Extractive production of microbial oil using hydrophobic adsorbents: A comparative study

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Abstract
Increased demand for vegetable oils has accelerated interest in producing its renewable alternate through microbial route. However, this process still awaits technological advancements to be executed as a commercially feasible operation. The oil productivity in this bioprocess is significantly challenged by the conventional recovery techniques used. To address this problem, we propose an extractive production system using hydrophobic adsorbents as oil capturing agents for simultaneous in situ oil recovery. Three polystyrene and two organo-silica-based capturing agents were studied on a “cell mimic system” to attest to an increase in the rate of oil recovery. High sorption capacity demonstrated by SEPABEADS, stalled the oil saturation in the aqueous medium and thereby, enhances the extracellular efflux of the produced oil. The efflux of oil, when matched with the substrate assimilation, allows for an increased production rate of oil. As a result, we observe and report oil production to 80% dry cell weight and volumetric productivity of 0.11 gL⁻¹ hour⁻¹. With SEPABEADS SP70 as an in situ oil capturing agent in the broth, the wild type “Yarrowia lipolytica” cells yield 0.33 goil g⁻¹ glucose, allowing us to propose a highly efficient system for extractive production of microbial oil.

KEYWORDS
extractive production, hydrophobic adsorbent, in situ product removal, microbial oil

1 | INTRODUCTION

Widespread applications of plant-derived oils in biofuels, foods, cosmetics, surfactants, and lubricant processing industries are expected to reach a global consumption of 100 million barrels/day. An exhaustive demand for these oils has ignited the “food vs fuel” debate, while also raising serious concerns over the land being dedicated for the oil crop cultivation. To pacify these arguments, the research interest has shifted towards the development and commercialization of edible oil equivalents. Particularly, oil production by oleaginous yeasts has gained impetus, as these organisms produce fatty acid profiles similar to the plant oils, and their cultivation is minimally dependent on environmental and spatial requirements.
However, these yeast-based technologies still await competent technological advancements for their commercial translation and are plagued by the substandard performance shown by the organism upon scale-up. While significant progress has been achieved to amplify intracellular oil accumulation in yeasts, the conventional down-stream techniques used, fail to achieve complete and well-timed recovery of the accumulated oil. The down-streaming thus accounts for major process losses and has been identified as a primary cost-driver in the economics of this process. These losses further contribute to lowered yields, volumetric productivities, and unwanted side reactions/lipid turnovers, that question the feasibility of this process.

Microbial oil recovery techniques practiced currently rely on solvent and energy-intense cell-disruptive oil extraction. These disruptive methods do not permit cell reuse and prohibit continuous operations in the bioprocess. Approaches to circumvent disruptive extraction have targeted production methodologies that allow the dynamics of intracellular oil out of the cell in an aqueous environment. Prominent strategies include either the addition of higher-chain alkanes to promote secretion or stabilizing the extracellular oil-in-water by a natural emulsifier. System engineering approaches also have been acknowledged to enhanced metabolic flux pull and realize an increased oil production and extracellular secretion of the produced oil. However, these methods are often associated with oil losses in liquid-liquid phase separations, leading to a problematic down-streaming and ending up in low extracellular oil titers.

Extractive fermentations have been testified for in situ product recovery of various fermentative products like lactic acid, antibiotics, flavors/fragrances, alcohol/solvents, and organic acids. The in situ product recovery approach has allowed product enrichment, improved yields, alleviated inhibitions, curtailed process flows, and costs. Heuristics by Urbanus et al presented different possibilities for devising an appropriate extractive production system. Although it is relatively easier to capture volatile products and charged unstable moieties, the isolation of microbial oil from fermentation broth requires more discerning approaches owing to the structural diversity of component lipids.

Phillips et al suggested the use of a heterogeneous phase in extraction procedures for hydrophobic natural products produced through fermentation. The challenge lies in developing an extractive fermentation for microbial oil production that allows continuous stable operations with (i) cell stability; (ii) maintenance of aerobic conditions for cells; (iii) matching oil production rate with the capture rate; and (iv) enabling complete assimilation of the substrate fed to the system.

Based on these requirements, an extractive production system has been envisaged in this study for the microbial oil production process, to synergize the oil production and its separation from the broth. This hypothesis will allow integration of the upstream and downstream operations. The present study evaluates the oil capturing capacity of three polystyrene-based matrices and two organo-silica-based resins. The mechanism of oil efflux, adsorption of oil dispersed in water, and its associated kinetics are simulated by a cell mimic system. A scalable model for simultaneous production and recovery of microbial oil has been presented.

2 MATERIALS AND METHODS

2.1 Materials

Yarrowia lipolytica NCIM 3590 was obtained from the National Collection of Industrial Microorganisms (NCIM)-National Chemical Laboratory, Pune, India. Olive oil was purchased from Figaro, Spain. Dialysis Membrane-110 (pore size of 2.4 nm), was procured from HiMedia, India. The adsorbent resins of Osorb with particle sizes of 0.125 mm (ss) and 0.2 mm (herein referred to as Osorb 1 and Osorb 2, respectively), manufactured by ABS materials Inc., Wooster, Ohio, were received as a kind gift from InNow LLC, Navi Mumbai, India. DIAION HP20 and SEPABEADS SP70 and SP700 were from Mitsubishi Chemical Corporation, India, respectively.

