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Production of sophorolipids from winterization oil cake by solid-state fermentation: optimization, monitoring and effect of mixing

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ABSTRACT

Sophorolipids (SLs) are a group of extracellular biosurfactants produced by the yeast *Starmerella bombicola*. The present study explored the use of winterization oil cake (WOC), a residual oil cake that comes from the oil refining industry, as a substrate for the production of SLs by solid-state fermentation (SSF). Sugar beet molasses (MOL) was used as a co-substrate and *C. bombicola* ATCC 22214 as the inoculum. Fermentation was performed on the 100-g scale and was optimized in terms of the ratio of substrates and the aeration rate using response surface methodology. The optimized SSF process (1:4 MOL:WOC mass ratio and 0.30 L kg^{-1} min^{-1} aeration rate), carried out under static conditions, was monitored for 10 days with a maximum SL yield of 0.179 g per g DM (dry matter), which corresponds to 19.1 g per 100 g of substrates (sum of the initial wet mass of WOC and MOL in the reactor). The effect of intermittent mixing on the process was also investigated. Mixing caused a 31% increase in SL production, with a total yield of 0.235 g per g DM, which corresponds to 25.1 g per 100 g of substrates. The Oxygen Uptake Rate (OUR) and the Cumulative Oxygen Consumption (COC) were used to monitor the biological activity of the fermentation processes. There were significant correlations between the SL yield and the oxygen and fats consumed. The SLs were characterized by FTIR and ^{1}H-NMR analysis.

Keywords: biosurfactant, oil cake, solid-state fermentation, sophorolipid, *Starmerella bombicola*

1. Introduction

Synthetic surfactants present several environmental burdens during their production and use [1, 2]. Surfactants represent a large growing market that is expected to reach $36.1 billion by 2020. The current world production is estimated at 15 million tonnes per year [3]. Biosurfactants, surface-active molecules produced by a variety of microorganisms, are an alternative to conventional surfactants because they are readily biodegradable, display low toxicity and can be produced from renewable feedstocks, or even wastes, by fermentation [4]. Biosurfactants can be applied in many fields, such as environmental management, improvement of oil quality, synthesis of new polymers and bioplastics, or in the pharmaceutical industry [5]. Sophorolipids (SLs) are a group of extracellular biosurfactants produced at relatively high yields by several non-pathogenic yeast species, with *Starmerella bombicola* being the most studied. SLs are glycolipids comprised of a sophorose moiety coupled with a hydroxylated fatty acid. They are produced as a mixture of different molecules with two major points of variation: acetylation in the sophorose, and lactonization (Fig. 1). Additionally, SLs have good anti-microbial, anti-inflammatory, anti-HIV and even anti-cancer effects, which allow these molecules to be used in the pharmaceutical sector [6].

SLs are currently produced for use as active ingredients in commercial products such as household cleaning products and cosmetics [7]. They are produced by submerged fermentation (SmF) with *Starmerella bombicola,* using glucose and vegetable oil as substrates [8]. Despite of the high industrial interest of SLs the major constraint for their market extension is the process productivity. Solid-state fermentation (SSF) is an alternative technology for SL production. SSF has proven successful for the production

of several compounds such as enzymes at high yields, often higher than SmF [9]. Some solid-state fermentations are already performed at the commercial scale in the food industry or for waste management and bioremediation processes. Knowledge on bioreactor design is strong and many novel processes have been performed in pilot scale bioreactors [10, 11].

Using SSF to produce SLs presents two important advantages over SmF [12]. First, SSF allows the use of inexpensive solid substrates, such as oil cakes from the food industry. Second, SSF avoids potential problems associated with foaming. However, there are several challenges that must be addressed before its use in large-scale processes. Some of the most important challenges are the design of a bioreactor that allows temperature and moisture control, the selection of efficient extraction methods and the monitoring of the cultivation process [13]. Little research has been performed on the production of SLs by SSF, and all the studies were performed using small amounts (5-10 g) of substrates [6,14,15].

The main goal of this work was to study the production of SLs through SSF of food-industry wastes by *Starmerella bombicola*. Winterization oil cake (a residual oil cake from the oil refining industry) and sugar beet molasses were used as substrates. The specific objectives were as follows: (i) to optimize the production of SLs in terms of the ratio of substrates and the aeration rate; (ii) to monitor the optimized process to establish the SL production curve; (iii) to assess the effect of intermittent mixing on SL production; and (iv) to investigate the correlation between SL yield and monitoring variables.

2. Materials and Methods

2.1. Materials

The winterization oil cake (WOC) was provided by the oil refinery LIPSA (Lípidos Santiga S.A., Barcelona, Spain). WOC is obtained by cooling sunflower oil to 5ºC for 24 h, followed by the removal of waxes by filtering with diatomaceous earth. The chemical composition of the WOC depends on the efficiency of the winterization process and the quality of the raw oil. Therefore, the content of WOC, in terms of waxes and other fats, can vary slightly among different batches. Sugar beet molasses (MOL) was supplied by the sugar company AB Azucarera Iberia S.L. (Madrid, Spain). The main characteristics of these materials are summarized in Table 1. Wheat straw, with a dry matter (DM) content of 96.0% and an organic matter (OM) content of 94.6% (dry basis), was provided by the experimental farms at Universitat Autònoma de Barcelona and was shredded and sieved and particles between 5 and 30 mm were used. All materials were autoclaved before use (121ºC, 30 min).

2.2. Yeast strain

Starmerella bombicola ATCC 22214 was obtained from the American Type Culture Collection (Manassas, USA). The strain was grown for 48 h at 30ºC on agar slants containing (g L^{-1}): dextrose, 10; peptone, 5; malt extract, 3; yeast extract, 3; agar, 20. The microorganism was conserved at -80ºC, and a new agar slant was cultivated when needed.

