (Table 1). The vessel was aerated using pressurized air at a flow rate of 1.5 nL min<sup>−1</sup>, controlled by a

mass flow controller (Brooks Instrument, Hatfield, United States); the temperature was maintained at 35 °C; and the stirring speed of a six-blade Rushton impeller of 45 mm diameter was kept at 1000 rpm. Aeration, temperature, and stirring speed were chosen to mimic typical fermentation conditions. To eliminate effects of any residual surfactants the vessel was cleaned with a regular dish soap, rinsed two times with demi-water, cleaned twice with 70% ethanol and rinsed again with demi-water.

## Droplet size analysis

Droplet images were recorded *in situ* by a SOPAT probe (SOPAT GmbH), and analyzed using the image analysis software provided by SOPAT GmbH<sup>40</sup> as described by Heeres *et al.*<sup>41</sup> A set of 100 pictures was taken 30 min after every oil addition, ensuring a stable droplet size and more than 1000 droplets per data point.

## Surface tension

Surface tension of water ( $\sigma_{wa}$ ), caryophyllene ( $\sigma_{oa}$ ), and dodecane ( $\sigma_{od}$ ) (Table 1) were measured using a Krüss ring tensiometer (model 01260).

## Modeling

### Droplet size and required separation area

In this work, the model proposed by Alopaeus *et al.*,<sup>42</sup> which applies to turbulent conditions, was chosen to estimate the droplet size ( $d_{oil}$ ) in the bioreactor, using the volume fraction of the dispersed phase ( $\phi_{oil}$ ), the power input per unit mass ( $e_G$ ), the o/w interfacial tension ( $\sigma_{ow}$ ), the viscosity of the continuous phase ( $\eta_w$ ), the densities of the dispersed ( $\rho_{oil}$ ) and continuous phases ( $\rho_l$ ), and a set of universal constants, which are independent of the operating conditions and design parameters ( $C_1 = 4.87 \times 10^{-3}$ ;  $C_2 = 5.52 \times 10^{-2}$ ;  $C_3 = 2.17 \times 10^{-4}$ ;  $C_4 = 2.28 \times 10^{13} \text{ m}^2$ <sup>43</sup>) (Eqn (3)):

$$\ln\left(10.8038 \cdot \phi \frac{C_3}{C_1}\right) = C_4 \frac{\eta_w \cdot \rho_l \cdot e_G}{\sigma_{ow}^2 (1+\phi)^3} \left(\frac{d_{oil}}{2}\right)^4 - C_2 \frac{\sigma_{ow} (1+\phi)^2}{\rho_{oil} \cdot e_G^{2/3} \cdot d_{oil}^{5/3}} \quad (3)$$

The required separation area for recovering the dispersed oil droplets of size ( $d_{oil}$ ) and density ( $\rho_{oil}$ ) in a disk-stack centrifuge was estimated based on the sigma factor ( $\Sigma$ ). This factor is the equivalent cross sectional area of a gravity settler and depends on the efficiency of the centrifuge

( $\xi$ ), the viscosity of the aqueous phase ( $\eta_w$ ), and the maximum capacity throughput (Q) (Eqn (4)):

$$\Sigma = \frac{Q}{\xi} \frac{18 \cdot \eta_w}{d_{oil}^2 \cdot (\rho_l - \rho_{oil}) \cdot g} \quad (4)$$

### Evaporation rate: L-V and L-L-V transfer models

The evaporation rate of sesquiterpene ( $R_{evap}$ ) can be estimated from its molar fraction in the gas phase ( $y$ ) and the total flow of gas leaving the bioreactor ( $F_G$ ) (Eqn (5)):

$$R_{evap} = y \cdot F_G \quad (5)$$

In this study, the maximum evaporation rate of sesquiterpenes at different aeration rates, fermenter volumes, and solvent volumetric fractions was evaluated by phase equilibrium models based on predicted physical properties (Table 1), and experimental data from Schuhfried *et al.*<sup>25</sup> Two possible transfer routes were considered:

