Article

Application of Extractive Fermentation on the Recuperation of Exopolysaccharide from *Rhodotorula mucilaginosa* UANL-001L

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Abstract: Exopolysaccharides (EPS) are high molecular weight biomaterials of industrial interest due to their variety of applications in the pharmaceutical, cosmetic, environmental, and food industries. EPS produced by *Rhodotorula mucilaginosa* UANL-001 L has sparked interest due to its bio-adsorbent and wide spectrum antimicrobial properties. However, full exploitation and commercial application of EPS has been restrained due to low yields and high production costs. In the present work, the production and separation of EPS from *Rhodotorula mucilaginosa* UANL-001L was attempted through extractive fermentation in order to increase EPS production while simplifying the recovery process. Extractive fermentation was implemented with a thermoseparating polymer for phase formation (EOPO 970 and EOPO 12,000); culture viability, biomass generation, EPS production, rheological system properties, and phase formation time and temperature were monitored throughout the process. Extractive fermentation of *Rhodotorula mucilaginosa* UANL-001L with EOPO 970 resulted in a 42% EPS and 7% biomass recovery on the top phase after 5 to 13-min phase formation time and temperatures between 30 and 40 °C. This is the first report of extractive fermentation application for EPS production by yeast of the genera *Rhodotorula*, resulting in an interesting strategy for EPS production and recovery, although further optimization is needed.

Keywords: extractive fermentation; exopolsaccharide; *Rhodotorula mucilaginosa* UANL-001L

1. Introduction

Exopolysaccharides (EPS) is a high molecular weight biomaterial excreted by various microorganisms [1], especially by the yeast of the following genus: *Aureobasidium*, *Bullera*, *Candida*, *Cryptococcus*, *Pichia*, *Phomopsis*, *Exophiala*, *Lipomyces*, *Rhodotorula*, *Sporobolomyces*, *Tremella*, and *Trichosporon* [2]. Various EPS are of industrial interest due to its variety of applications in pharmaceutical, cosmetic, environmental, and food industries [3], where some EPS, including xanthan, dextran, and hyaluronate,

are commercially produced [2]. EPS produced by *Rhodotorula mucilaginosa* UANL-001L has sparked interest due to its bioadsorbent [4] and wide spectrum antimicrobial [5] properties.

However, full exploitation and commercial application of EPS has been restrained due to low yields and high production costs. Several studies on cheaper fermentation substrates, fermentation process optimization, and even genetic engineering have surfaced in order to obtain higher titers [6]. Frequently, the recovery and purification of EPS starts with cell removal by centrifugation or filtration, followed by precipitation with solvents (ethanol, acetone, or isopropanol) and contaminants are removed by enzymatic or physical deproteinization [7]. Nevertheless, recuperation and purification of EPS has become an economical and technical challenge due to the application of unit operations such as centrifugation, sedimentation, and solvent extraction, where recoveries are reported to be not optimal [8].

Consequently, the implementation of effective fermentation and recuperation processes are highly needed. An alternative process is the implementation of extractive fermentation, where fermentation and separation of extracellular products takes place on the same environment at the same time [9]. Extractive fermentation consists in a fermentation process that takes place in an aqueous two-phase system, where the microorganism is able to reproduce on the bottom phase while the extracellular components can be extracted to the top phase. Moreover, extractive fermentation has the potential to increase production and recuperation of metabolites, increasing the overall productivity of the bioprocess [9,10]. Furthermore, extractive fermentation can be implemented with thermoseparating agents that allow phase formation depending on the system's temperature, being ethylene oxide and propylene oxide (EOPO) random copolymers the thermoseparating agents most widely used [11]. Then, the extracellular product can be retrieved from the system without the need of separation of solid–liquid unit operations, and without interrupting the fermentation process [9].