2.3. Preparation of the inoculum

Inoculum was prepared by adding a loopful of yeast, freshly grown on an agar slant, to a 250-mL Erlenmeyer flask with 50 mL of sterile medium containing (g L^{-1}): dextrose, 10; peptone, 5; malt extract, 3; yeast extract, 3. Subsequently, the culture was incubated in an orbital incubator shaker at 30ºC, 180 rpm for 48 h.

2.4. SSF experiments

Fermentation experiments were performed in 500-mL Erlenmeyer flasks containing 100 g of wet fermentation medium consisting of 45 g of substrates (WOC and MOL), 14 g of wheat straw (which was used as an inert support), plus 10 mL of inoculum and sterile distilled water to dissolve MOL to a final volume of 47 mL. The average bulk density of the substrate mixtures was 250 g L^{-1}, and the air filled porosity was 80%. A high value of air filled porosity enhances oxygen transfer and carbon dioxide and heat removal from the solid matrix [16]. Fig. 2 shows the experimental setup. The temperature was controlled by submerging the reactors in a water bath at 30ºC. Humidified air was continuously supplied to the reactors by means of a mass flow controller (Bronkhorst, Spain). The air line enters the reactor through the lid, with the air being injected below the bed. It passes through the solid bed and exits at the top. The oxygen concentration in the exhaust gases was recorded and used for the calculation of the Oxygen Uptake Rate (OUR) and the Cumulative Oxygen Consumption (COC) [17, 18]. OUR_{24h}, which is the average of OUR values over the 24 h of maximum activity, was also calculated [17].

At sampling times, the whole content of one reactor was collected and manually homogenized with a metal spatula. Then, 5 g was used to measure pH, 10 g was used to analyse SL content, and the rest of the sample was oven-dried at 105ºC to measure the moisture content (reported in wet basis). The fat content of the dry sample was then determined.

2.4.1. Optimizing the ratio of substrates and the aeration rate

The effects of the ratio of substrates and the aeration rate on the production of SLs were assessed by a full experimental design consisting of 12 experiments (3^2 experiments plus 3 replicates at the central point for statistical validation). The ratio of the substrates (MOL:WOC, w/w) was fixed at 1:2, 1:3 and 1:4 (normalized values -1, 0, 1, respectively), and the specific aeration rate was fixed at 0.05, 0.175 and 0.30 L air kg^{-1} total wet mass min^{-1} (normalized values -1, 0, 1, respectively). Table 2 summarizes the 15 experiments performed. The main characteristics of the mixtures are listed in Table 1. The yield of SLs at day 5 was selected as the objective function. Additional experiments to assess the influence of the aeration rate were performed in triplicate using a 1:4 MOL:WOC ratio.

2.4.2. Monitoring of the optimized process

The optimized SSF process was monitored for 10 days. Five reactors were prepared as described in section 2.4.1, and one of the reactors was sacrificed for analysis at each of the sampling points (0, 3, 5, 7 and 10 days). In consequence, analytical results are reported from one single reactor at each sampling point and average OUR and COC curves are obtained with the OUR and COC curves of all the reactors finishing at

different times: 4 OUR curves until day 3; 3 curves until day 5; 2 curves until day 7 and only one complete OUR profile for 10 days.

2.4.3. Effect of intermittent mixing on SL production

The optimized SSF process was also monitored for 10 days, but reactors were manually mixed at days 3, 5 and 7. For mixing, the whole content of a flask was discharged into a 1-L glass beaker, carefully mixed with a sterile metal spatula in a laminar flow chamber, and loaded again into the Erlenmeyer flask. One reactor was sacrificed, after mixing, at each of days 3, 5, 7 and 10.

2.5. Statistical analysis

The results of the full experimental design for process optimization were statistically analysed using a central composite design (CCD) with normalized values [19,20] and a one-way ANOVA followed by Duncan´s test ($p < 0.05$ confidence level). In addition, bivariate correlations (Pearson's coefficients) between SL yield and oxygen and fat consumed were determined. SPSS 15.0 software for Windows was used for all the statistical analysis.

2.6. Extraction of SLs and structural characterization

SLs were extracted from the fermentation mixture by mixing 10 g of material with ethyl acetate in a 1:4 (w/v) ratio at 250 rpm for 1 h. Three consecutive extractions were performed. The crude extract was centrifuged (2000 × g, 10 min), filtered through a 0.2-µm membrane filter, and the extract was vacuum-dried at 40ºC with a rotary evaporator.

The SLs were washed twice with 20 mL of *n*-hexane to remove any remaining oily residue and any hydrophobic substances such as fatty acids and alcohols formed during the fermentation. SLs are not soluble in n-hexane and remain in the flask after decantation. Partially purified SLs were obtained after evaporating the residual hexane at 40ºC under vacuum. The final product was amber coloured, honey-like, and semi-crystalline and was stored at 4ºC [21]. In this study, the SL yield is defined as grams of sophorolipid per g of total dry mass of fermentation. In the text, SL yield is also reported as grams of sophorolipid per 100 g of substrates (sum of the initial wet mass of WOC and MOL in the reactor) for comparison with yields reported in the literature.

The structural identity of the SLs produced was first confirmed by Fourier Transform Infrared Spectroscopy (FTIR) using a FTIR spectrophotometer (Tensor 27, Bruker). The instrument had an attenuated total reflection accessory (ATR Specac Golden Gate) which avoids the use of the KBr pellets. The infrared spectrum was recorded from 600 to 4000 cm^{-1}. Further characterization was carried out by ^{1}H-NMR using a $CDCl_3$ (Robot 250 MHz NMR Spectrometer, Bruker).