- Transfer from oil droplets to gas bubbles via aqueous phase (L-L-V): This model determines the molar fraction of the sesquiterpene in the gas phase ( $y$ ) in equilibrium with the aqueous phase as a function of the Henry's constant ( $k_H$ ), the total pressure ( $P_{tot}$ ), and the concentration of sesquiterpene in the aqueous phase (Eqn (6)):

$$y = \frac{k_H}{P_{tot}} C^w \quad (6)$$

Assuming equilibrium conditions between the oil and the aqueous phase, the concentration of sesquiterpene in the aqueous phase ( $C^w$ ) was estimated as the ratio between the concentration of sesquiterpene in the oil ( $C^{oil}$ ), and the predicted values of the sesquiterpene distribution coefficient between 1-octanol and water ( $P_{ow}$ ) (Eqn (7)):

$$C^w = \frac{C^{org}}{P_{ow}} \quad (7)$$

Direct transfer from oil to gas phase (L-V): This model assumes that oil droplets collide with gas bubbles allowing direct transfer of sesquiterpene from oil to the gas phase. Assuming ideal behavior, the gas phase composition ( $y$ ) in equilibrium with an oil phase of composition ( $x$ ) can be estimated by Raoult's law (Eqn (8)):

$$y = x \cdot (p^{vap} / P_{tot}) \quad (8)$$

Using the properties of farnesene as reference, the maximum evaporation rate of sesquiterpene in a bioreactor working at 35 °C, 1 atm and aerated at 1 vvm were esti-

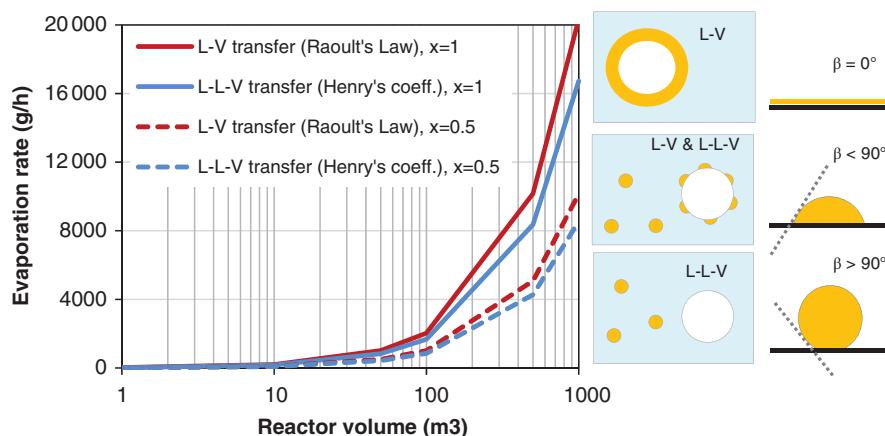


Figure 2. Maximum estimated evaporation rate at 1 vvm gas flow and 35 °C, as function of the fermenter working volume for two different routes: L-V modelled by Raoult's law, and L-L-V modelled by Henry's law.  $x = 1$  is the composition of an oil phase purely composed of sesquiterpene and  $x = 0.5$  represents a situation where 10% v/v of sesquiterpene is produced in a bioreactor containing 10% v/v of solvent. Distribution of oil phase on gas-water interface depends on the contact angle  $\beta$ : (i) When  $\beta = 0^\circ$  oil phase is fully spread onto gas surface (ii)  $\beta < 90^\circ$  oil beads with large contact area oil-gas are formed, and (iii) if  $\beta > 90^\circ$  oil beads with low oil-gas contact area are formed.

mated for both routes at different working scales (Fig. 2). A preliminary analysis considering interfacial tensions was performed to elucidate which route is more probable. The prevalence of one over the other depends on the interfacial properties of the three phases. Upon droplet-bubble collision, oil can remain on the bubble surface as beads, or can spread forming a layer (Fig. 2). The first situation would favor L-L-V transfer of sesquiterpene via the aqueous phase, whereas the formation of an oil layer on the bubble would promote direct L-V transfer of sesquiterpene. The values of interfacial tension ( $\sigma_{ow}$ ) for caryophyllene and dodecane (Table 1) were estimated following the method developed by Girifalco and Good<sup>23</sup> (Eqn (9)) using  $\Phi = 0.5595$  as indicated by Demond and Lindner<sup>19</sup> for aliphatic hydrocarbons:

$$\sigma_{ow} = \sigma_{oa} + \sigma_{wa} - 2 \cdot \Phi \cdot (\sigma_{oa} \cdot \sigma_{wa})^{1/2} \quad (9)$$