2.2 Analysis of the miscibility of oil in water and saturation of the oil production broth

Olive oil was added to water (10 g L⁻¹), in a continuously stirred vessel at 28 °C and 200 rpm. Samples (1 mL) were withdrawn periodically at an interval of 1 hour from the center of the vessel, using an extrusion needle. The oil from the aqueous sample was extracted using liquid-liquid extraction with chloroform and quantified gravimetrically. Oil
concentration in the aqueous phase, thus obtained at different intervals was used to plot a graph with respect to time, to obtain a saturation point.

2.3 | Adsorbent based oil recovery with a “cell mimic” system

A mixture of olive oil (8.5 g) and water (2.83 g) was filled in a Dialysis membrane bag, to have oil as was 75% (w w\(^{-1}\)) of the total bag weight. The bag was then suspended in 350 mL water in a stirred vessel at 28 °C and 200 rpm. The oil dispersion in water after 28 hours of stirring was studied by liquid-liquid extraction of the oil from the aqueous medium, using chloroform. Oil extracted in chloroform was quantified gravimetrically after distillation. Also, the weight of the bag before, after, and the oil retained inside the bag was noted.

A similar experimental set up with adsorbents resins was used for studying the influence of sorbents on the oil retention in the dialysis bag. The adsorbent resins used for the study include SEPABEADS SP70, SP700, DIAION HP20, and Osorb 1 and 2. These adsorbents were used separately within an effective resin loading of 10 g L\(^{-1}\). The oil adsorbed on the adsorbent and the oil in the aqueous media were estimated after desorption of the resins with chloroform and liquid-liquid extraction with chloroform, respectively.

2.4 | Assimilation of glucose for oil production in a two-stage system

2.4.1 | Growth phase

Stock cultures of Y. lipolytica NCIM 3590 were revived in 50 mL MGYP medium and incubated at 28 °C for 48 hours. The culture was used for preinoculum development in MGYP growth medium with composition per liter: 3 g malt extract, 20 g glucose, 3 g yeast extract, 5 g peptone. This seed culture was transferred to the experimentation flasks to make 10% (v/v) inoculum concentration and was incubated at 28 °C at 200 rpm for 48 hours. The growth phase was carried out in a chemostat mode to obtain the desired cell density, as described earlier.\(^7\) The moisture content of the wet cell biomass obtained after centrifugation of the broth was calculated on a Moisture Analyzer (METTLER TOLEDO, HE53) and dry cell weight (DCW) was estimated accordingly.

2.4.2 | Oil production phase

Cells of Y. lipolytica NCIM 3590 were inoculated in the oil production media of 5% glucose solution in a 100 mL Erlenmeyer flask to achieve a cell density of 50 g L\(^{-1}\). The fermentation was carried out at 200 rpm and 28 °C for 72 hours, as described by Warke et al\(^7\). Samples (10 mL) were collected every 24 hours from the broth. The withdrawn samples were subjected to centrifugation at 13 000g and 14 °C for 2 minutes to separate the aqueous supernatant and the cell biomass. The cell pellet was dried and weighed to estimate the DCW. The aqueous supernatant was filtered using a 0.2-micron filter and 2 mL of this filtered supernatant was analyzed for sugars using the high-performance liquid chromatography (HPLC) method described in Section 2.5. The remaining filtered sample was subjected to liquid-liquid extraction for estimating the extracellular oil gravimetrically. The intracellular oil was extracted directly from the wet cell pellet, as described in Section 2.7.

2.5 | Analysis of sugar and estimation of glucose uptake rate

Residual glucose concentration in the oil production broth was determined by HPLC (Agilent series 1200, Japan) system equipped with a refractive index detector. Two milliliters of the aqueous supernatant obtained after centrifugation and filtration of the harvested sample was used for this analysis. HPLC column used was Aminex 87H (Bio-Rad, Hercules, CA) and elution was carried out with 5mM sulphuric acid as the mobile phase at a flow rate of 0.6 mL min\(^{-1}\). The column temperature was maintained at 50 °C. The standard calibration curve was plotted for glucose and was used for the quantitative determination of unknown samples.
Consumption of glucose was thus estimated, and the Glucose uptake rate (GUR) was thus calculated over the given period.

2.6  |  In situ oil recovery using adsorbent resins

The hydrophobic adsorbents were introduced into the oil production broth at the onset (0 hour) of the fermentation to achieve a concurrent production and a simultaneous in situ oil capture inside the broth. Initial studies at shake flask level were carried out by adding 1 g of adsorbent resins to the oil production medium along with the inoculation of cell biomass obtained from the growth phase cells. The in situ oil recovery using adsorbent resins was also studied microscopically to visualize the phenomenon of oil capture. The adsorbent surface and its interaction with the oil in water and the oleaginous yeast cells were observed under a light microscope (with 10× magnification).