2.7. Analytical methods

The pH, moisture content and organic matter content were determined according to standard methods [22]. Moisture content is reported on a wet basis. Other variables are reported on a dry basis. The fat content was analysed using a standard Soxhlet method with *n*-hexane (≥ 95% pure, Sigma Aldrich) as the organic solvent (U.S. Environmental Protection Agency, Method 9071B). For determination of the carbohydrate content, three consecutive extractions were carried out by mixing a known amount of dried

sample with distilled water in a 1:10 (w/v) ratio. In each extraction, the mixture was incubated (50ºC, 15 min) in a water bath shaker, centrifuged (16000 × g, 10 min), and the supernatant was recovered. Water-soluble carbohydrate content was measured in the supernatant using the anthrone method [23].

3. Results and Discussion

3.1. Optimizing the aeration rate and the ratio of substrates

Winterization oil cake was found to be a feasible substrate for the production of SLs by SSF using sugar beet molasses as a co-substrate and *Starmerella bombicola* as the inoculum. The combined effect of the normalised aeration rate (A) and the normalised ratio of substrates (S) on the SL yield (Y, g g^{-1} DM) was determined by means of a factorial experimental design (Table 2), yielding the following equation with 6 significant terms and an R^2 of 0.913:

$$Y = 0.07 + 0.01A + 4.79 \cdot 10^{-3}S - 5.70 \cdot 10^{-3}A^2 - 6.66 \cdot 10^{-3}S^2 + 0.01AS \quad (1)$$

Fig. 3 shows the response surface obtained from Eq. (1). Increasing both the aeration rate and the ratio of substrates had a positive effect on the SL yield. The coefficient for A was approximately two-fold (2.08) higher than the coefficient for S, which indicates that aeration rate had a larger influence on SL yield than the substrate ratio. The interaction term highlights a simultaneous effect of both factors on SL yield. The optimal ratio of substrates was 1:4, the one with the highest amount of fat and the lowest amount of sugars (Table 1). This suggests that fats are the key substrate for SL

production in this process. The optimal aeration rate was the highest studied, 0.30 L kg^{-1} min^{-1}, confirming oxygen supply as an important parameter for this SSF. Additional experiments repeating the aeration rate of 0.30 L kg^{-1} min^{-1} and using higher aeration rates of 0.60 and 0.90 L kg^{-1} min^{-1} were performed at a fixed 1:4 substrate ratio to check if a further increase in the aeration rate would improve SL production (data not shown). However, there were no significant differences in the SL yield, OUR_{24h} and COC among the three different airflows. As demonstrated by Almeira et al. [24], low airflows can limit the biological activity, but increasing aeration over a given threshold does not affect the fermentation. Therefore, an aeration rate of 0.30 L kg^{-1} min^{-1} was selected for further experiments.

Significant positive correlations were found between the SL yield and both the OUR_{24h} and the COC (Pearson coefficients of 0.798 and 0.764, respectively, with $p < 0.01$). Additionally, SL yield and COC correlated significantly and positively with fat consumed (Pearson coefficients of 0.862 and 0.836, respectively, $p < 0.01$).

3.2. Time course of SL production in static and mixed fermentations

The SSF process was carried out over 10 days under the optimized conditions, namely a 1:4 WOC:MOL ratio and an aeration rate of 0.30 L kg^{-1} min^{-1}, so as to determine the time course of SL production. Figure 4 shows the results for the static (Fig. 4A) and mixed (Fig. 4B) fermentations.

SL production was detected since the first sampling on day 3. The maximum yield was reached on day 10, with a total amount of 0.179 g per g DM, which corresponds to 19.1

g per 100 g of wet substrates. Fig. 4A shows OUR and COC profiles over the 10 days. OUR reached its maximum value at 66 h (1.92 ± 0.21 mg O_2 g^{-1} DM h^{-1}, average ± standard deviation for 4 fermentations), and the oxygen consumed at the end of the fermentation was 330 g O_2 per g DM. Fig. 4A also shows the profile of fat content expressed as grams of fat per gram of total dry matter in the fermentation medium. Approximately half of the initial fats were consumed at the end of the fermentation. Unused fats remaining in the reactor may be composed of waxes not degraded by *C. bombicola* or fats that are not accessible to the yeast (for instance, in the inner part of the bigger particles) [19].

The pH dropped from an initial value of 6.4 to values between 3 and 3.5 after day 5. When SLs are produced by SmF, the pH also drops to acidic values [25]. The moisture content remained at values of approximately 45% wet basis during the whole process, which confirms the effectiveness of the humidified air in the control of the fermentation moisture.

To improve substrate bioavailability and reduce nutrients and biomass composition gradients in the solid mass [26], the effect of intermittent mixing on SL production by SSF was explored (Fig. 4B). Mixing increased the maximum SL yield by 31% compared with fermentation under static conditions (Fig. 4A), with a total production at day 10 of 0.235 g per g DM, which corresponds to 25.1 g per 100 g of wet substrates.

OUR reached a first maximum value prior to the first mixing and sampling (1.91 ± 0.18 mg O_2 g^{-1} DM h^{-1}, average ± standard deviation for 4 fermentations), this value being statistically identical to that of the static experiment. Mixing increased the OUR, and the

global maximum was reached at 168 h (1.97 ± 0.02 mg O_2 g^{-1} DM h^{-1}, average ± standard deviation for 2 fermentations). The total oxygen consumed at the end of the fermentation was 380 g O_2 per g DM. This is 15% higher than the value obtained in the non-mixed fermentation (Fig. 4), indicating a higher biological activity in the mixed reactors. By day 10, 22% more fat had been consumed in the mixed fermentation than in the static fermentation. As was the case in the experiments under static conditions, the pH dropped from an initial value of 6.6 to values between 3 and 3.5, and the moisture content remained at values near 45%.

We observed differences between the mixed and the static solids. Intermittent mixing reduced the size of the solid aggregates and visually enhanced the colonisation of the solids by the yeast. From these observations, we concluded that intermittent mixing increases the bioavailability of substrates to the yeast and, therefore, more oxygen and fats are consumed and more SLs are produced. The positive effect of intermittent mixing on the biological activity of solid-state processes has been previously described [26]. These findings indicate that the SSF process could be scaled up using bioreactors with mixing systems, such as rotating drums. These changes would not only improve the yield of SLs but would also help to reduce channelling and overheating problems that are typically found in unmixed systems [27].