The spreading coefficient ( $S$ ), indicating the wetting of a gas bubble by the oil phase in presence of water, and the contact angle ( $\beta$ ) between the three phases, were calculated from the interfacial tension values as described by Rowlinson and Widom (Eqns (10) and (11)).<sup>44</sup>

$$S = \sigma_{wa} - (\sigma_{oa} + \sigma_{ow}) \quad (10)$$

$$\cos(\beta) = ((\sigma_{wa})^2 - (\sigma_{ow})^2 - (\sigma_{oa})^2) / (2 \cdot \sigma_{ow} \cdot \sigma_{oa}) \quad (11)$$

## Process simulation: Basic assumptions

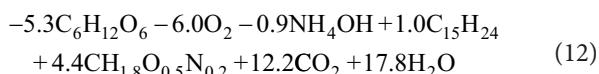
The techno-economic performance of a reference case (Fig. 1(B)) based on Tabur and Dorin<sup>9</sup> has been compared to a solvent-based process (Fig. 1(C)) by using the flowsheet simulation software SuperPro Designer™ (v. 9.5, build 3). Farnesene has been selected as a reference sesquiterpene due to its wide range of applications at different production scales (e.g., flavors, fragrances, and fuels), and due to the availability of some experimental data for product recovery. Several cases have been considered for the solvent-based process to account for the different roles that solvents can play (Table 2). In addition, a scenario in which sesquiterpene is used to enrich kerosene has been considered to represent an alternative in which solvents are compatible with the final product formulation. All cases included fermentation, primary recovery by centrifugation, demulsification and, when indicated, product/solvent separation. The basic assumptions per step are described below.

- Fermentation. Stoichiometric model based on metabolic pathway

In this work, 100 g L<sup>-1</sup> of sesquiterpene is produced in a continuous bioreactor by a recombinant strain of *S. cerevisiae* via glycolysis and mevalonate pathways according to the process reaction in Eqn (12):

**Table 2. Overview of process simulation parameters. Base case: TPI demulsification, Case 1: Dodecane in bioreactor and CPI demulsification, Case 2: condenser in bioreactor and CPI emulsification, Case 3: Dodecane in bioreactor and dodecane for CPI, Case 4: Farnesene for CPI, Case 5: Kerosene for CPI.**

Scale: 25 MT year <sup>-1</sup> (Flavors and fragrances, pharma, fine chemicals)								
		Base case	Case 1	Case 2	Case 2B	Case 3	Case 4	Case 5
Fermentation	V(m <sup>3</sup> )	1.16	1.27	1.16	1.17	1.21	1.17	0.06
	Evaporation Farnesene (g h <sup>-1</sup> )	24	15	24	24	15	24	1
	Evaporation dodecane (g h <sup>-1</sup> )	Na	75	Na	Na	72	Na	Na
	Condenser T(°C)	Na	Na	15	Na	Na	Na	Na
Centrifugation 1	o/w separation area (m <sup>2</sup> )	0.02	0.01	0.02	0.02	0.01	0.02	0.001
Demulsification	Demulsifier	Triton 0.5% w/w	MTBE 2:1 (v/v)	MTBE 2:1 (v/v)	MTBE 2:1 (v/v)	Dodecane 2:1 (v/v)	Farnesene 2:1 (v/v)	Kerosene 2:1 (v/v)
Distillation 1	V (m <sup>3</sup> )	Na	2.20	1.97	1.97	2.11	Na	Na
Distillation 2	V (m <sup>3</sup> )	Na	0.19	Na	Na	Na	Na	Na
Scale: 25 000 MT year <sup>-1</sup> (Biofuels and bulk chemicals)								
Fermentation	Vreactor(m <sup>3</sup> )	579(x2)	604(x2)	581(x2)	584(x2)	601(x2)	985(x2)	64
	Evaporation Farnesene (kg h <sup>-1</sup> )	12	7	12	12	7	12	1
	Evaporation dodecane (kg h <sup>-1</sup> )	Na	36	Na	Na	36	Na	Na
	Condenser T(°C)	Na	Na	15	Na	Na	Na	Na
Centrifugation 1	o/w separation area (m <sup>2</sup> )	21	12	21	21	12	21	1
Demulsification	Demulsifier	Triton 0.5% w/w	MTBE 2:1 (v/v)	MTBE 2:1 (v/v)	MTBE 2:1 (v/v)	Dodecane 2:1 (v/v)	Farnesene 2:1 (v/v)	Kerosene 2:1 (v/v)
Distillation 1	Number of distillation stages	Na	25	20	20	47	Na	Na
Distillation 2	Number of distillation stages	Na	34	Na	Na	Na	Na	Na