2.7  |  Ex situ oil recovery using adsorbent resins

The oil production broth (with and without cell separation) was externally contacted with the hydrophobic adsorbents, after the completion of fermentation. The postproduction broth (with and without cells) was repeatedly passed over a bed of adsorbents, to ensure complete removal of extracellular oil from the aqueous phase. The adsorbent surface and its interaction with the oil in water and the oleaginous yeast cells were observed under a light microscope (with 10× magnification).

2.8  |  Extraction of microbial oil from oleaginous yeast and estimation of oil production rate

Microbial oil from oleaginous yeast cells was extracted in the form of “Intracellular Lipid,” “Extracellular lipid” in the broth, and the “adsorbent bound oil.” The wet cell biomass, obtained as a pellet after centrifugation of the oil production broth at 8000 rpm in an ultracentrifuge at 20 °C for 15 minutes, was used for intracellular lipid extraction. The oil extraction from these wet cells was carried as per the method described by Yao et al by using Propan-2-ol:chloroform in a proportion of 1:1 (v v−1) as extracting solvents. The nonlipid cellular material was separated by centrifugal separation, and the supernatant was distilled in a preweighed flask to assess the intracellular oil content. The cell pellet obtained after centrifugation was weighed to estimate the wet cell weight. The DCW was thus calculated accounting for the moisture content in the wet cell pellet.

Extracellular oil extraction from the aqueous phase was carried by liquid-liquid extraction using chloroform as the extractant. The aqueous supernatant obtained after centrifugation of the oil production broth was also subjected to solvent extraction with chloroform in a ratio of 1:1 (v v−1), to obtain extracellular oil in the organic phase. After three times extraction from the aqueous phase, the raffinate (aqueous layer) was discarded, and the organic chloroform layer was used for further analysis. The extracellular oil was thus obtained and quantified after distillation of the extract organic solvent phase (chloroform layer) in a preweighed distillation flask.

The oil adsorbed on the resin beads was recovered after desorption of the resins with Propan-2-ol and chloroform, in a volumetric ratio of 1:1. The oil thus obtained after distillation of the solvent organic phases from each extraction was quantified gravimetrically. Extractive solvents used for analysis and extraction were of analytical grade or chromatographically pure and were obtained from SD Fine Chemicals Ltd., Mumbai, India. Oil production rate or productivity (g L −1 hour−1) was further calculated, by accounting the increase in the oil titer produced with respect to the time.

2.9  |  Evaluation of the binding capacity and sorption mechanism of adsorbent resins

The adsorptive capacity of resins for standard glucose and olive oil was calculated by interacting a known amount (1 g) of resins with an increasing concentration of the adsorbate in different tubes. All the tubes were placed on a rocker shaker for 2 hours and subjected to isothermal and isobaric conditions. The binding of the adsorbate on a given quantity of adsorbent, its saturation, and thus the binding capacity was calculated. The adsorption data were used to
study the sorption equilibrium, kinetics, and mechanism of adsorption by fitting into the reported isotherm and kinetic models.

2.10 | Microbial oil production using adsorbent resins at a fermenter scale

Microbial oil production with hydrophobic adsorbent resins as an oil capturing agents (OCAs) was carried out in a 5-L jacketed glass fermenter (BioFlo 120, Eppendorf, India), with 2 L working volume of oil production broth comprising 50 g L\(^{-1}\) glucose solution. Twenty grams of hydrophobic adsorbent resins were added to the fermenter at the onset of fermentation. Yeast cells harvested from the growth phase were inoculated to the fermenter at a cell load of around 50 g L\(^{-1}\). The oil production was carried out for 72 hours at 28 °C and 200 rpm, while the aeration was maintained at 1vvm. The regeneration of the resins was carried out before their addition to the broth, with repeated washes of propan-2-ol followed by at least three washes with sterile water, to ensure activation of the binding sites with complete removal of bound moieties and the eluting solvent propan-2-ol. The regeneration procedure also helped in sterilizing the adsorbent resin beads before their use.

3 | RESULTS AND DISCUSSION

3.1 | Miscibility of oil in water and saturation of the oil production broth

The oil dispersion was studied in a continuously stirred vessel containing water at 28 °C to determine the oil saturation limit of an aqueous medium. Olive oil (1% v/v water) was chosen as a representative triglyceride rich oil for this study, in order to relate the observations with microbial oil solubility in aqueous broth. It was observed that olive oil concentrations reached 3 g L\(^{-1}\) in the aqueous layer after 30 hours of continuous stirring (For details, see additional supporting information: Figure S1). No further increase was observed in the solubility of olive oil beyond this point, suggesting that the oil saturation limit of the aqueous phase was achieved. It was deduced that the rate of diffusion of oil in water for the given operating parameters was 0.098 g L\(^{-1}\) hour\(^{-1}\). The diffusivity “D” of oil in water was calculated based on the famous two-film theory\(^{19}\) and it was found to be 0.0193 cm\(^2\) hour\(^{-1}\) or 5.36E-10 m\(^2\) s\(^{-1}\) (For details see additional supporting information: Figure S2). The low value of “D” illustrates that the miscibility of the oil in the water phase was limited to 3 g L\(^{-1}\), and its diffusivity (\(D = 5.36E-10 \text{ m}^2 \text{ s}^{-1}\)) was very low. These values can be increased by optimizing the parameters like operating temperature, the ionic/salt concentration in the broth, and mixing regimes in the vessel. As these parameters effect responses on viability and production capacity of the oil-producing microbe, these cannot be altered in a fermentative process.