3.3. Sophorolipid production by SSF: monitoring of process variables

The monitoring of SSF processes for biosurfactant production is a challenge that must be addressed in the scale-up of such processes to commercial production scale [13]. Respiration parameters, such as OUR and COC, are useful for the monitoring of the

biological activity in the fermentation medium. However, respirometry has not yet been explored for the production of biosurfactant by SSF.

All the experimental results reported in this paper plus the additional experiments with high aeration mentioned in section 3.1 were gathered together to explore the correlations between oxygen and fats consumed and SLs produced. As illustrated in Fig. 5A, SL yield (g g^{-1}) was directly proportional to the COC (g g^{-1}), with a proportionality constant of 0.502 (g SL per g O_2). (n = 35, R^2 = 0.946, p = 0.001). This suggests that the respiration parameter COC can be used as an indirect measurement of the production of SLs for the on-line monitoring of SSF. Additionally, SL yield (g g^{-1}) was directly proportional to the fats consumed (g g^{-1}), with a proportionality constant of 0.805 (g SL per g fat consumed) (n = 35, R^2 = 0.970, p = 0.001) (Fig. 5B). The fats in the WOC act as the hydrophobic carbon source for SL synthesis.

3.4. Sophorolipid production by SSF: yields

As described before, the yield of SLs in this work was 19.1 g per 100 g of wet substrates, which increased to 25.1 g per 100 g of wet substrates when intermittent mixing was applied. Our results are similar to those of Parekh and Pandit [14], who obtained a maximum SL yield of 18 g per 100 g of substrates using glucose and oleic acid as pure substrates (blended with wheat bran). Additionally, similar yields were obtained when the oleic acid was substituted by mango kernel fat as the hydrophobic carbon source for SL production [15]. Rashad et al. [6] used a medium based on a mixture of sunflower oil cake and soybean oil moistened with a nutrient medium. These authors obtained a maximum yield of 22 g per 100 g of substrates when using the same

ethyl acetate extraction method as that used by Parekh and Pandit [14] and in this work. However, they obtained 49.5 g per 100 g of substrates when they improved the extraction by using a new concept based on two consecutive extractions with two different solvents: methanol and ethyl acetate. This highlights that the quantification of SL production strongly depends on the extraction method. In consequence, the efficient extraction of SLs from the solid matrix must be further studied.

These three studies of SL production by SSF were performed using 5-10 g of substrates in 15-20 g of total wet mass. In our study, we increased the scale of operation five-fold to 100 g of total wet mass and used wastes instead of pure substrates, yet we still obtained yields similar to those previously reported [6, 14, 15]. Additionally, these authors either moistened the media with a pH buffer or added nutrients to the solid medium (e.g., yeast extract). In our study, we avoided the use of additives to simplify and cheapen the SSF process in view of its prospective scale-up.

3.5. Structural characterization of the SLs obtained

The SLs obtained were initially identified and characterized by FTIR (Fig. 6A). The spectrum revealed a broad band at 3396 cm^{-1}, which corresponds to the O-H stretch (Fig. 1). Asymmetrical stretching (ν_{as} CH_2) and symmetrical stretching (ν_s CH_2) of methylene were observed at 2924 cm^{-1} and 2854 cm^{-1}, respectively. The C=O absorption band at 1742 cm^{-1} may include contributions from lactones, esters or acids. The stretch of the C-O band of C(=O)-O-C in lactones appeared at 1165 cm^{-1}. The C=O absorption band at 1235 cm^{-1} of acetyl esters and the band at 1367 cm^{-1} for the symmetrical bending of the methyl groups of the acetyl esters indicate that our SL mixture contains

acetylated sophorose moieties. The band at 1456 cm^{-1} corresponds to the C-O-H in-plane bending of carboxylic acid (-COOH) and may indicate the presence of small quantities of unused fatty acids that were left after hexane washings or the contribution of acid SLs. The spectra also show the absorption for C=C at 721 cm^{-1}. Finally, a C-O stretch from C-O-H groups of sugars was observed at 1034-1075 cm^{-1}.

The ^{1}H-NMR spectrum of the SLs obtained is typical of a glycolipid-type structure (Fig. 6B). The protons of the two glucoses resonated at 3.44 - 4.57 ppm. It was possible to identify the signals of the protons of glucose-H-1´ (4.45 and 4.48 ppm) and glucose-H-1´´ (4.54 and 4.57 ppm). These signals correspond, respectively, to the glycosidic bond between the sophorose molecule and the fatty acid chain and the glycosidic bond between the two glucoses. The protons of the ($–COCH_3$) group resonated at 2.09 ppm. Multiple signals of protons at 1.30 ppm indicate the presence of a fatty acid chain moiety, and peaks at 5.34 ppm correspond to the vinyl group (-CH=CH-) of the fatty acid chain.

The structural characterization obtained by FTIR and ^{1}H-NMR analysis was similar to the structural characterization reported previously by other authors [6, 25], which confirmed that the product obtained belongs to the SLs.

4. Conclusions

Winterization oil cake is a feasible substrate for producing sophorolipids by SSF using sugar beet molasses as the co-substrate and *Starmerella bombicola* as the microorganism. The sophorolipid yield at 10 days was 0.179 g per g of DM, which

corresponds to 19.1 g per 100 g of wet substrates. Intermittent mixing (at 3, 5 and 7 days) increased SL yield 31% to 0.235 g per g of DM, which corresponds to 25.1 g per 100 g of wet substrates. SL levels were linearly correlated with the cumulative oxygen consumption, suggesting that respirometry can be a useful tool for monitoring SL production by *C. bombicola* in SSF.