The previous equation assumes a production of 12 mol ATP per mol of sesquiterpene (based on metabolic pathway), a yield of 16.5 g cells per mol of generated ATP,<sup>45</sup> a maintenance coefficient of 0.05 mol ATP C-molX<sup>-1</sup> h<sup>-1</sup>,<sup>46</sup> and a specific growth rate of 0.04 h<sup>-1</sup>.

The evaporation rate of sesquiterpene in the bioreactor was estimated by the L-V model presented in Eqn (5), considering fermenters without off-gas condensers. To evaluate the use of solvent for reducing product evaporation, case 1, using 10% v/v of dodecane in the fermentation, is compared to case 2 and case 2B. Case 2 implements an off-gas condenser at 15 °C to recover the evaporated sesquiterpene, followed by a settler to separate the sesquiterpene

from condensation water. Case 2B does not incorporate product recovery from the off gas.

No effects in fermentation performance have been reported in the sesquiterpene literature for oil fractions of 0.1–0.2 v/v. At these oil fractions it is expected that it only affects the stirring and aeration requirements for maintaining enough dissolved oxygen in the fermentation broth. In consequence, the current work does not consider any impact of the solvent in the fermentation model. Potential improvements in oxygen transfer would be reflected in utilities requirements (i.e. power consumption for stirring and aeration). However, our results show that, in all cases, the utilities contribution to the operating costs are less than 8% (see Table 3 and supplementary material). Hence, no significant economic impact is expected.

- Recovery of dispersed oil phase from aqueous broth: Disk-stack centrifuge

The dispersed organic phase is separated from the aqueous phase and the cells using a disk-stack centrifuge. Based on data reported by Tabur and Dorin,<sup>9</sup> it is assumed that 90% w/w of the oil phase is recovered in the form of an emulsion containing 75% w/w oil, 5% w/w cells, and 20% w/w water. The droplet size of the dispersion entering the centrifuge was calculated using the model presented in Eqn (3).

To evaluate the impact of solvent in oil recovery by promoting coalescence and creaming in the reactor, case 1, using 10% v/v of dodecane in the fermentation, is compared to case 2, which does not incorporate any solvent in the fermentation.

- Demulsification and recovery of clear oil phase: Disk-stack centrifuge

In the base case, the o/w emulsion is inverted by TPI by adding 0.5% w/w of Triton x-114 as reported by Tabur and Dorin.<sup>9</sup> In cases 1 and 2 the o/w emulsion is inverted by CPI by adding to the emulsion 2 volumes of MTBE per volume of fermentation broth leaving the reactor as reported in laboratory-scale protocols.<sup>8</sup>

For evaluating alternative solvents to MTBE for CIP, cases 3–5 were developed using 2:1 v/v of dodecane, recycled farnesene and kerosene respectively.

In all cases, the continuous oil phase is separated from the water phase by centrifugation in a disk-stack centrifuge assuming 98% of clear oil recovery, a cell diameter of 5 µm<sup>47</sup> and a cell density of 1050 g L<sup>-1</sup> (SuperPro Designer™ database).

- Solvent-product separation: Distillation

Solvent-product separation is simulated in SuperPro Designer™ using a distillation column. An additional column was considered when using more than one type of solvent. Due to the large number of separation stages required, the use of a continuous distillation column at a small scale would lead to an unfeasible high aspect ratio, and therefore distillation is simulated in a batch column.

