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Real-time dynamic analysis with low-field nuclear magnetic resonance of residual oil and sophorolipids concentrations in the fermentation process of *Starmerella bombicola*

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ABSTRACT

The yeast *Starmerella bombicola* is known to produce sophorolipids (SLs) by fermentation method. In the fermentative production of SLs, at least three phases i.e. hydrophilic phase of glucose, ions and acidic-form SLs; hydrophobic phase of oil and lactone-form SLs; solid phase of cell biomass and SLs crystals are present in the broth. Therefore, a rapid and real-time detection of residual oil and SLs can provide valuable information in the regulation and optimization of the process to better produce SLs effectively. Looking into the importance, in this study, a rapid, accurate and precise method to quantify the concentrations of oil and SLs using low-field nuclear magnetic resonance (LF-NMR) was developed. Compared to the traditional weighing method, all the parameters for the evaluation of accuracy, precision and relative recovery presented better performances. Moreover, the proposed LF-NMR method could completely avoid using organic solvents that are commonly employed in the weighing method, besides shortening the pre-treatment and detection times from 24 h to just < 20 min. Finally, LF-NMR method has been successfully used to monitor residual oil and SLs during the fermentation of *Starmerella bombicola* to obtain SLs and it has been observed that the production of SLs would be effectively improved by appropriately controlling the concentration of oil in terms of titer, productivity and yield.

1. Introduction

Sophorolipids (SLs) are very promising biologically derived surfactants of high biodegradability and low toxicity and thus have wider applications in the food, cosmetic, pharmaceutical and oil fields (Jezierska et al., 2018). Many microorganisms have been reported to synthesize SLs such as *Candida* (Konishi et al., 2018), *Wickerhaniella* (Chen et al., 2005), *Pichia* (Thaniyavarn et al., 2008) and *Rhodotorula* (Sen et al., 2017). Among them, *Starmerella bombicola* belonging to *Candida* is a most widely used specie due to its highly efficient capacity to produce SLs (Shah et al., 2017).

SLs are the mixtures of numerous structural derivatives consisting of two parts, i.e. a hydrophilic disaccharide sophorose and a hydrophobic hydroxyl fatty acid with 16–18 carbon atoms (Saerens et al., 2011). The carboxyl group of fatty acid can be esterified with 4″ hydroxyl group of sophorose to obtain lactone-form SLs, whereas the 6″ hydroxyl group of sophorose can be acetylated to obtain acetyl-form SLs (Fig. 1) (Hu and Ju, 2001a). Moreover, the hydrophobic part is differentiated based on the position of hydroxyl (ω or ω-1), the chain length of fatty acid and the degree of saturation (Van Bogaert et al., 2011b). High performance liquid chromatography-mass spectrometry (LC-MS) and high performance liquid chromatography (HPLC) methods were applied to isolate 20 different structures of SLs (Davila et al., 1994; Hu and Ju, 2001b).

In general, the physicochemical properties of SLs are dependent on their structures (Van Bogaert et al., 2013; Van Bogaert et al., 2011a; Roelants et al., 2016). Lactone-form SLs have better surface activity and antibacterial properties, while acidic-form SLs have better foaming capacity and solubility (Lang et al., 2000; Díaz De Rienzo et al., 2015; Zhang et al., 2017a, 2017b). Also, oil as a source of fatty acid is continuously fed into the broth during the SLs fermentation, which further complicates the composition of broth. At least three phases, i.e. hydrophilic phase of glucose, ions and acidic-form SLs; hydrophobic phase of oil and lactone-form SLs; solid phase of cell biomass and SLs crystals, are present in the broth. The traditional measurements of oil and SLs are based either on weighing or HPLC method which have been considered to be time-consuming and labor intensive processes for the pre-treatment and quantification of samples, in addition they require a large amount of organic solvents that are harmful to both the operator

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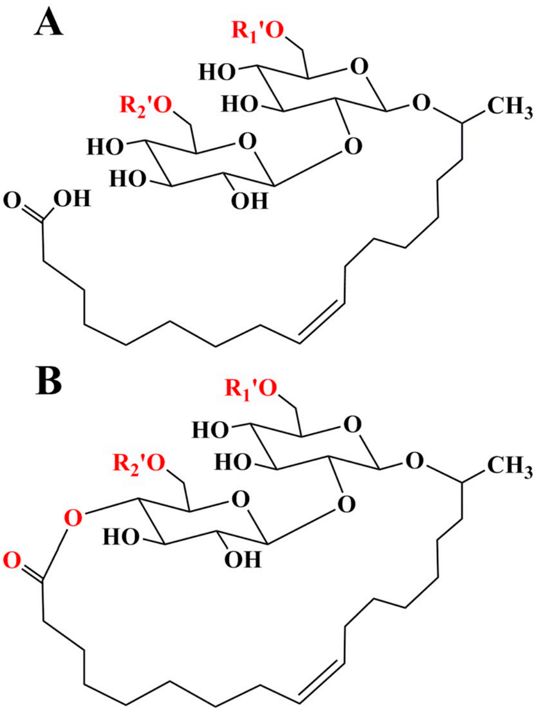