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Tables

Table 1

Main characteristics of the substrates and the initial mixtures.

	Substrates		Initial Mixtures		
	Winterization Oil Cake	Sugar Beet Molasses	MOL:WOC Ratio 1:2	MOL:WOC Ratio 1:3	MOL:WOC Ratio 1:4
pH	4.75 ± 0.01	6.79 ± 0.06	6.14 ± 0.02	6.26 ± 0.06	6.54 ± 0.03
Moisture (%)	0	16.9 ± 0.8	43.7 ± 0.8	43.8 ± 0.9	44.7 ± 0.9
Organic matter (%, db)	67.5 ± 2.5	87.3 ± 2.1	78.1 ± 0.1	75.9 ± 0.3	74.7 ± 0.4
Fat content (%, db)	67.5 ± 2.5	n.d.	33.9 ± 0.8	36.1 ± 0.2	39.0 ± 0.2
Carbohydrate content (%, db)	n.d.	78.4 ± 2.8	16.1 ± 1.0	13.4 ± 0.8	10.6 ± 1.1

Abbreviations: db, dry basis; n.d., not detected. Data presented as mean ± standard deviation of the sample.

Table 2

Aeration (normalized value)	Substrate ratio (normalized value)	Aeration (L air kg^{-1} WM)	Substrate ratio (w/w)	Sl yield (g g^{-1} DM)	Predicted SL yield (g g^{-1} DM)
-1	-1	0.05	1:2	0.0519	0.0507
-1	0	0.05	1:3	0.0523	0.0519
-1	1	0.05	1:4	0.0382	0.0398
0	-1	0.175	1:2	0.0553	0.0572
0	0	0.175	1:3	0.0732	0.0687
0	1	0.175	1:4	0.0704	0.0668
0	0	0.175	1:3	0.0695	0.0687
0	0	0.175	1:3	0.0670	0.0687
0	0	0.175	1:3	0.0634	0.0687
1	-1	0.30	1:2	0.0532	0.0524
1	0	0.30	1:3	0.0753	0.0741
1	1	0.30	1:4	0.0804	0.0824

Abbreviations: WM: wet mas;, DM: dry mass.

Figures

Fig. 1. Structure of natural sophorolipids (A) lactonic form and (B) free acid form.

Fig. 2. Experimental setup of the SSF experiments.

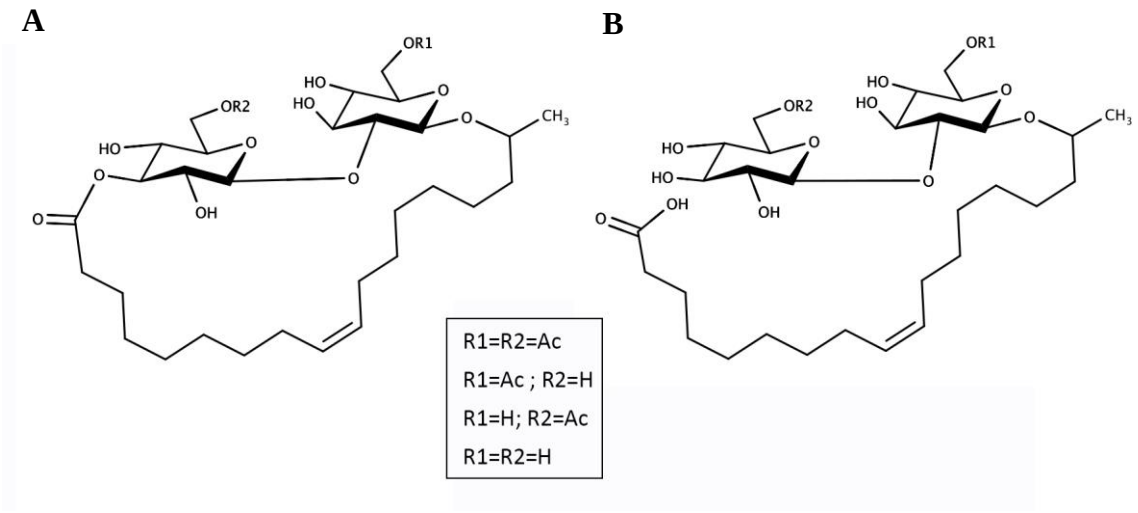
Fig. 3. Response surface for SL yield (g per g DM) obtained at different aeration rates: 0.05 (-1), 0.175 (0) and 0.30 (1) L air kg^{-1} min^{-1}; and the different ratio of substrates: 1:2 (-1), 1:3 (0) and 1:4 (1) MOL:WOC.

Fig. 4. Solid-state fermentation profile of the process under static (A) and intermittently mixed (B) conditions: OUR and COC profiles and time course of SL yield and fat content.

Fig. 5. Correlations between SL yield and Cumulative Oxygen Consumption (COC) (A) and between SL yield and fat consumed (B).

Fig. 6. Spectra of the SL produced by FTIR (A) and ^{1}H-NMR (B).