The vapor pressure of the light key ( $p_i^{vap}$ ) and heavy key ( $p_j^{vap}$ ) components was evaluated at the molar averaged temperature of the bottoms (Eqn (13)) and used to calculate their relative volatility ( $\alpha_{ij}$ ) (Eqn (14)). The Antoine coefficients for the components mentioned in this work are obtained from the SuperPro Designer™ database and Tochigi *et al.*<sup>48</sup>

$$\log_{10}(p^{vap}) = A - B / (T + C) \quad (13)$$

$$\alpha_{ij} = p_i^{vap} / p_j^{vap} \quad (14)$$

## Economic model and environmental impact

Cases were compared on economic performance and environmental impact. Economic performance was assessed on the basis of the unit cost (\$ kg<sup>-1</sup>), calculated according to the SuperPro Designer™ built-in model for a new plant, considering materials cost (e.g., glucose, nutrients, and solvents), utilities cost (e.g., heating, cooling, and power), and facility-dependent cost (e.g., depreciation and maintenance), and excluding labor-dependent and waste-treatment costs. These economic estimates are expected to have an accuracy of 25%–40%, as usual in the conceptual design stages. To evaluate the environmental impact of the process, the E factor (kg waste kg<sup>-1</sup> product) has been estimated.<sup>49</sup> This E factor accounts for the fermentation off-gas emissions, and the bottom streams of the centrifuges containing cells, residual sesquiterpene, and residual solvent. As indicated by Sheldon,<sup>49</sup> water was excluded from the calculations of the aqueous waste streams.

More details on the economic model can be found in the supplementary material.

## Results and discussion

The base cases, corresponding to the current state of the art, resulted in unit costs of \$49.0 kg<sup>-1</sup> and \$3.2 kg<sup>-1</sup> at 25 MT year<sup>-1</sup> and 25 000 MT year<sup>-1</sup>, respectively (Table 3). The unit cost obtained at 25 000 MT year<sup>-1</sup> is within the range publicly reported ([www.amyris.com](http://www.amyris.com)) in 2012 and 2015 (\$9.6 kg<sup>-1</sup> and \$2.15 kg<sup>-1</sup>, respectively). Note that, at both scales, unit cost is dominated by the fermentation section. At 25 MT year<sup>-1</sup> fermentation costs represent 74% of the unit cost, already accounting for \$36.3 kg<sup>-1</sup>. At 25 000 MT year<sup>-1</sup> this increases to 99%, or \$3.17 kg<sup>-1</sup> (see supplementary material).

## Lowering evaporation rate of sesquiterpene

The sesquiterpene caryophyllene has a negative spread coefficient  $S < 0$ , and an oil-gas-water contact angle of  $\beta = 56^\circ$ . In this situation, both L-L-V and L-V transfer routes seem feasible (Fig. 2). Similar results are expected for other sesquiterpenes based on their comparable properties (Table 1). The lower contact angle for dodecane  $\beta = 34^\circ$  suggests that some solvents could promote the spreading of the oil phase onto the gas bubble and consequently direct transfer of sesquiterpene from the oil to the gas phase. Evaporation rates estimated at 35 °C, 1 atm, and 1 vvm were similar for both routes (Fig. 2), ranging from ~g h<sup>-1</sup> in 1 m<sup>3</sup> reactors to ~kg h<sup>-1</sup> in 1000 m<sup>3</sup> reactors. In the simulation of a continuous fermentation these

**Table 3. Overview of techno-economic performance. Base case: TPI demulsification, Case 1: Dodecane in bioreactor and CPI demulsification, Case 2: condenser in bioreactor and CPI emulsification, Case 3: Dodecane in bioreactor and dodecane for CPI, Case 4: Farnesene for CPI, Case 5: Kerosene for CPI.**

	Scale: 25 MT year <sup>-1</sup> (Flavors and fragrances, pharma, fine chemicals)						
	Base case	Case 1	Case 2	Case 2B	Case 3	Case 4	Case 5
E factor (kg waste kg <sup>-1</sup> product)	5	5	4	5	5	5	0,3
purity % w/w	99	96	100	100	95	100	5
Unit cost (\$ kg <sup>-1</sup> ) total stream	49.0	106.0	82.4	80.3	84.0	79.7	37.7
Glucose (%)	5	2	3	3	3	3	0
Other raw materials (%)	0	1	1	1	3	40	1
Utilities (%)	0	0	0	1	0	0	0
Depreciation and facility costs (%)	94	97	96	96	94	57	98
	Scale: 25 000 MT year <sup>-1</sup> (Biofuels and bulk chemicals)						
E factor	5	5	4	5	5	5	0,3
purity % w/w	99	95	100	100	96	100	5
Unit cost (\$ kg <sup>-1</sup> ) total stream	3.2	4.1	3.8	3.7	5.6	5.3	0.7
Glucose (%)	78	59	67	68	43	48	19
Other raw materials (%)	5	24	15	15	42	42	69
Utilities (%)	4	5	7	6	8	2	1
Depreciation and facility costs (%)	13	11	12	12	8	8	11