Fig. 1. Sophorolipids produced by *S. bombicola*. A: acidic-form SLs; B: lactone-form SLs. These structures can be non-(R_1, R_2 = –OH), mono-(R_1 = –OH, R_2 = –OCH$_3$), or di-acetylated (R_1, R_2 = –OCH$_3$).

and the environment. A real-time and dynamic monitoring of oil and SLs always a prerequisite in case of an efficient fermentation process for SLs production, as both oil and SLs in the broth will significantly affect the rheological properties and subsequently influence the mass transfer and mixing, which are found to be important for cell growth and metabolism. Therefore, it is of utmost importance and brings a critical challenge to quantify oil and SLs quickly and accurately.

Nuclear magnetic resonance (NMR) is a multi-functional analysis technique, not only used for the analysis of chemicals, but also widely applied in medical imaging, materials science and biochemical engineering (Chaughule et al., 2002; Mahawanich and Schmidt, 2004). Low-field nuclear magnetic resonance (LF-NMR) technique utilizes magnetic resonance phenomenon where a constant intensity of magnetic field of < 0.5 T is applied. It has the advantages of using small sample size, simple pre-treatment and operation, and the determination is quick and non-invasive (Lv et al., 2018). The quantification using LF-NMR method is based on different relaxation times of hydrogen nuclei in different phases of the sample (Santos and Colnago, 2018; Srikaeo and Rahman, 2018). Generally, carbohydrate and protein exhibit the shortest relaxation times in the order of microseconds. In contrast, the relaxation times for bound water, lipid and free water are in the orders of microsecond, millisecond and second, respectively (Sørland et al., 2004; Todt et al., 2005; Zhang et al., 2017a, 2017b). LF-NMR method has been adopted as a robust approach to detect fat in minced meat with convenient data processing (Jia et al., 2016). Otherwise, LF-NMR has been used to realize rapid, real-time and accurate dynamic analysis of cellular lipids during microalgal fermentation process using *Chlorella protothecoides* (Wang et al., 2016). The dynamic states of water in shrimp during dying process was integratedly analyzed by LF-NMR method and magnetic resonance imaging where a significant decrease in the transverse relaxation time of immobilized and free water has been found out (Cheng et al., 2018). However, all the above studies were based on utilizing relatively simple samples with single phase and seldom have been applied for the samples with complex constituents, such as the case of SLs fermentation broth.

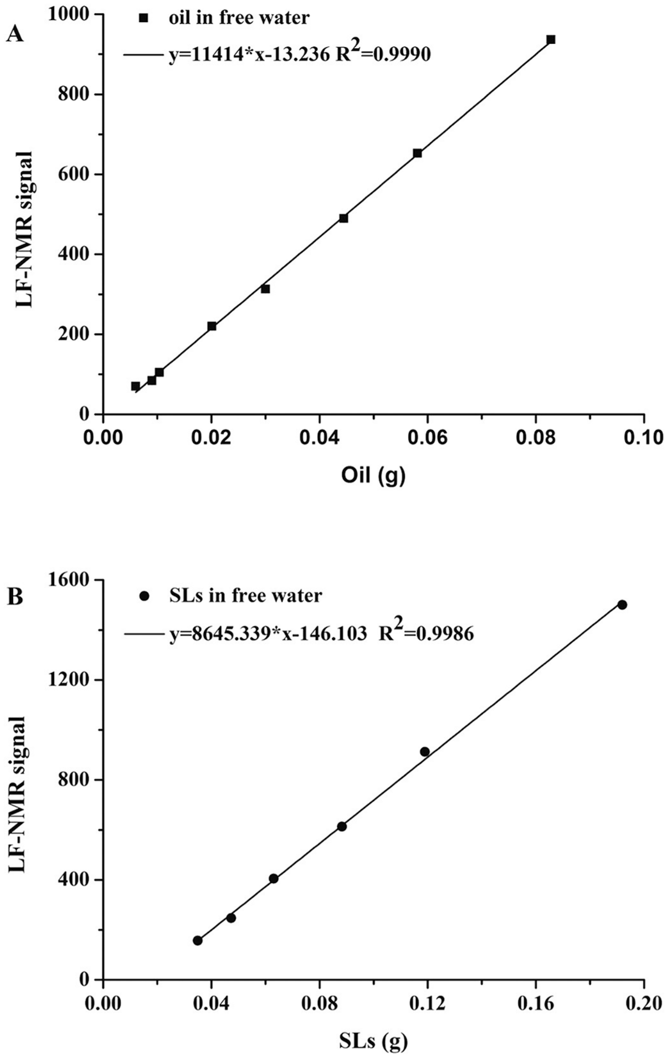

Fig. 2. The standard curves of oil (A) and SLs (B) by LF-NMR method. The standard curve was retested every month (*n* = 3). LF-NMR signal means the response value of substance under the CPMG pulse sequence.