In the present work, the production and separation of EPS from *Rhodotorula mucilaginosa* UANL-001L was attempted through extractive fermentation in order to increase EPS production while simplifying the recovery process. In order to achieve this goal, two main strategies were implemented. First, media composition (Yeast Malt Broth (YM) medium vs mineral medium) was studied to determine if EPS production on submerged fermentation was affected. Then, extractive fermentation was implemented with a thermoseparating polymer for phase formation (EOPO 970 and EOPO 12,000), monitoring culture viability, biomass generation, and EPS production. Finally, three factors such as phase formation time, temperature and EOPO concentration were studied for the thermoseparating polymer that yielded the highest biomass and EPS production, in terms of EPS and biomass recovery on each phase.

2. Materials and Methods

2.1. Media Composition Effect on EPS Production

2.1.1. Cultivation Procedures in Submerged Fermentation

Rhodotorula mucilaginosa UANL-001 L was provided on solid medium by Laboratorio de Biología Sintética y de Sistemas at CIBYN, México. In order to work with liquid cultures, a colony from the plate was taken with an inoculation loop, inoculated on 50 mL Yeast Malt Broth (YM) medium (Sigma Aldrich, Toluca, México) on sterile conditions, and kept in incubation for 3 days at 30 °C and 250 rpm (standard conditions). Later, 10 mL of culture were transferred to fresh YM medium (100 mL) to obtain the starting culture. YM medium composition is shown in Table 1.

Table 1. Yeast Malt Broth (YM) medium composition.

Component	Concentration (g/L)
Peptic digest of animal tissue	5.0
Yeast extract	3.0
Malt extract	3.0
Dextrose	10.0

Mineral medium was supplemented with saccharose and prepared according the formulation on Table 2. Cultures with 100 mL were used for media composition studies. 20 mL of starting culture were inoculated in both YM and mineral medium and kept for 7 days on standard conditions. Every 24 h, 5 mL samples were taken for biomass and EPS quantification.

Table 2. Mineral medium composition.

Component	Concentration (g/L)
Ammonium chloride (NH_4Cl)	2.0
Potassium phosphate (KH_2PO_4)	1.0
Magnesium sulphate ($MgSO_4$)	0.5
Sodium chloride (NaCl)	0.1
Calcium chloride (CaCl)	0.1
Zinc sulphate ($ZnSO_4$)	0.005
Saccharose ($C_{12}H_{22}O_{11}$)	100.0

2.1.2. Cultivation Procedures in Presence of EOPO

For extractive fermentation, EOPO 970 and EOPO 12,000 (Sigma Aldrich, Toluca, México) were used. In order to initiate extractive fermentation, 10 mL of an starting culture were transferred to 100 mL of YM or mineral medium with 20 or 30% (*v*/*v*) EOPO 12,000 or 10, 20, 30, 40, or 50% (*v*/*v*) EOPO 970. As a control, 10 mL of an starting culture on 100 mL YM or mineral medium without EOPO was used.

2.1.3. Biomass and EPS Recuperation in Submerged Fermentation

A 5 mL sample from the submerged fermentation was retrieved and centrifuged at 9500 rpm for 20 min at 4 °C. The supernatant was reserved at 50 mL in falcon tubes and the pellet was reserved at −20 °C for further biomass quantification. The supernatant was filtered through a 0.2 µm cellulose membrane. Two volumes (5 mL approximately per volume) of 96% ethanol were added and incubated for 12 h at −20 °C to precipitate EPS. After incubation, the mixture was centrifuged at 9500 rpm for 20 min at 4 °C, discarding the supernatant and washing the pellet twice with 70% ethanol. Between washes, the EPS was centrifuged at 9500 rpm for 20 min at 4 °C, discarding the supernatant. Finally, the resulting pellet was dried by ethanol evaporation at room temperature for 15 min.

Once both biomass and EPS pellets were obtained, the water content was evaporated to determine biomass and EPS dry weight. The drying process was performed in an Integrated Speed Vac (ThermoFisher, Waltham, MA, USA) at 45 °C, 5 Torr pressure for 6 h. After this process, dry weight was determined by difference of the dry weight and the original sample weight.