rates represented about 1% of the total product (Table 2). However, the current state of the art in microbial sesquiterpene fermentations is fed-batch operation. In this case lower productivities are achieved (e.g., 0.2–0.4 g L<sup>-1</sup> h<sup>-1</sup><sup>4,5</sup>) and evaporation could result in 5% to 10% of product loss. This estimation agrees with reported loss of 3% farnesene in a 2 L scale bioreactor operating in fed-batch at 30 °C and 1 vvm.<sup>6</sup>

The addition of 10% v/v of solvent in the bioreactor can reduce the evaporation rate by 50% (Fig. 2; Table 2) but it increases process complexity by requiring more unit operations (Fig. 1(C)). The need for an additional distillation column led to higher unit costs (Case 1, \$106.0 kg<sup>-1</sup> at 25 MT year<sup>-1</sup> or \$4.1 kg<sup>-1</sup> at 25 000 MT year<sup>-1</sup>) than recovering the sesquiterpene from the off-gas using a condenser (Case 2, \$82.4 kg<sup>-1</sup> /\$3.8 kg<sup>-1</sup>), or even higher than not recovering the sesquiterpene from the off-gas at all (Case 2B, \$80.3 kg<sup>-1</sup> /\$3.7 kg<sup>-1</sup>) (Table 3).

### Enhancing coalescence and creaming of the oil phase

Sesquiterpenes and dodecane have similar interfacial tension (Table 1) and thus estimated droplet sizes for dispersions of sesquiterpene in water and dodecane in water were comparable (Fig. 3). Despite this, adding solvent in the bioreactor results in higher oil fraction and lower

oil-phase density, leading to larger droplet size and lower required centrifugation area (Fig. 3) for a given recovery percentage. Experimental droplet size values were ~50 µm lower than predicted values and Eqn (3) could only predict experimental data when interfacial tension was lowered to about 15 mN m<sup>-1</sup>. These interfacial tension values are similar to data reported for biosurfactants,<sup>50</sup> which suggests that residual surfactants were present despite the thorough cleaning procedure of the mixing vessel (Fig. 3). Although the required o/w separation area has probably been underestimated, the required o/w separation areas are very small (Table 2). Using the cost-model available in SuperPro Designer™ the economic results are not affected unless areas above 10 000 m<sup>2</sup> are needed, which would correspond to droplet sizes smaller than 10 µm. The disk stack centrifuge also accounts for less than 15% of the total equipment cost and therefore this underestimation does not have a remarkable effect on the overall techno-economic performance.

### Demulsification of the oil phase by phase inversion

At 25 MT year<sup>-1</sup> commercial demulsifiers like Triton-X114 yielded significant lower unit cost (\$49.0 kg<sup>-1</sup>) than using low-boiling point solvents, like MTBE, as demulsifiers (\$82.4 kg<sup>-1</sup>) (Table 3). At large scale both alternatives