Figures



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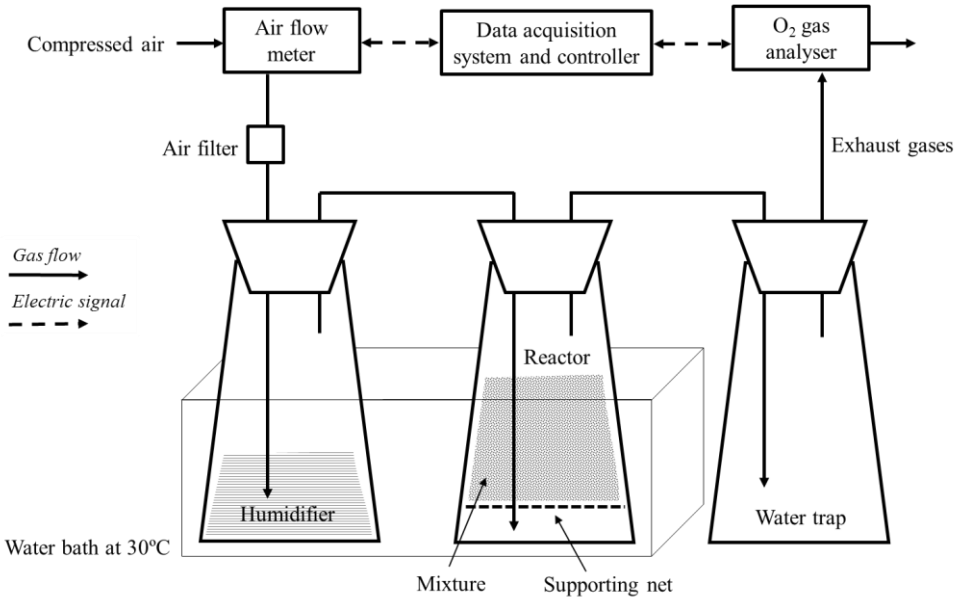

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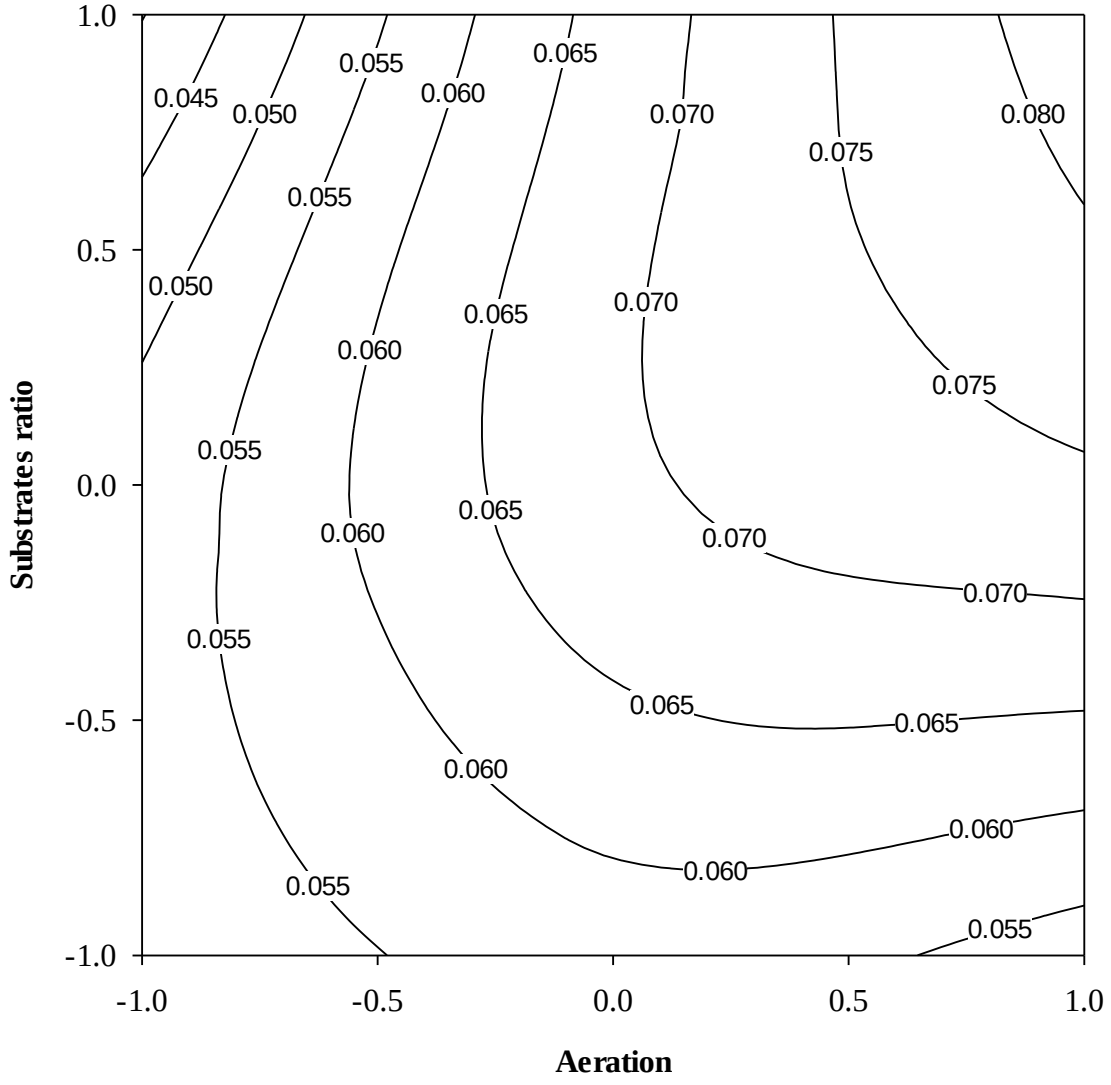