An alternate strategy to address this predicament would be to delay the saturation by continuously removing produced oil from the system.

3.2 | Adsorbent-based oil recovery

3.2.1 | Oil recovery with a “cell mimic” system

An aqueous mixture of oil packed in a dialysis bag and suspended in water (350 mL) was used as a “cell mimic” system to study the influencing parameters that could aid transport of oil to the aqueous exterior environment. A ratio of 3:1 for oil:water in the dialysis bag was used for this study to depict 75% oil present in the cell.

The pore size of the dialysis bag (2.4 nm and molecular weight cut off around 12000-14000 Da), should permit free movement of the oil (triglycerides with molecular wt 800-1200 g/mol) in the aqueous system. However, the majority of the oil was observed (Figure 1A) to be restrained in the dialysis bag, even after 28 hours. Slow diffusion was observed through the semipermeable membrane over the period of time tested, based on estimations of oil extracted from the aqueous media. Thirteen percentage of the total oil was recovered from the aqueous phase (Figure 2). This suggests that the concentration of the oil in the aqueous phase was lower than 3 g L\(^{-1}\) as obtained earlier.

Adsorbent matrices in the form of spherical resin beads exhibit as compatible conformations by offering a larger effective surface area for selective adsorption of dispersed moieties from an aqueous medium. Synthetic styrene-based
Aromatic macroporous adsorbents like SEPABEADS SP70, SP700, and DIAION HP20 are widely used for adsorption of hydrophobic compounds by pore diffusion.\textsuperscript{16} Whereas organo-silica-based matrices like Osorb, which swell in presence of hydrophobic compounds, have been employed for oil remediation.\textsuperscript{20,21} These hydrophobic adsorbents, displaying different capturing mechanism, were therefore considered and compared in this study for oil recovery from the aqueous phase.

The hydrophobic adsorbents were added into the aqueous stirred environment in which the semipermeable dialysis bag was suspended. Studies on the aqueous phase over the time period showed the presence of oil in the water. However, visual observations indicated a loss of oil from the dialysis bag. The hydrophobic adsorbents stuck to the bag at multiple points and were observed to adsorb oil from the bag. This aided the transport of oil from the internal environment of the bag to the external aqueous environment. Thus, in other words, the addition of an in situ OCA in the form of hydrophobic resins was observed to enhance the efflux of oil through the semipermeable membrane. As displayed in Figure 2, SEPABEADS SP700 exhibited the highest (55\%) recovery of the total oil entrapped in the bag. It adsorbed >40\% of the total oil that was initially present in the bag. Similarly, Osorb was observed to capture >50\% of the total oil in 28 hours. The amount of oil adsorbed by SEPABEADS SP70 was comparable to SP700. However, a lesser amount of oil outside the bag and in aqueous phase suggests that the rate of adsorption and thus the rate of oil efflux or diffusion was slower in case of SP70. The performance of HP20 was lowest amongst all the adsorbents tested.

In another individual experiment, incorporation of the adsorbents at the beginning itself caused further oil sequestration from the dialysis bag, in addition to the oil recovery from aqueous medium. The resins were found to have adsorbed the oil and were closely associated with the dialysis bag. These observations authenticate the physical transfer of oil from the bag to the outside environment and onto the adsorbent surface.

It was interesting to note that the “overall weight of the bag” remained unchanged, despite significant oil transfer from the bag and its capture on the adsorbent. Notably, this effect can be ascribed to the inflow of an equal amount of water inside the bag, maintaining an equilibrium. Also, it was observed that the rate of oil efflux, in this case, was 0.189 g L\(^{-1}\) hour\(^{-1}\) when adsorbent resins were incorporated in the system (Table 1). The 2-fold increase in the rate of oil efflux in the presence of adsorbents can allow equal increment in the oil productivity or oil production capacity of the organism.

Therefore, the dialysis bag mimics the membrane surface for oleaginous yeast cells and the model demonstrates a robust mechanism that can be used for oil recovery in a noninvasive manner.
TABLE 1 Rate of extracellular oil secretion and oil productivity in different microbial oil production systems