2.2. *EOPO Effect on Cell Viability and Biomass Generation*

2.2.1. Cell Viability

In order to verify if *Rhodotorula mucilaginosa* UANL-001L viability was maintained when EOPO was added to the medium, a cell viability assay was performed. Cells were stained with a methylene blue solution as stated in Table 3. Briefly, 1 mL culture sample was mixed with 1 mL of methylene blue solution. The mixture was incubated for 15 min at 25 °C and a 300 µL sample was then observed in a Neubauer chamber for cell count. Both stained and not stained cells were counted in order to determine cell viability with Equations (1) and (2).

$$[C] = \frac{CC}{Cq} \times TQ \times FD \times 10,000 \quad (1)$$

$$V = \frac{([C] - CA)}{[C]} \times 100 \quad (2)$$

where:

CCell concentration
CC: Counted cells
Cq: Counted quadrants
CA: Counted blue cells
TQ: Total quadrants
FD: Dilution Factor
V: Cell viability

Table 3. Methylene blue solution composition.

Component	Concentration (g/L)
Methylene blue	0.025
Sodium chloride (NaCl)	0.90
Calcium chloride ($CaCl_2$)	0.04
Potassium chloride ($CaCl_2$)	0.04

2.2.2. Biomass Generation

100 mL cultures where prepared as stated in Section 2.1.2. Every 24 h, a 10 mL sample was retrieved for 7 days. Biomass was determined as previously stated in Section 2.1.3

2.3. Extractive Fermentation Parameter Study: Rheological Characterization and Phase Formation Time and Temperature

2.3.1. Rheological Characterization of Two-Phase Systems

Extractive fermentation is an aqueous two-phase system (ATPS) that requires the interaction and characterization of each phase; however, the properties of the aqueous two-phase systems used in this study are not reported in the literature. Therefore, the experimental measurement of dynamic viscosity, superficial tension, and interfacial tension were performed by varying the EOPO concentration for each media. Dynamic viscosity measurement was carried out with an Ostwald viscometer CANNON 200 AA58 (Cannon Instrument Company, State College, PA, USA) at 25 °C using water as a reference liquid. Surface tension and interfacial tension were both measured with a DuNouy tensiometer (CSC Scientific, Fairfax, VA, USA). For surface tension, the measured system consisted of the EOPO + media with air at 25 °C; while for the interfacial tension measurement, the system was heated at the formation phase temperature to form both phases (top: aqueous phase and bottom: EOPO rich phase). Three measurements were performed resulting in a standard error below 5%.

2.3.2. Two-Phase Generation in the Prescence of EOPO 970

For different concentrations of EOPO 970, time and temperature were monitored in order to determine the conditions to generate two-phase systems. First, 50 mL starting cultures were prepared and maintained in standard conditions for 5 days. Later, 5 different concentrations of EOPO 970 (10, 20, 30, 40, or 50% (*v*/*v*)) were added to cultures. Then, each culture was put in a water bath for culture temperature modification (30, 35, 40, and 45 °C), by heating the water on the bath as needed. Once the desired temperature was reached, the phase generation time was recorded at the time the two phases were visibly distinctive. Culture temperature was monitored by an infrared thermometer.

2.4. Biomass and EPS Production and Recovery on Extractive Fermentation with EOPO 970

After EOPO 970 concentration determination for phase formation, viability retention and phase formation temperature, the biomass and EPS produced were quantified for the top and bottom phase.

First, 100 mL cultures were prepared with 20, 30, 40, or 50% (*v*/*v*) EOPO 970. After phase formation, the top phase was removed with a pipette and treated as described in Section 2.1.3 for biomass and EPS quantification. Likewise, the bottom phase of each culture was treated as described in Section 2.1.3 in order to separate and quantify the biomass and EPS retained on the phase.

2.5. Statistical Analysis

Statistical analysis was performed using Microsoft Excel (Redmon, WA, USA). One-way Analysis of Variance (ANOVA) was used to determine significant differences (p-value < 0.05) between treatments. All experiments were performed in biological duplicates and the mean is reported with the corresponding standard deviations.