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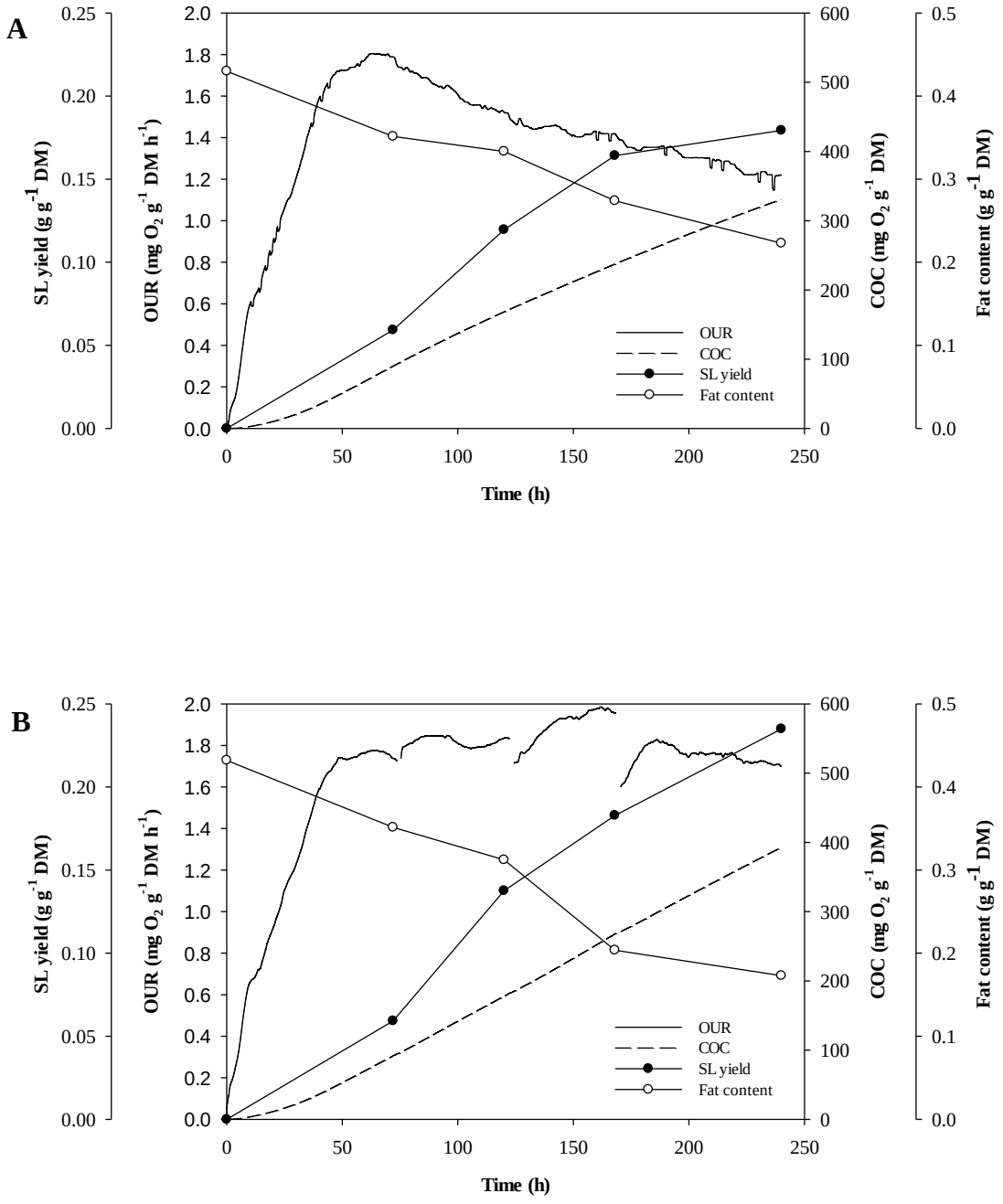

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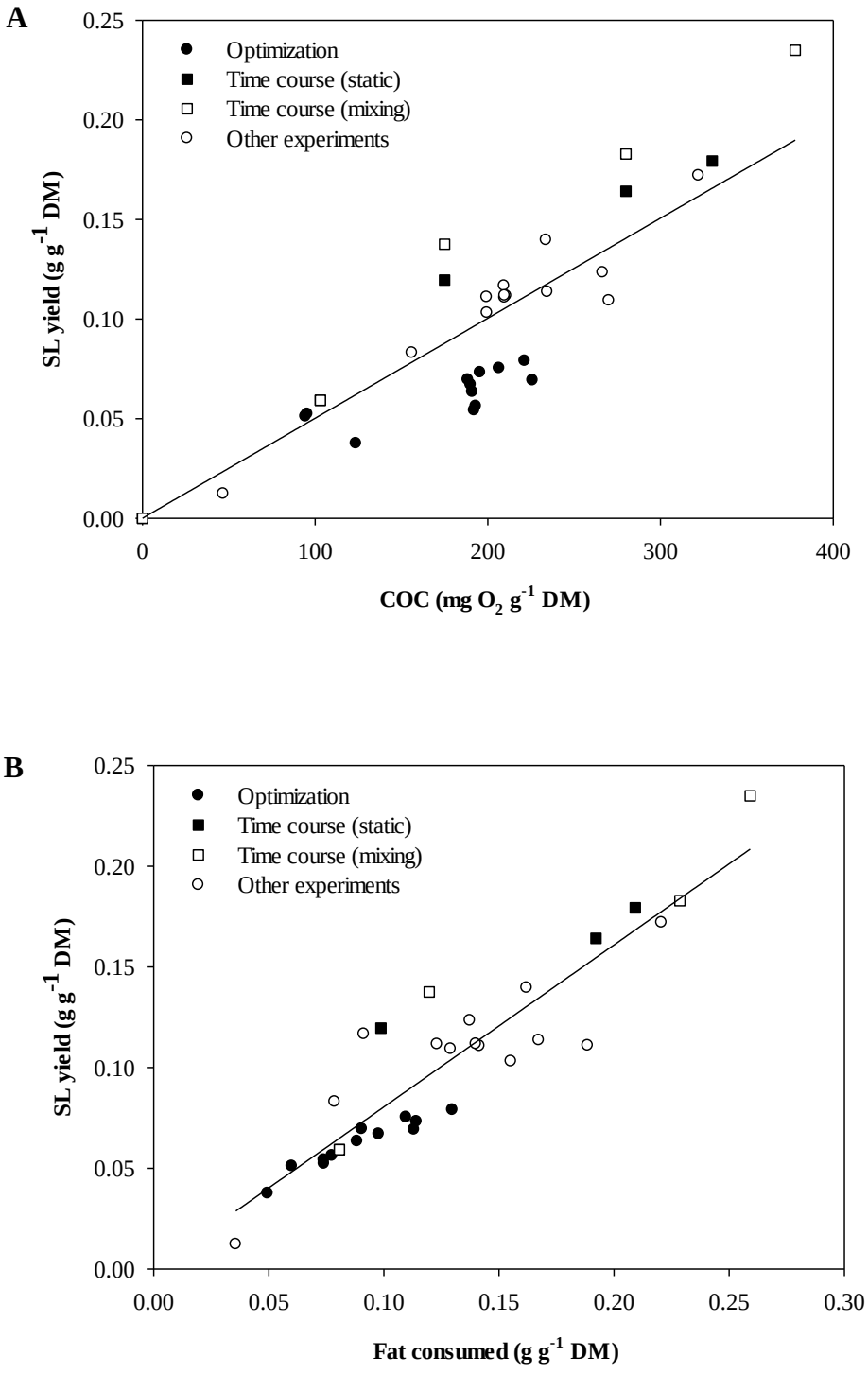