In this context and in this study, the determination of oil and SLs by the LF-NMR method was explored through optimizing the pre-treatment and operation processes. Consequently, the developed method was successfully adopted to real-time monitoring and to regulate the residual oil level in the fermentation of *S. bombicola* for the optimized production of SLs. To the best of our knowledge, this is the first report for real-time and dynamic quantification of residual oil and SLs simultaneously in the fermentation process of *S. bombicola*.

2. Material and methods

2.1. Microorganism, media and culture conditions

S. bombicola ATCC 22214 was bought from Guangdong Culture Collection Center (China) and stored at −80 °C in 20% glycerol solution.

The seed medium contained glucose 50 g/L, KH$_2$PO$_4$ 1 g/L, (NH$_4$)$_2$SO$_4$ 4 g/L, MgSO$_4$·7H$_2$O 0.5 g/L, corn steep liquor 10 g/L. The initial fermentation medium contained glucose 100 g/L, KH$_2$PO$_4$ 1 g/L, (NH$_4$)$_2$SO$_4$ 4 g/L, MgSO$_4$·7H$_2$O 0.5 g/L, corn steep liquor 10 g/L. All the media were sterilized for 30 min under 115 °C.

The seed was cultured in a 1 L baffled shake flask with 200 mL working volume for 48 h. The shaker rotation and culture temperature were 200 rpm and 25 °C, respectively.

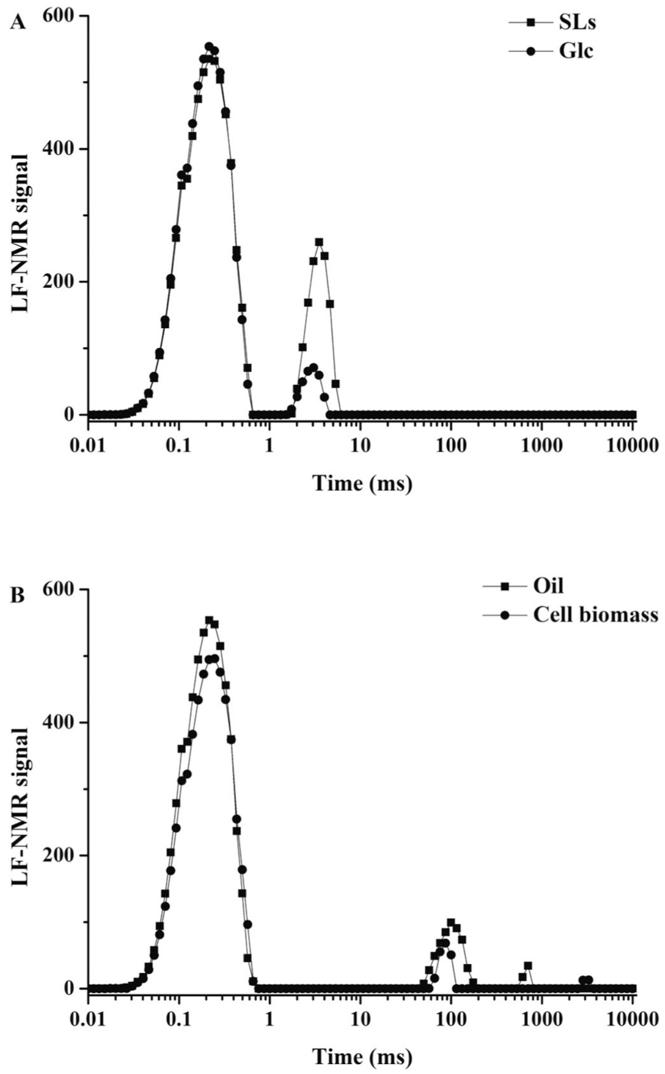

Fig. 3. T_2 distribution of different components by LF-NMR method detection. A: SLs and glucose. B: oil and cell biomass. Transverse relaxation time (T_2) is the speed of attenuation of the transverse magnetization intensity M_{xy}, which mainly depends on the proximity of the proton.