3. Results

3.1. Media Composition Effect on EPS Production

YM and mineral Medium were compared in terms of biomass and EPS production on submerged fermentation. As shown in Figure 1, biomass (a) and EPS (b) production is similar in both YM and mineral media. After 240 h of cultivation, biomass concentration was 0.0360 g/L in YM medium while a 0.0298 g/L biomass concentration was obtained from mineral medium. Moreover, it is important to note that EPS production is very low at the first 72 h of cultivation, resulting in a higher production after 120 h of culture in both media, being the highest EPS concentration obtained from YM medium (0.0052 g/L). Both biomass and EPS maximum concentration from both media resulted not significantly different and proved to be fairly constant after 168 h of culture.

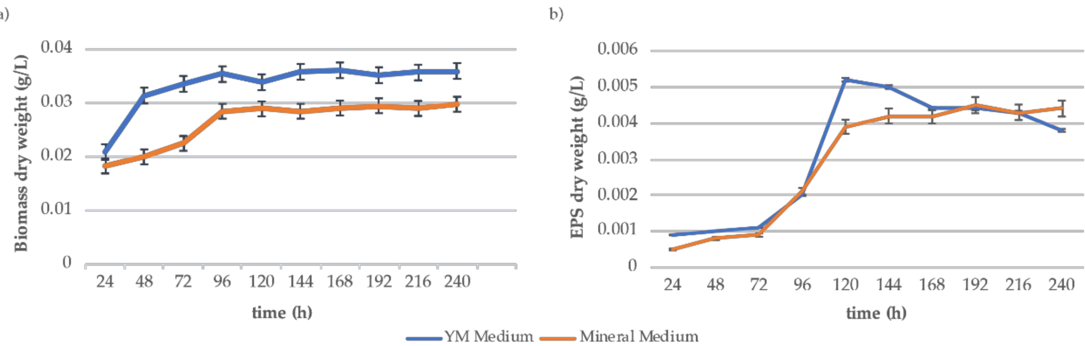

Figure 1. Biomass (**a**) and EPS (**b**) production in submerged fermentation of *Rhodotorula mucilaginosa* UANL-001L cultures in YM and mineral media. Cultures were maintained in the corresponding liquid medium on sterile conditions and kept in incubation for 3 days at 30 °C and 250 rpm.

3.2. EOPO Effect on Viability and Biomass Generation

Culture viability in presence of EOPO was evaluated by cell count in a Neubauer chamber and Methylene Blue coloration. Concentrations from 10% to 50% (*v*/*v*) were tested for EOPO 970, while 20% and 30% (*v*/*v*) were tested for EOPO 12,000. As shown in Figure 2, cultures in presence of EOPO 12,000 (a) or EOPO 970 (b) showed an important decrease in viability after 96 h. More importantly, culture viability decreased by 35% after 168 h in comparison to YM and mineral media controls. Higher EOPO concentrations in both experiments signified higher drops in culture viability, where concentrations higher than 30% EOPO signified less than 50% cell viability after 96 h of culture.

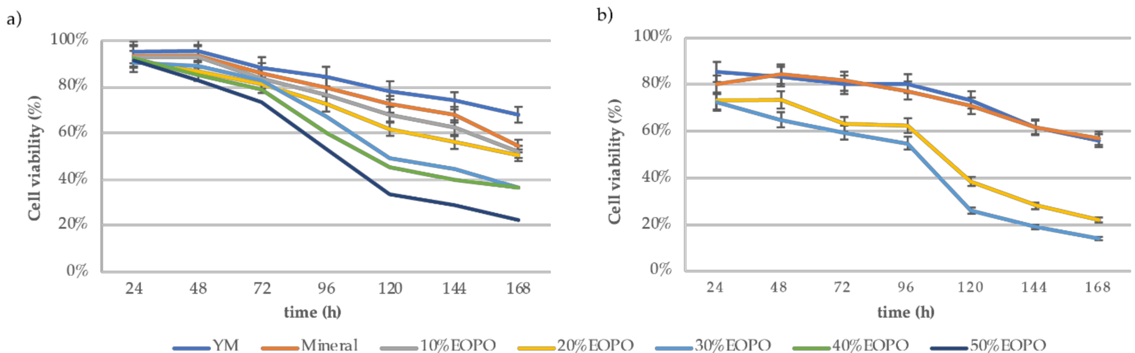

Figure 2. Cell viability in submerged fermentation of *Rhodotorula mucilaginosa* UANL-001L cultures in presence of (**a**) EOPO 970 or (**b**) EOPO 12,000. Cultures were maintained in liquid medium on sterile conditions and kept in incubation for 3 days at 30 °C and 250 rpm.