| Mode of operation                                                                 | Rate of oil diffusion/efflux (g L\(^{-1}\) h\(^{-1}\)) | Oil productivity (g L\(^{-1}\) h\(^{-1}\)) | Reference |
|----------------------------------------------------------------------------------|-------------------------------------------------------|------------------------------------------|-----------|
| Interfacial diffusion through the two layers in a CSTR                           | 0.098                                                 | Not applicable                           | Present study |
| Growth associated single stage fermentation 40 g L\(^{-1}\) acetic acid as a stimulant | 0.1042                                                | 0.0208                                   | 22         |
| Two-stage fermentation 5% Glucose                                                | 0.1036                                                | 0.066                                    | 7          |
| Solvent-mediated extractive fermentation 7.5% (v v\(^{-1}\)) Dodecane            | 0.1077                                                | 0.0169                                   | 8          |
| 15% (v v\(^{-1}\)) Dodecane                                                     | 0.1386                                                | 0.1386                                   | 10         |
| Adsorbent                                                                        |                                                       |                                          |            |
| SP70                                                                             | 0.1196                                                | 0.1095                                   | Present study |
| SP700                                                                            | 0.1893                                                | 0.1519                                   | Present study |
| Osorb 1                                                                          | 0.1471                                                | 0.0833                                   | Present study |
| Osorb 2                                                                          | 0.1406                                                | 0.0679                                   | Present study |
| HP20                                                                             | 0.1170                                                | 0.0555                                   | Present study |

Abbreviations: CSTR, continuous stirred tank reactor.

FIGURE 3 Microscopic images of adsorbent resins observed in phase contrast mode of a light microscope (10× magnification). (A) Macroporous resin with a smooth and opaque surface. (B) Hydrophobic interaction of oil dispersed in water with adsorbent in an ex situ oil capture mode. (C) Translucent and rough adsorbent surface, suggesting light refraction through adsorbed oil and cell adherence onto the hydrophobic surface.

3.2.2 Oil recovery from the oil production broth

Oil capture by hydrophobic adsorbents, in the presence of 5% sugar, was studied; to understand their applicability in the fermentation broth. Polystyrene-based SEPABEADS SP70 captured 121 mg\(_{\text{oil}}\) g\(_{\text{SP70}}^{-1}\), whereas 145 mg\(_{\text{oil}}\) g\(_{\text{Osorb}}^{-1}\) was adsorbed by the organo-silica matrices of Osorb when assessed separately for their oil binding capacity in this aqueous broth containing 3 g L\(^{-1}\) olive oil and 50 g L\(^{-1}\) sugar. The oil binding capacity of both the adsorbents was observed to be significantly higher than the binding capacity for glucose in the broth. The amount of the glucose bound to the adsorbents was 4.64 mg\(_{\text{glucose}}\) g\(_{\text{SP70}}^{-1}\) and 16 mg\(_{\text{glucose}}\) g\(_{\text{Osorb}}^{-1}\), respectively. Thus, if these adsorbents are added to a fermentation broth, the decrease in glucose concentration in the broth can solely be attributed to the glucose consumption by oleaginous yeast cell and not to its binding on the adsorbent resin.

The microscopic evaluation (Figure 3A) showed that the adsorbent resin beads were smooth and opaque prior to their addition to the broth. When the oil-containing aqueous phase (post-fermentation and after cell separation), was contacted to these OCAs, the dispersed oil (micelle) in water was found to interact with these adsorbent resins, as seen in Figure 3B. Interaction with the cell surface was also seen (Figure 3C), when these adsorbents were directly contacted with the
fermentation broth without cell separation. In the case of postfermentation ex situ oil capture, a complete recovery of the extracellular oil was observed with both types of hydrophobic adsorbents used (Figure 4).

The cell adherence observed on the adsorbents (Figure 3C) suggests a probability of physical surface-surface interaction of the yeast cell surface and also the surface bound lipids with the hydrophobic adsorbents. In our previous report, we characterized the oleaginous yeast cells for the lipids bound to their cell surface. These surface lipids were observed to affect the flux across the cell membrane and also the substrate uptake kinetics. Negative feedback elicited by these lipids also inhibits the biosynthesis of microbial oil inside the cells and regulates cellular homeostasis. Thus, it becomes important to stall this extracellular oil saturation in the aqueous phase as well as on the cell surface.

To evaluate the possibility of continuous oil stripping from the aqueous phase and from the cell surface, the adsorbent resins were added into the production broth with the onset of fermentation. SEPABEADS SP70, SP700, DIAION HP20, and organo-silica matrices of Osorb were used separately with a load of 10 g L⁻¹ into the oil production broth. As seen in Figure 4, the total oil produced in this in situ oil capture mode was distributed in three forms, viz., “Intracellular oil”, “Extracellular oil”, and “Extracellular oil captured by the resins or “Resin bound oil”. Different resins, based on their binding capacities and properties, showed alterations in this oil distribution pattern. An increase in the total lipid content and oil titers indicate an enhancement in microbial oil production, in the presence of in situ adsorbents. The highest oil titer of 8 g L⁻¹ was observed with SEPABEADS SP70, wherein the total lipid produced was 80% of DCW. The intracellular oil content, in this case, was found to be very low (4.42% DCW), whereas the extracellular (37.17% DCW) and resin-bound oil (37.19% DCW) were equally distributed.

The decrease in the intracellular oil content in presence of in situ adsorbents, implies a possibility of direct sequestration of intracellular oil from the cells into the external environment (i.e., cell surface and aqueous medium) and its subsequent capture by the in situ adsorbents. A variation in oil distribution was also observed with the use of different in situ adsorbent resins. This variation indicates capabilities of individual adsorbents, for pulling out the intracellular oil from the cells.