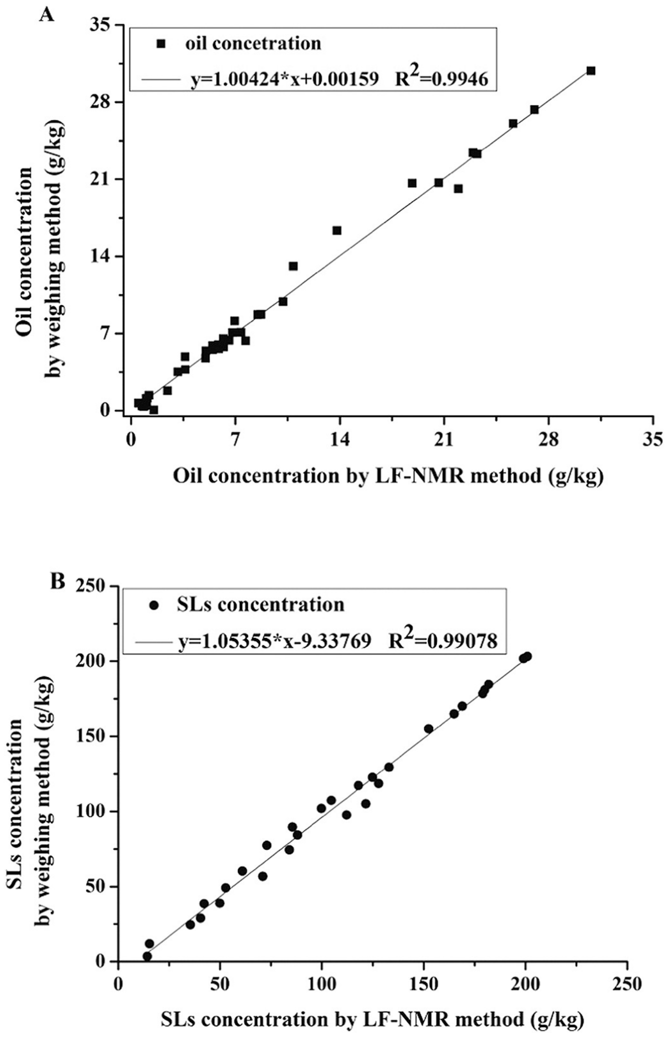

Fig. 5. Comparison of residual oil (A) and SLs (B) detection by LF-NMR method and weighing method.

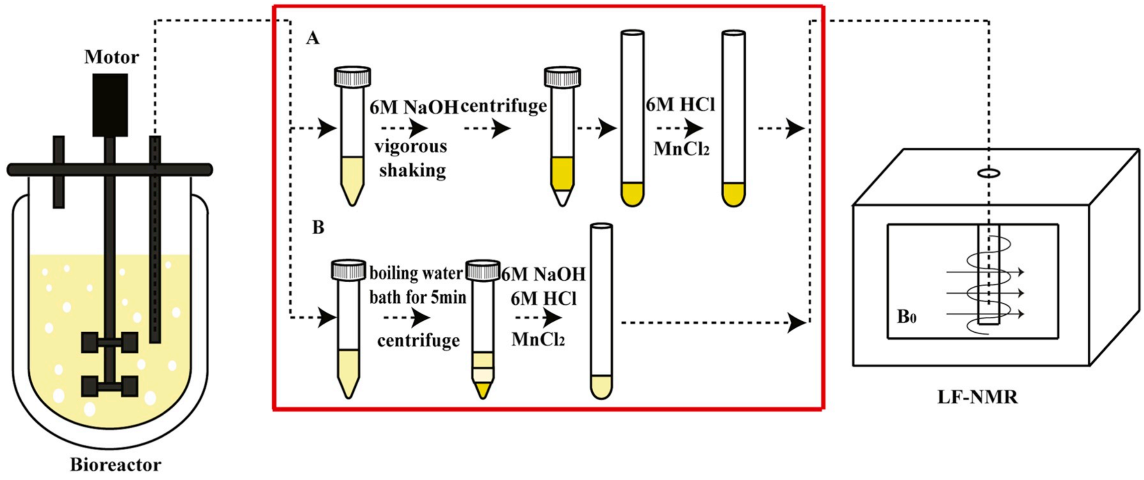

Fig. 4. Schematic diagram of sample pre-treatment process.

Table 1
Evaluation parameters of LF-NMR method and weighing method used for oil and SLs quantification.

Samples	Parameter				
	Limit of detection (LOD)[a]	Limit of quantification (LOQ)[b]	Accuracy[c]	Precision[d]	Relative recovery[e]
Oil by LF-NMR method	0.002 g	0.004 g	3%	$< 5\%$	$> 96\%$
SLs by LF-NMR method	0.009 g	0.03 g	3%	$< 5\%$	$> 96\%$
Oil by weighing method	–[f]	–[f]	6%	$< 10\%$	$< 93\%$
SLs by weighing method	–[f]	–[f]	6%	$< 10\%$	$< 93\%$

[a] The lowest oil and SLs quantity in relevant sample which can be detected by LF-NMR method with a signal of background signal (S/N) ratio > 3 (n = 18).
[b] The lowest oil and SLs quantity in sample which can be quantified by LF-NMR method (oil and SLs signal of background signal (S/N) ratio > 10).
[c] Indicated by percent difference of measurement obtained by LF-NMR method (weighing method) and HPLC.
[d] Indicated by relative standard deviation (RSD) of measurements.
[e] Indicated by percent difference of measurements obtained by LF-NMR method and weighing method with the actual values of addition in the samples.
[f] Not detected.