In terms of biomass generation, Figure 3a shows that concentrations of up to 40% EOPO 970 retrieved similar biomass concentrations as the control after 96 h of culture. However, biomass concentration in cultures with EOPO 12,000 was significantly lower (about 30%) than the control, even at early culture stages. Therefore, further studies for extractive fermentation were carried out only with EOPO 970.

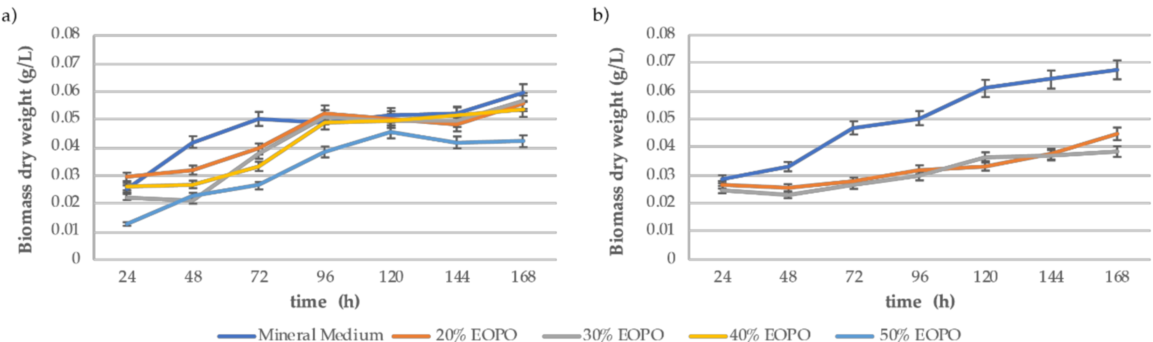

Figure 3. Biomass production in submerged fermentation of *Rhodotorula mucilaginosa* UANL-001L cultures in presence of (**a**) EOPO 970 or (**b**) EOPO 12,000. Cultures were maintained in liquid medium on sterile conditions and kept in incubation for 3 days at 30 °C and 250 rpm.

3.3. Extractive Fermentation Parameter Study: Rheological Characterization and Phase Formation Time and Temperature

3.3.1. Rheological Characterization of Two-Phase Systems

Experimental measurements of dynamic viscosity, superficial tension, and interfacial tension of the systems, with and without *Rhodotorula mucilaginosa* UANL-001L, at various EOPO concentrations are presented in Table 4**.** These results are highly valuable since no data for this system is available in the literature, and extractive fermentation depends on the analysis of the two-phase system.

Viscosity linearly increases with increasing EOPO concentrations in both media, with and without the presence of *Rhodotorula mucilaginosa* UANL-001L. More importantly, media viscosity increases about 133% when *Rhodotorula mucilaginosa* UANL-001L is growing on mineral medium, while an increase of 22% is observed when YM medium is used.

On the other hand, superficial tension decreases almost linearly with increasing concentrations of EOPO. In systems with mineral medium and no microorganisms, superficial tension only decreases from 1.47 to 3.44%, while it decreases from 10% to 14.3% in systems with YM medium and no microorganisms. However, superficial tension is higher in systems comprised of mineral medium than in systems with YM medium. When *Rhodotorula mucilaginosa* UANL-001L is present on the system, superficial tension changes significantly only with YM medium.

Contrary to the superficial tension behavior, the interfacial tension increases with an increase in EOPO concentration. The systems with YM medium present lower interfacial tension than the systems with mineral medium. However, interfacial tension measurements showed that, liquid–liquid phase formation (immiscible system) do not take place with EOPO concentration below 20%, therefore, interfacial tension measurement was not possible. Consequently, extractive fermentation could not take place at concentrations below 20% EOPO concentration since immiscible phases are not formed.

Table 4. Two-phase system property measurements.