3.3 Extractive fermentation using in situ adsorbent resins

The oil production capacity of the oleaginous yeast, Y lipolytica NCIM 3590 was examined in presence of in situ hydrophobic resins of HP20, SP70, SP700, and Osorb in two size variants (herein referred as Osorb 1 [or Osorb small size (ss)] and Osorb 2). The effect of the adsorbents on the lipid content (w.r.t DCW), oil yield, oil production rate, and GUR was studied and has been depicted in Figure 5.

It was observed that the oil production rate was significantly enhanced to 0.15 g L⁻¹ hour⁻¹ in presence of SEPABEADS SP700. The oil productivity with SP70 (0.11 g L⁻¹ hour⁻¹) was closer to SP700 and was in accord with the oil efflux rate calculated by the “cell mimic” model (Table 1). The oil production can be obtained continually at this rate, so long as the
**FIGURE 5** Comparative study of the effect of in situ hydrophobic adsorbent resins on microbial oil production in oleaginous yeasts

![Graph showing lipid content, oil yield, glucose uptake rate, and oil production rate for different adsorbents.](image)

**TABLE 2** Properties of the hydrophobic adsorbents

| Properties         | Polymeric matrix of poly styrene divinyl benzene | Organo-silica-based matrices |
|--------------------|-----------------------------------------------|-------------------------------|
|                    | Specifically treated aromatic copolymer | Unsubstituted aromatic crosslinking | Bis (trimethoxysilyl) benzene |
| Hydrophobic resin  | SP70                                        | HP20                          | Osorb 1 Osorb 2 |
| Particle size (μm) | 850                                         | 250                           | 900             | 125 200 |
| Surface area (m² g⁻¹) (m² mL⁻¹) | 870                        | 1200                         | 590             | 736 NA |
| Pore volume (mL g⁻¹) | 1.5                                 | 2.2                           | 1.3             | 0.65 NA |
| Pore radius (Å)    | 70                                           | 90                            | 290             | 60 NA |
| Mechanism of capture | Physical adsorption on porous polymer beads showing π-π interaction | Swelling of silica nanostructure matrix in presence of nonpolar moieties |

NA, not available.

The maximum sorption capacity of the adsorbent is reached. However, the oil production with DIAION HP20 did not show any enhancement and was comparable to the inherent oil production capacity of *Y. lipolytica*. Organo-silica based resins of Osorb (ie, Osorb 1 and Osorb 2) also showed an effective increase in oil production but not as much as the aromatic polystyrene-based resins.

The glucose uptake was also found to increase in the presence of all the in situ adsorbents tested. With SEPABEADS SP70 and Osorb 1 adsorbents, this increase in glucose uptake corresponded to theoretical oil yields of 0.33 g oil g glucose consumed⁻¹.

The efficacy of these hydrophobic resins as OCAs in aqueous medium and their hydrophobicity, is associated with their chemical structure, surface area, pore radius/size, binding capacity, and mechanism of adsorption (Table 2).

The use of synthetic crosslinking polymers offers a wide range of precise pore size, based on the polymerization. These adsorbents are conferred with different physical and adsorptive properties. The high specific surface area of SEPABEADS shows high oil adsorption as compared to others, which is also supported by the increased oil titers in their presence. However, the oil production rate of SEPABEADS SP700 does not correlate with the GUR, resulting in a lower oil yield than that of the theoretical maximum. The small particle size (250 μm) of SP700 has been identified to hinder the material dynamics, diffusion, and thereby affect their applications in batch adsorption process (DIAION Technical Manual, No. 01-01-D-0103).
DIAION HP20 resins, with pore radius, three to four times larger than that of SEPABEADS, exhibit a comparatively smaller surface area for adsorption. Their larger pores are prone to cell deposition, which further decreases the effective available surface for adsorption.\textsuperscript{16} This effect can be ascribed for the lesser oil production in their presence. A similar effect was also noticed in another in situ extractive production process with HP20,\textsuperscript{24} where mutually competing mechanisms associated with the process impeded the metabolite production.

A similar trend was observed for Osorb resins with different sizes.\textsuperscript{21} The nanoporous matrix of Osorb is known to swell about eight times of their dry weight upon an encounter with organic moiety. These provide for reversible and selective adsorption of the nonpolar molecule dispersed in an aqueous phase.\textsuperscript{25} These features endorse Osorb with a high binding capacity and linearity in adsorption.\textsuperscript{20} However, high glucose binding observed with Osorb implies that the nutrient binding affects the substrate uptake rate and, therefore, the production and yield in the presence of organo-silica matrices.\textsuperscript{26}

Furthermore, it has also been reported earlier that organo-silica matrices are known to hinder mass transfer by limiting the rate of diffusion.\textsuperscript{27} The pore radius of 90 Å in the case of SP70 offers it with an extensive surface area, in contrast to 60 Å of Osorb. Also, the material strength provided by the styrene divinyl-based polymer makes it amenable for its use in CSTR conditions. Most importantly, Osorb, due to its tendency to shrink in the aqueous phase and swell on exposure to organic solvents, offers a limited entry for hydrophobic moieties in the aqueous broth, in spite of its excellent desorption characteristics. Thus, Osorb matrices are not comparable with the polystyrene resins in an in situ extractive microbial production process. Considering these aspects, and the US FDA compliance, the SEPABEADS SP70 was used in further scale-up studies.