The SLs fermentation was carried out in a 5 L bioreactor (Shanghai Guoqiang Bioengineering Equipment Co., Ltd., China) with 2.5 L initial working volume. The operation conditions were as follows, inoculum of 2.9% (optical density of about 80), fermentation temperature of 25 °C, aeration of 0.5 vvm, initial agitation of 200 rpm. The process pH was maintained at 3.5 by adding 4 M NaOH solution. The dissolved oxygen (DO) was controlled above 40% saturation concentration during 0–36 h and above 25% saturation concentration after 36 h by adjusting the agitation step-wisely. The rapeseed oil was continuously fed into the broth and maintained the level lower than 10 g/L during the SLs fermentation. Lastly three different levels of residual oil concentration, i.e. lower concentration of oil (< 4 g/kg), medium concentration of oil (4–8 g/kg) and higher concentration of oil (> 8 g/kg), were used to optimize the SLs fermentation process. The solid glucose was added per 24 h to keep the level between 30 g/L and 80 g/L.

2.2. Analytical methods

2.2.1. Determination of oil and SLs concentrations by weighing method

Concentrations of oil and SLs in the broth were determined by weighing. Briefly, three parallel broth samples were extracted twice using the same volume of n-hexane for oil content determination. The upper layer was then transferred to another tube and dried for 24 h to constant weight by an oven. After oil extraction, the broth samples were extracted twice with ethyl acetate. The upper layer was transferred and dried as described above for SLs content determination (Youngbum et al., 2009).

2.2.2. Determination of cell biomass and glucose concentrations

Concentration of glucose was analyzed using an enzymatic bioanalyzer (SBA—40C, Shandong Academy of Sciences, China). After the extraction of SLs from the broth, the lower layer solution was centrifuged at 4000 rpm for 2 min. The precipitant was then washed twice by distilled water and dried at 80 °C for 24 h to measure dry cell weight (DCW).

2.2.3. Determination of oil and SLs concentrations by LF-NMR method

Proton NMR measurements of oil and SLs concentrations were performed on a PQ001 Benchtop Pulsed NMR analyzer (Niumag Corporation Ltd., Shanghai, China) equipped with a permanent magnet and a 25 mm diameter probe, operating at 18.166 MHz, the temperature was maintained at 32.00 °C. The Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence experiments were then conducted, with 18,000 echoes recorded for each transient, an inter-echo interval of 2000 ms, and a total of 8 added transients to increase the signal-to-noise ratio (SNR). The pulse durations used were 5.00 and 10.00 μs for the 90° and 180° pulses, respectively.

The broth samples were withdrawn every 12 h and then pre-treated as described in Section 3.2. Sample A, 3 mL broth was withdrawn at a certain fermentation time. Then, 0.4 mL of 6 M NaOH solution was added and mixed vigorously for about 20 s. After centrifugation at 4000 rpm for 5 min, the precipitation of cell biomass was discarded and 0.4 mL of 6 M HCl solution was added into the supernatant to neutralize the pH to approximately 5.0. Sample B was directly treated in boiled water for 3 min and then cooled to room temperature. After centrifuging sample B for 5 min at 4000 rpm, the water phase supernatant was separated and detected by LF-NMR method and the signal for glucose could be acquired. Before oil and SLs determination by LF-NMR method with a test tube (25 * 200 mm) of maximum 4 mL volume, 0.2 mL of 40% $MnCl_2$ solution was also added before the measurement by LF-NMR method to eliminate the influence of solvent water (Wang et al., 2016).

2.2.4. Determination of oxygen uptake rate (OUR) and carbon dioxide evolution rate (CER)

OUR and CER were determined by a process mass spectrometer (MAX300-LG, Extrel, USA). The OUR and CER during the fermentation process were determined by Eqs. (1) and (2) (Wang et al., 2016).

$$\mathrm{OUR} = \frac{F_{in}}{V}\left[C_{O_2in} - \frac{C_{inert\,in}\bullet C_{O_2out}}{1-(C_{O_2out}+C_{CO_2out})}\right]\bullet\frac{273}{273+t_{in}}\bullet P_{in}\bullet\frac{1}{1+h}\bullet 10^{-5} \tag{1}$$

$$\mathrm{CER} = \frac{F_{in}}{V}\left[\frac{C_{inert\,in}\bullet C_{CO_2out}}{1-(C_{O_2out}+C_{CO_2out})} - C_{CO_2in}\right]\bullet\frac{273}{273+t_{in}}\bullet P_{in}\bullet\frac{1}{1+h}\bullet 10^{-5} \tag{2}$$

Where, F_{in} represents intake flow rate (mmol/L), V represents initial working volume of bioreactor (L), $C_{inert\ in}$ represents the concentration of inert gas in the inlet (mmol/L), $C_{O2\ in}$ and $C_{O2\ out}$ represent O_2 concentration of inlet and exhaust gas (mmol/L), respectively, $C_{CO2\ in}$ and $C_{CO2\ out}$ represent CO_2 concentration of inlet and exhaust gas (mmol/L), respectively, t_{in} represents inlet temperature, P_{in} represents inlet pressure, h represents inlet humidity. The units of OUR and CER are mmol/L/h.