Medium	**EOPO 970**	**Microorganism**	**Viscosity**	**Superficial Tension (σ)**	**Interfacial Tension (γ)**
	[% *v*/*v*]	**(Yes/No)**	**[Pa·s]**	**[N/m]**	**[N/m]**
Mineral	10	No	0.00227	0.0680	ND
Mineral	20	No	0.00285	0.0680	0.0337
Mineral	30	No	0.00329	0.0680	0.0367
Mineral	40	No	0.00378	0.0670	0.0380
Mineral	50	No	0.00422	0.0670	0.0387
Mineral	0	Yes	0.00135	0.0677	ND
Mineral	10	Yes	0.00237	0.0680	ND
Mineral	20	Yes	0.00422	0.0670	0.0310
Mineral	30	Yes	0.00605	0.0660	0.0330
Mineral	40	Yes	0.00771	0.0660	0.0350
Mineral	50	Yes	0.00987	0.0653	0.0367
YM	10	No	0.00291	0.0467	ND
YM	20	No	0.00385	0.0443	ND
YM	30	No	0.00490	0.0430	0.0300
YM	40	No	0.00696	0.0420	0.0317
YM	50	No	0.00835	0.0420	0.0350
YM	0	Yes	0.00168	0.0537	ND
YM	10	Yes	0.00426	0.0500	ND
YM	20	Yes	0.00510	0.0500	0.0280
YM	30	Yes	0.00850	0.0490	0.0287
YM	40	Yes	0.00841	0.0470	0.0300
YM	50	Yes	0.01019	0.0460	0.0330
YM	0	No	0.00154	0.0403	-
EOPO	100	No	0.11363	0.0547	-

ND: non-detected, or phase separation did not take place.

3.3.2. Two-Phase Generation in the Prescence of EOPO 970

Phase formation time and temperature was studied by heating the cultures with different EOPO 970 concentration (20, 30, 40, and 50% *v*/*v*) in a water bath until reaching 30, 35, 40, and 45 °C culture temperature, and recording the time needed at each temperature to visualize phase formation. In Figure 4, the results of such analysis are depicted, where it is clear that, at higher temperatures, lower times are needed for phase formation regardless EOPO concentration in the culture. However, it is clear that higher EOPO concentrations require less time and temperature for phase formation, being at 40% and 50% EOPO the most attractive alternatives, where 4 to 6 min are needed in order to observe phase formation at 30 to 45 °C. More importantly, after 6 min of phase formation time, higher temperatures do not provide conditions for significantly lower formation times in cultures with 40% and 50% EOPO concentration.

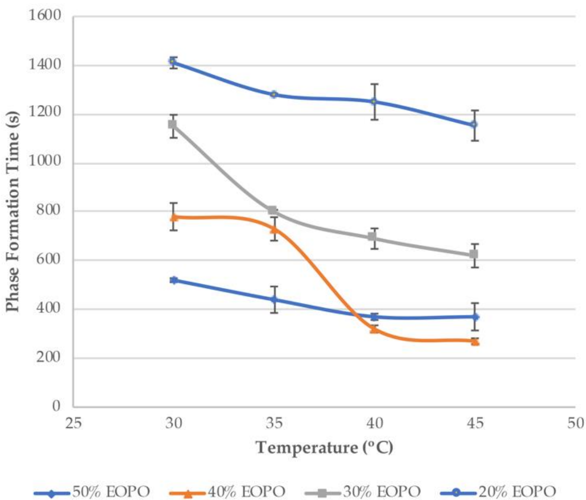

Figure 4. Phase formation time and temperature needed for different EOPO 970 concentrations (20, 30, 40 and 50 % *v*/*v*) on *Rhodotorula mucilaginosa* UANL-001L cultures in mineral medium.