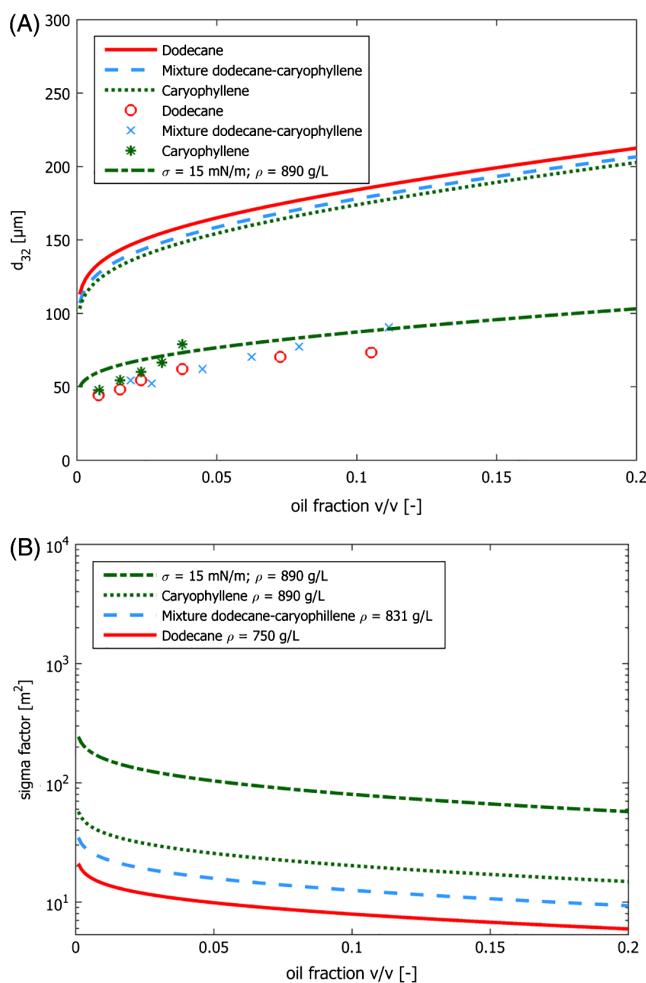


Figure 3. Theoretical values of droplet size (A) and required sigma factor (B) for recovering oil droplets in a disk stack centrifuge of 30% efficiency and  $11 \text{ L s}^{-1}$  capacity as function of oil fraction estimated at a constant power input of  $2.4 \text{ W kg}^{-1}$  (corresponding to 1.5 kg fermenter, stirring rate of 1000 rpm, and 1 vvm of gas flow) compared to experimental droplet size values (indicated as markers) at power input ranging from  $2.2$  to  $2.7 \text{ W kg}^{-1}$  (corresponding to 1.275 L of water and different amounts of caryophyllene and dodecane). Potential effect of lowering interfacial tension to  $15 \text{ mN m}^{-1}$  by surface active components in a caryophyllene/water dispersion is also shown.

presented similar costs ( $\$3.2 \text{ kg}^{-1}$  in the base case and  $\$3.8 \text{ kg}^{-1}$  in case 2), and similar environmental impact in terms of E factor ( $5 \text{ kg waste kg}^{-1}$  product in both cases). The E-factors of all the analyzed cases are in the order of values expected for bulk chemicals.<sup>49</sup>

Some alternatives to MTBE were proposed. Using dodecane to reduce evaporation and for demulsification (Case 3,  $\$84.0 \text{ kg}^{-1}/\$5.6 \text{ kg}^{-1}$ ) could not compete with the base case ( $\$49.0 \text{ kg}^{-1}/\$3.2 \text{ kg}^{-1}$ ). Although recy-

cling farnesene as solvent in CPI presented lower cost (Case 4,  $\$79.7 \text{ kg}^{-1}/\$5.3 \text{ kg}^{-1}$ ) than MTBE or dodecane, it was still less competitive than the base case. The main reason is the partial loss of farnesene in the second centrifugation step, which requires a considerable amount of farnesene as make-up of the recycle stream. On the other hand, enrichment of kerosene with 5% of farnesene ( $\$37.7 \text{ kg}^{-1}/\$0.7 \text{ kg}^{-1}$ ) is a promising option with lower unit costs than the base case at any scale.

Finally, this work employed 2 volumes of solvent per volume of generated broth, as reported in literature. However, in the studied processes aqueous broth and cells were partially removed prior to the CPI. The actual volume ratios of solvent:emulsion are about 10:1, and a possible reduction in solvent cost seems feasible. As an example, reducing the amount of solvent by 50% in case 2, leads to 7% of unit cost savings at  $25 \text{ MT year}^{-1}$  and 3% savings at  $25\,000 \text{ MT year}^{-1}$ .