2.3. Statistical analysis

All experiments were performed in triplicate and all data were presented as the mean with standard deviation (SD). Statistical analysis was performed using One-way Analysis of Variance (ANOVA) and *t*-test ($P < 0.05$) was used to test whether there is any significant difference among treatments (SPSS 22.0, SPSS Inc., USA).

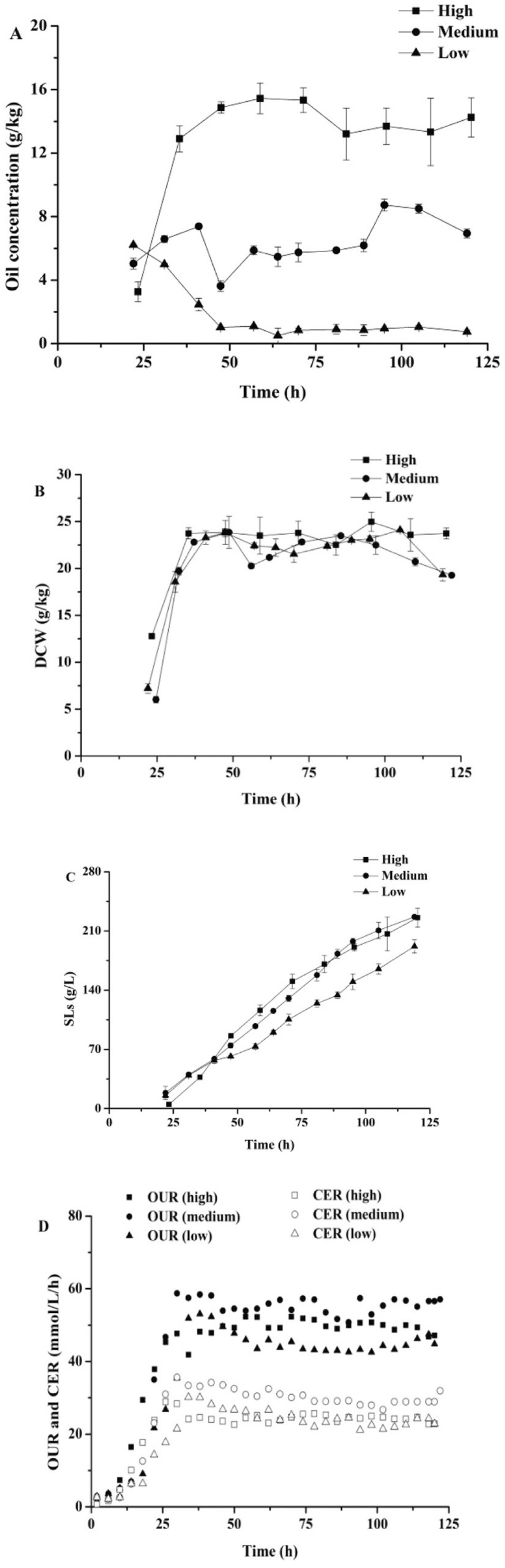

Fig. 6. Time courses of residual oil concentration (A), cell growth (B), SLs production (C) and, OUR and CER (D) with three different oil controlling strategies (n = 3).

3. Results and discussion

3.1. The detection of relaxation characteristics of broth components by LF-NMR

The components of real SLs broth are complex consisting of glucose, oil, cell biomass, SLs, ions, etc. Based on the experiments of a single factor, it was found that except glucose and cell biomass, other components did not show any influence on the determination of SLs and oil by the LF-NMR method (Fig. 3). The signals from LF-NMR presented good linearity to the concentrations of SLs and oil with R^2 higher than 0.99 (Fig. 2). However, it should be noted that the relaxation times of glucose and cell biomass were very close to SLs (1–10 ms) and oil (> 10 ms), respectively (Fig. 3). Therefore, it is essential to remove the influence of glucose and cell biomass through pre-treatment process before the detection of SLs and oil.