3.4. Biomass and EPS Production and Recovery on Extractive Fermentation with EOPO 970

After phase formation with 20, 30, 40, and 50% *v*/*v* EOPO 970 concentration, each phase (bottom and top phase) was separated for biomass and EPS quantification. Figure 5 shows both biomass and EPS recovery % for each separated phase. Cultures with 30, 40, and 50% EOPO 970 concentration showed a biomass separation predominantly to the bottom phase with recoveries from 88% to 95%, while EPS also partitioned predominantly to the bottom phase with recoveries from 37 to 45%. It is important to note that cultures with 40% and 50% EOPO 970 provided the best conditions for biomass and EPS separation on different phases. For systems with 40% EOPO concentration, 7% of biomass and 42% of EPS is recovered on the top phase and 93% of biomass and 58% of EPS can be recovered in the bottom phase. For 50% EOPO 970, a similar behavior was observed, since 5% of biomass and 37% of EPS is recovered on the top phase; while 95% biomass and 63% EPS can be recovered in the bottom phase. Although said systems do not provide conditions for full EPS and biomass partition to different phases, said EOPO 970 concentrations have potential for biomass and EPS separation on continuous flow extraction, since EPS could be recovered on the extracting phase and further separated with other methods.

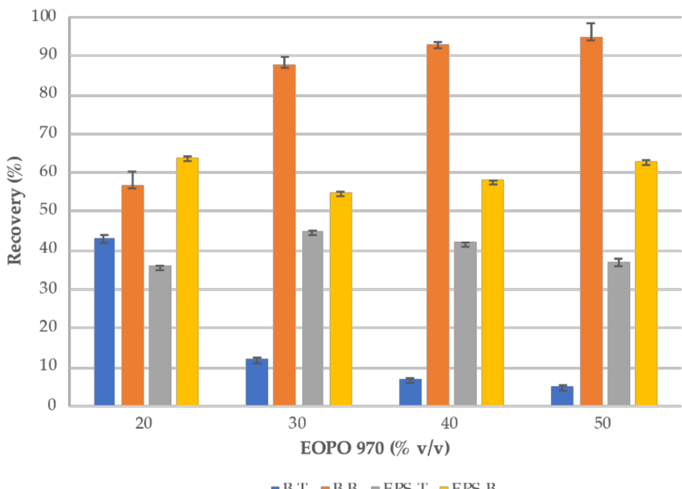

Figure 5. *Rhodotorula mucilaginosa* UANL-001L biomass (B) and exopolysaccharide (EPS) recovery in top (T) and bottom phase (B) of extractive fermentation with EOPO 970 at 35ºC.

4. Discussion

Biomass and EPS production are highly affected by media composition and other culture conditions such as temperature, pH, and agitation [12]. In the present study, both biomass and EPS generation was not significantly affected by media composition (glucose in YM medium vs saccharose in mineral medium), but biomass production resulted lower than the reported in different studies [13–16], where production reached up to 10.2 g/L biomass concentration. However, it has also been reported that nitrogen content and source are important factors for biomass formation [17–19], and that yeast extract and ammonium nitrate are generally the best sources for yeast cultivation. For mineral medium, nitrogen was provided by ammonium chloride, so its substitution should be studied in order to determine if biomass production could be increased.

EPS production has been previously reported in other *Rhodotorula* strains, such as *Rhodotorula mucilaginosa* YR-2, where EPS production was observed after 120 h culture [20], which coincides with our results. However, EPS production has also been reported to be higher, since titters up to 7.8 g/L have been reported [20]. Such difference on EPS production could be attributed to the low *Rhodotorula mucilaginosa* UANL-001L biomass concentrations obtained, as well as differences in the *Rhodotorula mucilaginosa* strain. More importantly, it has been reported that even on the same yeast strains, EPS production can result in different production rates or EPS composition, due to culture conditions and carbon source [21,22], so further investigation on culture conditions and carbon source is needed in order to obtain higher concentrations of EPS.

Since the aim of the present investigation was to apply extractive fermentation on the recuperation of EPS, it was of importance to first determine if EOPO added to the culture affected cell viability. EOPO is a polymer, therefore it adds viscosity to the medium, affecting the rheological attributes of the culture. Therefore, cultures with EOPO require higher mixing times [23], present lower oxygen diffusion due to coalescence [24] and a diminution of pH control [25], and microorganism metabolism rates [26]. According to Table 4, viscosity increases with increasing EOPO concentrations in both media, and that effect intensifies in mineral medium. Therefore, cell viability can be affected negatively due to the high culture viscosity when EOPO is used for extractive fermentation. In this scenario, *Rhodotorula mucilaginosa* UANL-001L biomass production was lower than the production previously reported for *Burkholderia cepaci* [27] in extractive fermentation with thermoseparating polymers, where polymer concentration reduced the microorganism growth rate. In other studies, polymers such as polyethylene glycol were proven to be toxic to *E. coli* depending on the polymer molecular weight and concentration, where lower molecular weights (200–600 MW) and concentrations (30% *v*/*v*) resulted in lower cell viability losses (up to 20% loss) than higher molecular weights and concentrations (up to 70% loss) [28].