### 3.4 Mechanism and kinetics of oil sorption

#### 3.4.1 Adsorption equilibrium studies for understanding sorption mechanism

The adsorptive oil capture with SEPABEADS SP70 was studied further to understand the mechanism associated with it and to derive the parameters about maximal adsorptions. One gram of adsorbent beads were contacted with 10 mL of olive oil-in-water emulsions to study the oil sorption equilibrium. The oil concentration in these emulsions was varied from 6.3 mg to 200 mg in the different individual tubes, which were placed on the rocker shaker for 120 minutes. In this study, six well-known two-parameter sorption isotherm models viz, Langmuir, Freundlich, Temkin, Harkin-Jura Isotherm, Jovanovic Isotherm, and Dubinin-Radushkevich were used to illustrate the adsorption isotherm of oil onto the adsorbent. The adsorption data were plotted based on these isotherm models to find the model parameters. (Figure 6) (For details see additional supporting information S3: Table S3).

The adsorbent showed the best fit to Langmuir adsorption model (coefficient of determination; $R^2 = 0.977$); accounting for a monolayer surface coverage by balancing a dynamic adsorption-desorption equilibrium. The Langmuir maximum adsorption capacity “$q_m$,” was found to be 121.77 (mg g$^{-1}$), whereas the Langmuir constant $K_L = 11.71$(mL/g) complemented the binding capacity with the sorption surface area and porosity of the adsorbent. This implies the relative surface of the adsorbent covered by the adsorbate and that available for adsorption.\textsuperscript{28} Furthermore, the dimensionless separation factor, $R_L = \frac{1}{1+K_L C_0}$ = 0.0004 suggested a favorable adsorption as, $0 < R_L < 1$. Lower values of $R^2$, in case of Freundlich and Dubinin-Radushkevich isotherm implied lesser fit to the possibility of adsorption onto a heterogeneous adsorbent surface and multilayered adsorption. This is also evident by the lower values of $K_F$ and $1/n$, which are indicative of adsorption capacity/intensity or the relative energy distribution and the heterogeneity of adsorbate sites.\textsuperscript{29}

Nevertheless, the value of mean adsorption energy, $E = 0.1038$ kJ/mol denotes physical adsorption ($E \leq -8$) conceivably by pore filling mechanism with the involvement of Van Der Waal’s interactions.\textsuperscript{30,31} However, the Temkin isotherm fit pointed towards the possibility of secondary adsorbate-adsorbate interactions in the adsorption process; assuming an effect of molecular contact between the layers as a result of increased surface coverage.\textsuperscript{32} The probability of multilayered sorption onto a heterogeneous and porous adsorbent was therefore evaluated by Harkin-Jura isotherm model. The “goodness-of-fit” to this model over Freundlich and Temkin isotherms (which are based on similar assumptions), aided in solving the ambiguity associated with the adsorption process.\textsuperscript{33} Moreover, the risk of mechanical fit of adsorbate onto an adsorbent was overruled by noncoherence with the Langmuir based Jovanovic Isotherm model.\textsuperscript{34}
FIGURE 6  Adsorption isotherm plots of (A) Langmuir isotherm, (B) Freundlich isotherm, (C) Temkin isotherm, (D) Dubinin-Radushkevich, (E) Harkin-Jura Isotherm and (F) Jovanovic Isotherm model. The adsorption data for oil sorption on adsorbent resins were fitted in each of the models to evaluate the mechanism of sorption and determining the maximum sorption capacity.

3.4.2 | Kinetics of oil adsorption

The mechanism of oil sorption and the actual mass transfer kinetics involved in the oil sorption was evaluated using the pseudo-first-order kinetic model, pseudo-second-order kinetic model, Elovich model, Weber’s Intraparticle diffusion model, Bangham’s model for pore diffusion, and Boyd’s diffusivity model for a batch contact process. The adsorption kinetics data for oil adsorption showed a better fit to the Pseudo-first-order Kinetic Model, stating for physical adsorption of the adsorbate involving weaker sorbate interactions with the adsorbent surface. Figure 7. The value of $q^*e$ obtained after solving the model equations was found closer to the equilibrium adsorbed oil concentration. The reduced regression observed with Pseudo-second-order model inferred that there was no chemical bonding involved between the adsorbate and adsorbent. It was observed that the rate of adsorption, “K”, decreased with an increase in the oil concentration, in both the models, presumably due to increased hydrophobicity and slower diffusion through the pores of adsorbents. The Elovich Kinetic Model, based on chemisorption, also underlined a similar trend of decreasing Elovich parameters; “a” (initial adsorption rate) and “b” (desorption constant), with an increasing adsorbate concentration.