3.2. Optimization of pre-treatment process

SLs are mainly divided into two types: acidic-form SLs and lactone-form SLs. The acidic-form SLs exhibit better hydrophilic characteristics than the lactone-form SLs. In real fermentation broth, four layers of oil in the top, followed by water soluble and cell biomass layers as well as SLs layer in the bottom, would be formed after centrifugation. However, it should be noted that oil would be dispersed in the SLs layer to some extent. When the solution pH is higher than 11, the lactone-form SLs could be hydrolyzed spontaneously to water soluble acidic-form SLs (Fig. S1). Therefore, by centrifugation the bottom layer of cell biomass could easily be removed. Subsequently, the oil content and the total amount of glucose and SLs could be directly quantified by the LF-NMR method. In order to eliminate the influence of glucose on the detection of SLs, another sample of broth was prepared and heated to separate SLs phase from water phase as both acidic-form SLs and lactone-form SLs would be precipitated at high temperatures. Thus, the concentration of glucose in the supernatant after discarding the SLs layer could be measured by LF-NMR method and then the concentration of SLs was further determined from the difference between two samples.

As illustrated in Fig. 4, the pre-treatment process for the determination of oil content was conducted by the following steps. Two samples (sample A and B) of each of 3 mL broth were withdrawn at a certain fermentation time. Then, 0.4 mL of 6 M NaOH solution was added into sample A and mixed vigorously for about 20 s. After centrifugation for 5 min, the precipitation of cell biomass was discarded and 0.4 mL of 6 M HCl solution was added into the supernatant to neutralize the pH to approximately 5.0. Also, 0.2 mL of 40% $MnCl_2$ solution was added before subjecting to LF-NMR method to eliminate the influence of solvent water. The signal representing oil with the relaxation time > 10 ms and the total signal for glucose and SLs with the relaxation time between 1 and 10 ms could be obtained. On the other hand, at first, sample B was directly treated in boiled water for 3 min and was then cooled to room temperature (Fig. 4). It was found that the upper water phase with glucose and cell biomass and the lower SLs phase with some oil would be separated. Through centrifuging the sample B for 5 min, the layer consisting of cell biomass layer formed in the middle. Subsequently, the supernatant water phase was separated and detected by LF-NMR method and the signal of glucose could be acquired. Consequently, the concentration of SLs could be indirectly calculated from the difference between sample A and B.

The concentrations of oil and SLs were calculated based on the following Eqs. (3) and (4):

$$C_{oil}(g/kg) = \frac{(S_{oil} + 13.236)}{11414 \bullet M_s(kg)}(g) \tag{3}$$

Table 2
Comparison of fermentation performances with three different oil controlling strategies.

Oil concentration	SLs titer (g)	SLs concentration (g/L initial volume)	SLs productivity (g/h)	oil consumption (g)	Glc consumption (g)	$Y_{SLs/Oil}$ (g/g)[a]	$Y_{SLs/Glc}$ (g/g)[b]	$Y_{SLs/S}$ (g/g)[c]
Lower (< 4 g/kg)	479.50 ± 20.00	191.8 ± 8.0	4.03 ± 0.16	380.2 ± 9.2	699.8 ± 9.9	1.26 ± 0.042	0.69 ± 0.022	0.44 ± 0.011
Medium (4–8 g/kg)	566.75 ± 10.75*	226.7 ± 4.3*	4.76 ± 0.090*	446.7 ± 7.3*	698.2 ± 9.7	1.27 ± 0.0037	0.81 ± 0.021*	0.49 ± 0.010*
Higher (> 8 g/kg)	564.25 ± 23.25*	225.7 ± 9.3*	4.70 ± 0.19*	542.9 ± 6.9*	743.1 ± 10.3*	1.04 ± 0.027*	0.76 ± 0.0015	0.44 ± 0.013

*Significant differences at $P < 0.05$ with lower concentration of oil as the control.

[a] $Y_{SLs/Oil} = \frac{\text{SLs production (g)}}{\text{Oil consumption (g)}}$

[b] $Y_{SLs/Glc} = \frac{\text{SLs production (g)}}{\text{Glc consumption (g)}}$

[c] $Y_{SLs/S} = \frac{\text{SLs production (g)}}{\text{Total substrate (g)}}$

$$C_{SLs}(g/kg) = \frac{(S_{total} - S_{glucose}) + 146.103}{8645.339 \bullet M_s(kg)}(g) \tag{4}$$

where, C_{oil} and C_{SLs} represent the concentrations (g/kg) of oil and SLs, respectively. M_S represents the amount of sample (kg)and S_{oil} represents the LF-NMR signal of oil peak in sample A. S_{total} represents the sample from LF-NMR between 1 and 10 ms in sample A and $S_{glucose}$ represents LF-NMR signal of glucose peak in sample B.

