Continuous Aqueous Two-Phase System Extraction using Oscillatory Flow Reactor

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Motivation

Aqueous two-phase extraction (ATPE) have been used for a half a century, but its industrial implementation have been poor. However, with the continuous processes taking place in biotechnology industry

– very fast reaching the equilibrium;

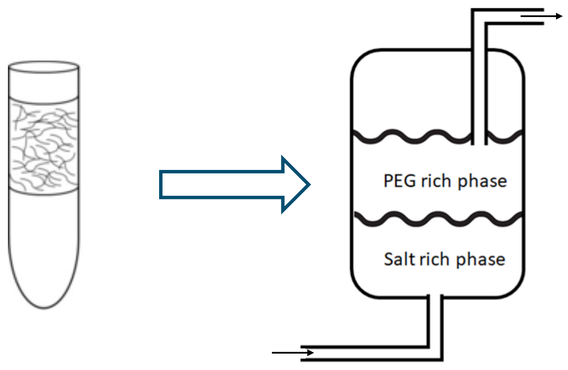
– high biocompability;

– low cost;

– recycling phase forming components;

– easy to scale up;

– prone to process integration.



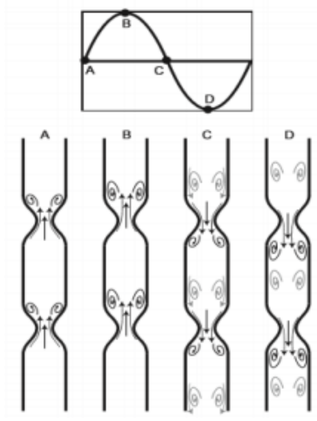
Oscillatory flow reactor (OFR) can be the winning design for continuous ATPE, since it has been proved as significant enhancement in processes such as heat transfer, mass transfer, particle mixing and separation, and it overcomes the main disadvantages of the previous column contactors used for this type of liquid-liquid extraction.

Goal: proof-of-concept of OFR as continuous ATPE extractor using pure myoglobin, comparing the yields obtained with the batch assays

Background

Oscillatory flow reactor is a cylindrical column that contains spaced baffles and where the fluid is oscillated in the axial direction, at one or both ends, developing a uniform mixing created by the generation and cessation of eddies when flow interacts with the restrictions. The fluid moves across the reactor with an intensity controlled by the oscillation amplitude (x_0) and frequency (f).

This type of reactor has been used in processes such as: crystallization; flocculation; liquid-liquid reaction; polymerization.

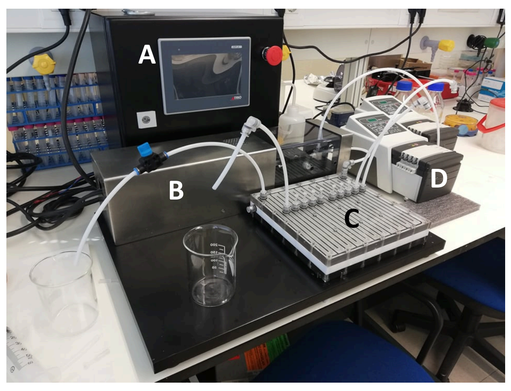


Inside **oscillatory flow reactor,** the fluids operate under plug flow, i.e., perfect mixing in the radial direction and all flow elements reside for the same length of time. These conditions mean that the residence time is the same for all fluid elements, which overtake the main problems faced by continuous ATPE processes – flooding, backmixing, emulsification – however, there is a lack of studies comparing this type of reactor with the batch ATPE.

In present work a modular oscillatory flow plate reactor (patent WO2017175207A1) was used. This technology is commercialized by OFRTech (Portugal).

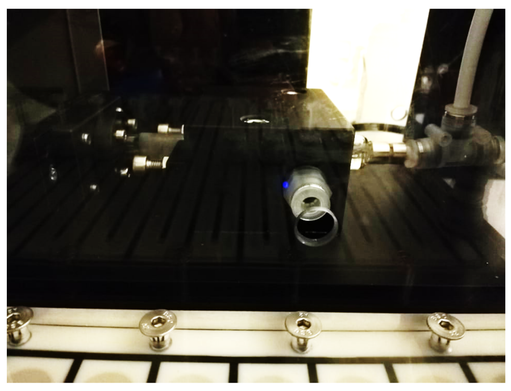
Materials and Results

General apparatus



OFR-Plate apparatus (OFRTech, Portugal): A- control unit; B-oscillatory unit; C- OFR-Plate; D - pumps

Syringe apparatus



– To oscillate the fluid in the reactor the syringe tip is linked to the first inlet of the reactor and the syringe piston to an linear motor, that control the oscillatory movement (frequency (f) and amplitude (x_0))

– Dimensions of the syringe: **internal diameter = 12.1 mm~~; (Volume = …)~~**

– Conditions used in the assays: **x_0 = 4 mm; f = 3 Hz**

Plate apparatus (patent WO2017175207A1)