Further insights of sorption kinetics were obtained by Weber’s diffusion model, which considers the different mass transfer processes involved in adsorption of an adsorbate on the adsorbent surface. Herein, the value of $C_{i,}$ showed that the boundary layer effect over the adsorbent surface was prominent at the initial oil concentrations between 35 mg to 55 mg mL$^{-1}$; beyond which it was insignificant. The linearity of the plot was affected with an increase in the oil concentration, suggesting that multiple rate controlling diffusion steps regulated the oil adsorption process beyond 66 mg. Boyd’s Diffusivity Plot was used to calculate the rate of diffusion, and it was found that the highest rate was at the oil concentration of 36 mg mL$^{-1}$. Further increase in oil concentration decreased the linearity as well as the rate indicating hindrance to the phenomenon of pore diffusion. These results are understandable with that obtained by Weber’s model and therefore imply that surface diffusion is more significant than the pore diffusion in the given concentration range. Moreover, the values of diffusion coefficient obtained by Boyd’s diffusivity for adsorption processes and that derived by aqueous two film theory for oil dispersion in water corroborate the prevalence of mass transfer by film diffusion, as both the values are in between 10$^{-6}$ and 10$^{-11}$ m$^2$ s$^{-136}$ (for details, see additional supporting information S4: Table S4). Bangham's equation was used to investigate more about the pore diffusion. However, the values of $\sigma$ were lesser than unity denoting a poor representation of the model.
Nevertheless, as seen from the results, it is evident that the rate of oil sorption is much faster, and the time required for reaching the maximum sorption capacity of around 0.12 g g\(^{-1}\) is less than 2 hours. The adsorption can thus be considered as an instantaneous phenomenon (since the rate of oil adsorption \(\gg\) rate of oil efflux). This suggests that the addition of in situ adsorbents can ensure an immediate and simultaneous oil recovery from the aqueous production medium and thereby avoid the attainment of oil saturation conditions in the aqueous broth and on the cell surface. Moreover, the physical interaction of the adsorbents with the yeast cell surface can overcome the slower oil diffusion rates in the aqueous phase and allow us to hypothesize a possibility of direct oil sequestration from the cell.

Therefore, the use of adsorptive resin as “OCAs” can actually promote the microbial oil production by tuning its productivity with the rate of oil removal.

### 3.5 Microbial oil production and applicability of the adsorbent based in situ oil recovery on a fermenter scale

Microbial oil production was carried in a 2 L batch fermenter; with and without in situ adsorbents in the oil production broth, to validate the results obtained in the shake flask study. Both the studies were carried with a cell load of 50 g L\(^{-1}\) (17 g DCW L\(^{-1}\)) and a sugar concentration of 50 g L\(^{-1}\), to evaluate the effect of an in situ adsorptive recovery on the oil production over the period of time. SEPABEADS SP70 adsorbent resins were used in this study based on their performance on the shake flask level and their oil binding capacity.

As seen in Table 3, the cell biomass was found viable, and its concentration remained constant throughout the 72 hours of batch fermentation in both the cases. An increase in the oil production and glucose uptake was seen in presence of the adsorbents. The oil titer increased with in situ oil recovery in the batch fermenter and the total lipid content was about 80% of DCW. This increase in total lipid content was on account of increased extracellular oil secretion, observed in presence of in situ oil adsorbents. An oil yield of 0.33 g g\(^{-1}\) glucose consumed; corresponding to theoretical maximum oil yield on glucose was achieved in this case, with complete consumption of the glucose fed to the system. The comparative study of glucose assimilation, cell biomass viability, and oil production over 72 hours in an oil production phase, thus revealed that the simultaneous adsorptive oil capture had an advantage in every aspect (Table 3).
4 CONCLUSION

Microbial oil production processes are highly limited for continuous commercial applications as oil produced is primarily intracellular, and its low solubility (~3 g L$^{-1}$) in aqueous medium limits extracellular recovery methods. The present study describes an extractive production system, using hydrophobic adsorbents for in situ oil recovery from the aqueous phase and attempts to modify fluxes of stored oil into the aqueous environment. Three polystyrene and two organo-silica-based capturing agents demonstrate an increase in oil recovery rate. The oil efflux rate from the cells complemented the substrate uptake rate proves to be a positive inducer for increased oil production. With SEPABEADS SP70 and Osorb adsorbents, the wild strain of Y lipolytica resulted in theoretical oil yields of 0.3 g g$^{-1}$ over complete consumption of 5% glucose. These OCAs effect a total oil production of about 80% DCW with the oil productivity of 0.11 g L$^{-1}$ hour$^{-1}$, suggesting a methodology wherein, concomitant nondisruptive oil recovery from the cells and extracellular broth can be used for continuous sequestration of oil from nonconventional yeasts (Y lipolytica). The developed process thus offers a model set up for extractive production of a wide range of renewable oleo-chemicals through fermentation and for other hydrophobic products.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Pratik P. Pawar contributed to the conceptualization, data curation, formal analysis, methodology, writing of the original draft, review, and editing. Rajeshkumar N. Vadgama contributed to the conceptualization, supervision, validation, visualization. Arvind M. Lali contributed to the project administration, supervision, visualization. Annamma A. Odaneth contributed to the conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, supervision, visualization, writing of the original draft, review, and editing.

DATA ACCESSIBILITY

The data used to support the findings of this study are available from the corresponding author upon request.
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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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