The concentrations of SLs and residual oil in the broth could be accurately detected by LF-NMR method after the pre-treatment process. Moreover, through comparing the data sets of LF-NMR and weighing methods with the concentrations of oil (0.7–30 g/kg) and SLs (15–200 g/kg), the slopes of the linear relationship were 1.004 and 1.054 respectively (Fig. 5). This indicated that LF-NMR method could completely substitute the weighing method in the real-time determination of oil and SLs contents in the broth. More importantly, the time for sample pre-treatment and measurement could be greatly shortened from > 24 h by the weighing method to < 20 min by the LF-NMR method. Moreover, organic solvents, such as n-hexane and ethyl acetate, could also be completely avoided by following the LF-NMR method.

3.3. Accuracy and precision analyses in the quantification of oil and SLs by LF-NMR

The major parameters in the evaluation of LF-NMR method for the oil and SLs have been summarized in Table 1. The limits of detection (LOD) for oil and SLs were 0.002 g and 0.009 g respectively. Whereas, the limits of quantification (LOQ) for oil and SLs were 0.004 g and 0.03 g, respectively. The very low LOD and LOQ values indicate that LF-NMR method is extremely sensitive to the concentrations of oil and SLs. On the other hand, to assess the accuracy and precision of LF-NMR method, 6 samples each for the measurements of oil and SLs were carried out using LF-NMR method and the results were compared to those obtained by the weighing method (Table 1). The accuracy of LF-NMR and weighing methods were 3% and 6% respectively. The precision and relative recovery of LF-NMR method were over 95% and 96% respectively, both of which were better than the values of 90% and 93% obtained through weighing method. Therefore, it is reasonable to apply LF-NMR method to replace the traditional weighing method for the determination of oil and SLs in the fermentation process of *S. bombicola* for SLs.

3.4. Dynamic monitoring of the contents of oil and SLs in real-time fermentation process

It is of significance to monitor the concentrations of substrate and product in real-time during the fermentation process, whereby some process control strategies could be implemented to achieve high titer, productivity and yield of the desired product. Dynamic monitoring of oil and SLs is based on LF-NMR method which has been successfully realized during the fermentation of *S. bombicola* to obtain SLs.

Oil as an important substrate significantly affects the synthesis of SLs. As shown in Fig. 6, although the cell growth presented almost no dependence on the level of oil in the broth, the physiological states of cellular processes could be reflected by the significantly different physiological parameters of OUR and CER. Compared to lower concentration of oil (< 4 g/kg), higher OUR and CER levels could be kept when the concentration of oil was maintained at a relatively higher values (4–8 g/kg and > 8 g/kg) (Fig. 6A and D), and consequently the final titer value of SLs also demonstrated a significant difference (Fig. 6C). Through relating the fermentation performances with three controlling strategies of oil, it could be found that compared to the lower amount of oil, medium and higher amounts of oil could improve the production of SLs by 18.2% and 17.7% respectively. Although the oil consumption under medium oil conditions was higher than low oil conditions, it led to obtaining similar yields on the production of SLs to oil consumption ($Y_{SLs/Oil}$), and it could be noted that the consumption of glucose under these two conditions are comparable. Thus, both the yields of the production of SLs to the consumption of glucose ($Y_{SLs/Glc}$) and to the total consumption of substrate ($Y_{SLs/S}$) were enhanced by 17.4% and 11.4% respectively. In terms of higher oil conditions, though the titer value of SLs at 120 h was higher than low oil conditions, the consumption of both oil and glucose were larger, which consequently resulted in similar $Y_{SLs/S}$ and even a slightly lower $Y_{SLs/Oil}$ (Table 2). On the basis of these results, it could be concluded that an appropriate concentration of oil in the broth could effectively improve the production of SLs by *S. bombicola*. Furthermore, it is believed that the real-time and dynamic quantification of oil and SLs by the LF-NMR method could ensure regulating the process precisely.

4. Conclusions

In this study, the LF-NMR method was used to quantitatively determine the concentrations of SLs and residual oil in the fermentation broth of *S. bombicola*. In comparison to the traditional weighing method, the LF-NMR method not only possesses better accuracy and precision, but also completely avoids the use of organic solvents during the pre-treatment and detection processes. More importantly, the LF-NMR method could reduce the detection time from > 24 h by the weighing method to approximately just 20 min, which realizes real-time and dynamic monitoring and controlling. Consequently, three strategies in controlling the concentration of oil by the real-time detection with LF-NMR method were followed. The results demonstrated that as compared to the condition of low oil concentration, medium oil concentration of 4–8 g/kg in the broth could significantly improve the production of SLs and the yield to substrate consumption. To the best of our knowledge, this is the first time LF-NMR technology has been adopted to simultaneously determine oil and SLs in the broth having complex physiochemical characteristics. It is believed that the proposed detection method as established in this work could not only be applied for further development of regulating the process strategy, but may be extended to other fermentation processes involving bio-surfactants, such as rhamnolipids.

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Competing interests

The authors declare that they have no competing interests.

Ethical statement

This article does not contain any studies with human participants or animals performed by any of the authors.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mimet.2018.12.007.

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