Parameters such as viscosity, superficial tension, interfacial tension, phase formation time, and temperature for extractive fermentation are not frequently reported simultaneously. The most reported parameter in the literature is polymer concentration and temperature needed for phase formation in a 20 to 30 min time range [27,29,30]. More importantly, the temperature needed for phase formation is generally high (30–50 °C) in comparison to the optimal growth temperature for the microorganism, which can ultimately affect growth rates. For example, extractive fermentation implemented on *Burkholderia cepaci*, which grows in temperatures around 30 °C, require a 50 °C temperature for 15 min in order to observe phase formation [27]. Said conditions can be of importance for not only for cell viability, but also for EPS production and recovery. In our study, lower temperatures were needed for EOPO to induce phase formation, which resulted in a range of 4 to 12 min needed for phase formation. Said conditions results in low differences between optimal growth temperature and phase formation temperature, which could be beneficial for *Rhodotorula mucilaginosa* UANL-001L in extractive fermentation. This is of importance, since phase formation temperatures should be compatible to ideal temperatures for microorganism growth, in order to not affect growth rates, production rates, oxygen diffusion and even production costs [26]. Moreover, phase formation temperatures should be short in order to maintain cell viability and cell culture stability [31].

Extractive fermentation has proven to be an adequate technique for the recovery and purification of different metabolites, such as enzymes [27,32], obtaining enzyme purity up to 90% applying temperatures high as 50 °C for 20 min, and 87% purity with 65 °C for 15 min, respectively. However, since the products are of protein nature, it is hard to compare such operation conditions and purities obtained in said studies with our work. EPS is a complex high molecular weight product, therefore, its partition behavior, and conditions needed for its recovery and purification can be expected to be different than other products reported.

To our knowledge, biomass and EPS production and recovery in extractive fermentation has not been reported previously for any species of *Rhodotorula*. Recently, it was reported the implementation of extractive fermentation for the production of pullulan, an EPS produced by *A. pullulans*, in PEG-dextran or PEG-mono/bi-sodium systems [33]. This study reported that biomass was partitioned to the PEG rich top phase and that the pullulan was partitioned towards the bottom phase. In our study, biomass and EPS partition was dependent on EOPO concentration, where both products were partitioned predominantly to the bottom phase in various proportions. Although partition behavior is different for both microorganisms and extractive fermentation systems, it is clear that extractive fermentation can result in an important strategy for recovery and even purification of EPS.

Finally, we observed that extractive fermentation performance could be enhanced in a continuous flow extractor by taking advantage of the liquid–liquid system. Future work should be focused on the interaction between each extractive phase (EOPO + Medium + Microorganisms) to control mass transfer and provide operational conditions for a full EPS and biomass separation. Since interfacial tension increases with mineral medium and with EOPO concentration, experiments could be designed to favor different flow patterns, such as slug flow or drop flow, to increase the interaction between phases, and thus enhance the extraction and recovery.

5. Conclusions

Fermentation parameters such as EOPO concentration, and phase formation temperature and time were identified as first approaches to understand and determine the application of extractive fermentation for EPS production and recovery, with interesting results. This is the first report of extractive fermentation applied on EPS production and recovery from a yeast of the genera *Rhodotorula*, proving to be an interesting strategy, although further optimization is needed in order to increase biomass and EPS production by this technique. Since *Rhodotorula mucilaginosa* UANL-001L EPS is of special interest due to its bioadsorbent and wide spectrum antimicrobial properties, further study of extractive fermentation as a technique for production, recovery and purification is highly encouraged.

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