Outlet: Aqueous two-phase system bottom phase

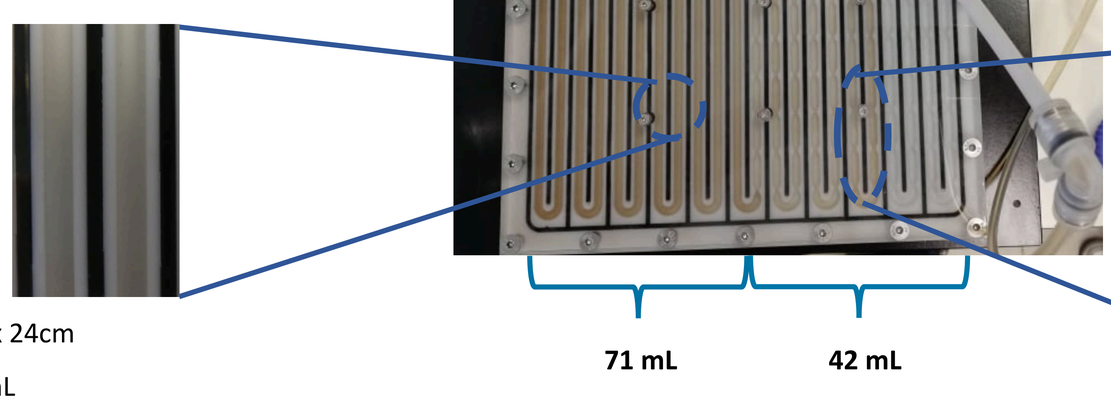
Outlet: Aqueous two-phase system top phase

Possible additional inlets and/or outlets

Inlet: Potassium phosphate (K_2HPO_4/KH_2PO_4) buffer, pH 7.4 40% (w/w)

Inlet: Polyethylene glycol (PEG) solution, 50% (w/w)

Inlet: Protein Sample (2 mg/mL of Myoglobin)



– No restrictions

– Phase separation

– Restrictions/baffles

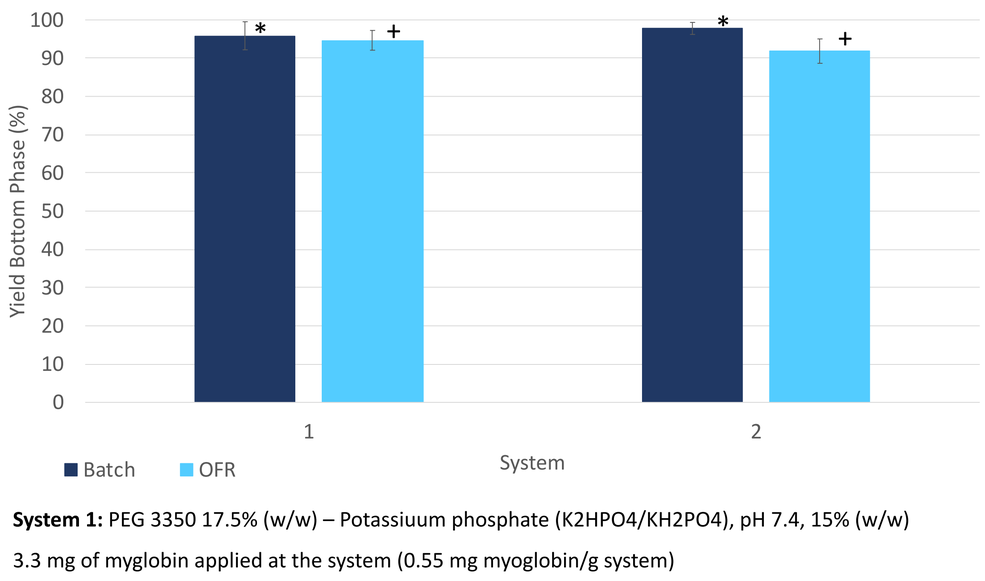
– Mixing of the phases

– Dimensions: D = 4 mm; d = 1.65mm; V = 0.344 mL

Plate dimensions: 24cm x 24cm

Total Volume: 113 mL

Results



System 1: PEG 3350 17.5% (w/w) – Potassiuum phosphate (K2HPO4/KH2PO4), pH 7.4, 15% (w/w)

3.3 mg of myglobin applied at the system (0.55 mg myoglobin/g system)

System 2: PEG 6000 20% (w/w) – Potassiuum phosphate (K2HPO4/KH2PO4), pH 7.4, 14% (w/w)

3 mg of myoglobin applied at the system (0.5 mg myoglobin/g system)

* **Batch ATPE** were performed in tubes, with a total mass of 6g

+ Oscillatory flow reactor ATPE were performed using a total mass flow of 30 g/min

The results were obtained with size exclusion chromatography analysis, using TSK gel® HPLC Column, measuring the absorbance at 280 nm

Myoglobin was the protein used in the assays, because its distinctive brown colour; it has a molecular weight of 17 kDa and isoelectric point between 6.8 and 7.3

✓ The partition of myoglobin was, statistically, the same in both batch and OFR aqueous two-phase system

✓ Fast reaching the steady state: 2 – 3 renovations ≡ 8 – 13 minutes

✓ The formation of the phases occurs inside the reactor: in the case of the system 2, in the first exit was possible to recover 50% of the top phase in the first exit (in the system 1, the recovery of the phases were done outside)

✓ No one of the problematic phenomena – flooding, backmixing, emulsification – were observed

– Volume ratio of the aqueous two-phase system in the OFR was slightly higher comparing with the batch ATPE, however more studies are needed in order to prove if this is constant in this type of continuous reactor

Conclusions

→ Conditions inside oscillatory flow reactor are shown to be suitable for continuous aqueous two-phase extraction, overtaking problems described in previous designs, and with the advantage of fast reaching steady state

→ In our proof-of-concenpt approach, the yields of partition of myoglobin are statiscally the same in the batch and continuous assays

→ OFR will be ultimate goal of a scale-up process for continuous ATPE

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