### Impact of scale in techno-economic performance

The main advantages of using solvent at process scale are: (a) reducing product evaporation and consequently glucose consumption; (b) avoiding the presence of surfactants in the final product; (c) enhancing product recovery by reducing o/w separation area in the disk stack centrifuge; and eventually (d) reducing the power input requirements in the fermentation. On the other hand, extra investment in solvent-product separation is needed.

When a new plant is considered, as in this work, at the small scale typical of the flavors and fragrances market, equipment costs dominate over operating costs (see Table 3 and supplementary material). As a result, savings in raw material when reducing product evaporation cannot overcome the extra investment in equipment required for solvent-product separation (case 1). A solvent-based process can only compete with the current state of the art when considering options that do not require extra separation units, like product recycling (case 4) or using solvents compatible with final product formulation (case 5). These options did not bring any remarkable economic advantage compared to the base case; however, they yielded higher product purity and resulted in lower environmental impact, respectively (Table 3).

At larger scales, typical of bulk chemicals and fuels, however, unit operating costs are significantly reduced. Furthermore, raw materials have a much higher contribution to the costs than the equipment (e.g., about 80% in the base case), and consequently the advantages of using solvents in the fermentation become more relevant. In

addition, some equipment, like distillation columns, can be operated in continuous mode allowing for a more efficient solvent-product separation. As a result, unit costs of solvent-based processes ( $\$3.7\text{--}5.6\text{ kg}^{-1}$ ) are comparable with the current state of the art ( $\$3.2\text{ kg}^{-1}$ ), or even lower in the context of a kerosene enrichment process ( $\$0.7\text{ kg}^{-1}$ ). Larger savings in solvent-based sesquiterpene process would require improving the CPI demulsification efficiency by reducing the required amount of solvent and reducing the loss of solvent and product in the aqueous streams of the centrifuges.

## Conclusions

In this work a solvent-based process for microbial sesquiterpene production was evaluated at different scales. Although several simplifications were made and absolute values should be considered with care, trends and comparisons among cases are expected to be correct. Solvents reduce sesquiterpene evaporation in fermentation and enhance product recovery. However, solvent selection should consider compatibility with final product formulations to avoid extra separation costs. Further reduction in product recovery costs and environmental impact can be achieved in sesquiterpene production by lowering the amount of demulsifiers (e.g., solvent, surfactants) or by implementing alternative recovery methods with higher yields and less unit operations.

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## Nomenclature

$A$	Antoine coefficient
$\alpha_{LH}$	relative volatility
$B$	Antoine coefficient
$\beta$	L-L-V contact angle
$C_D$	drag coefficient
$C$	Antoine coefficient
$C^{oil}$	concentration of sesquiterpene in the oil
$d_{oil}$	droplet size
$\Delta C_{O_2}$	difference in oxygen concentration
$e_G$	power input per unit mass

$F_G$	Gas flow leaving the bioreactor
$\phi$	volume fraction of oil
$\Phi$	molecular interaction parameter
$g$	gravitational constant
$\eta_w$	viscosity of the continuous phase
$k_H$	Henry's constant
$k_{La}$	overall mass transfer coefficient
$L$	liquid phase
OTR	oxygen transfer rate
$p^{vap}$	vapor pressure
$p_i^{vap}$	vapor pressure of the light key
$p_j^{vap}$	vapor pressure of the heavy key
$P_{tot}$	total pressure
$P_{ow}$	distribution coefficient between 1-octanol and water
$Q$	maximum capacity throughput
$\rho_l$	aqueous phase density
$\rho_{oil}$	oil density
$R_{evap}$	evaporation rate
$\sigma_{wa}$	surface tension of water
$\sigma_{oa}$	surface tension of oil
$\sigma_{ow}$	oil/water interfacial tension
$\sigma_{oa}$	oil/air interfacial tension
$S$	spreading coefficient
$\Sigma$	sigma factor
$T$	temperature
$v_d$	creaming velocity
$V$	volume
$V$	vapor phase
$x$	molar composition of oil phase
$\xi$	efficiency of the centrifuge
$y$	molar fraction in the gas phase

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Susana Pedraza-de la Cuesta has combined her background in chemical engineering and biotechnology to pursue her PhD on the integration of fermentation and recovery of sesquiterpenes at Delft University of Technology. Currently, she researches bioprocess and membrane separation as R&I process technologist for Danone-Nutricia.