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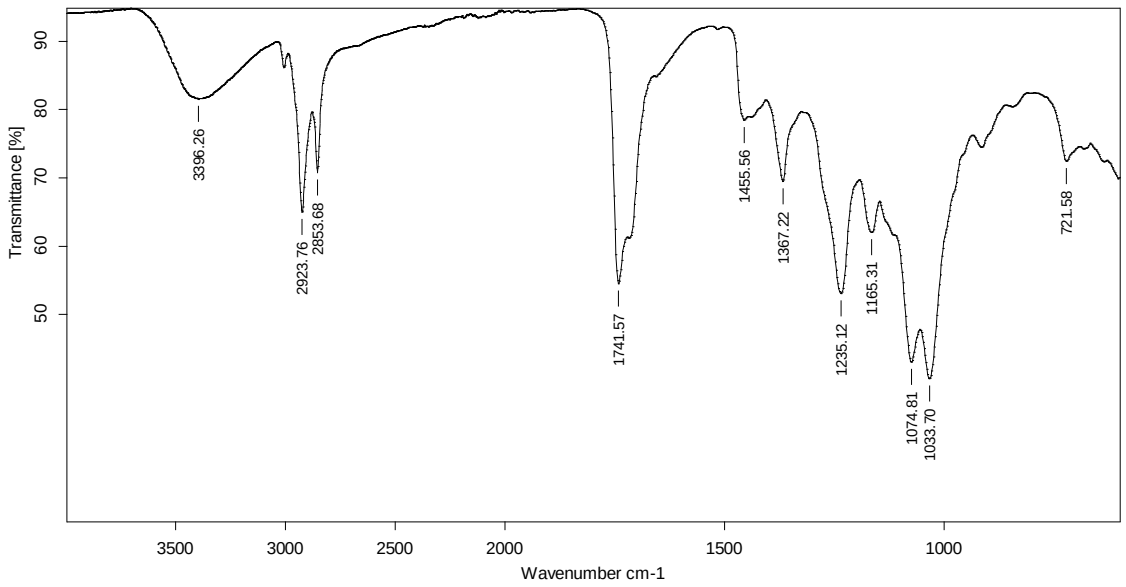
A



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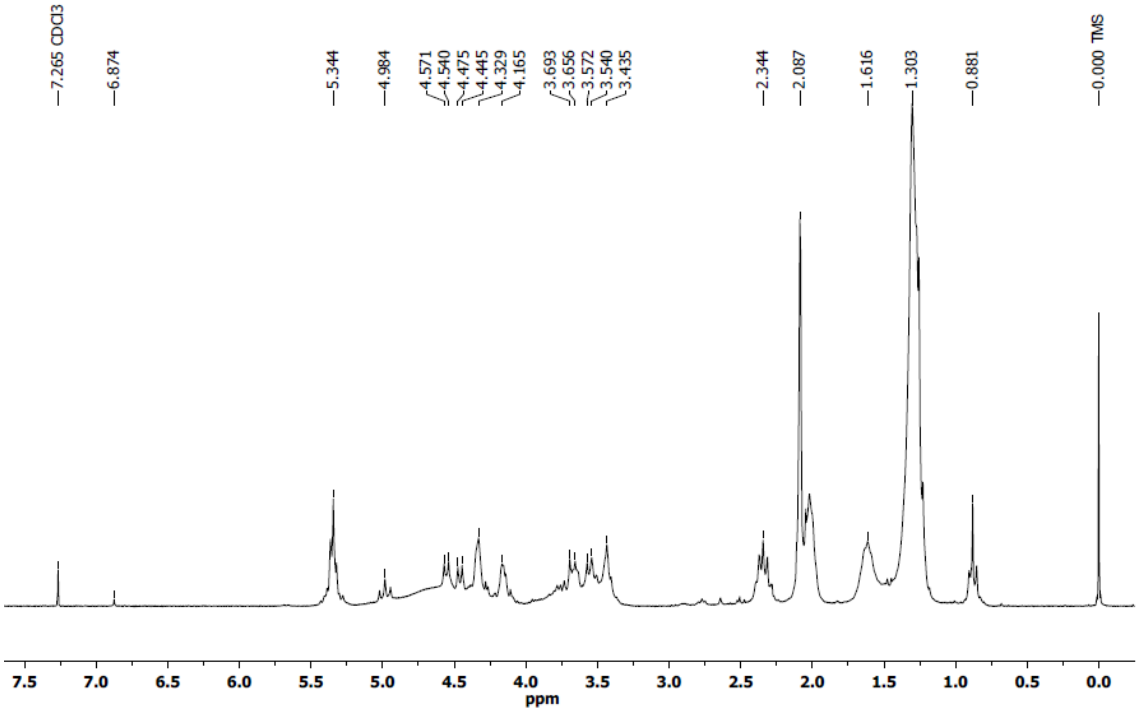

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