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Potential application of perfusion and pertraction for in situ product removal in biocatalytic 2-phenylethanol production

Ivan Červeňanský, Mário Mihal', Jozef Markoš *

Institute of Chemical and Environmental Engineering, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, 812 37 Bratislava, Slovakia

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ABSTRACT

Bioproduction of 2-phenylethanol from L-phenylalanine using *Saccharomyces cerevisiae* is affected by strong product inhibition and thus the maximum reachable concentration in an ordinary batch or fed-batch bioreactor is only about 4 g L^{-1}. To minimize the effect of product inhibition and to prolong the production period, continual removal of 2-pehnylethanol can be applied. For product removal, adsorption in a fixed bed column can be used but to prevent the column from biomass pollution, a separation method for obtaining a cell free aqueous phase had to be employed before the adsorption step. In this work, the applicability of perfusion and pertraction for obtaining the cell free fermentation medium is studied. Performance of both processes was studied in a series of kinetic measurements with varying concentrations of biomass in a model solution. In case of pertraction, long term stability of supported liquid membrane created by octane was examined. Also, mathematical models of pertraction and perfusion have been verified with experimental measurements. In perfusion experiments, membrane fouling occurred due to the biomass present in feed phase. On the contrary, the biomass did not affect the mass transfer rate during the pertraction. Pertraction was found to be more stable since pores of the membrane are protected from fouling by octane which creates a supported liquid membrane. Octane also forms a barrier for other compounds found in the fermentation medium and can be used for selective transport of 2-phenylethanol to a cell free aqueous solution.

1. Introduction

Natural aromas are often compounds with higher added value; they are widely used in cosmetics, perfumery and food industry. An example of such compounds is e.g. 2-phenylethanol (PEA), which is a higher aromatic alcohol with rose like fragrance. Natural PEA can be produced in a reasonable extent only by a few yeast species utilizing PEA via the Ehrlich pathway with L-phenylalanine as a precursor of growth associated biotransformation [1]. Main limitation in natural PEA production is its strong inhibitory effect on the growth of biomass [2]. Majority of research is focused on the production of PEA using yeasts *Saccharomyces cerevisiae*, which can sustain concentrations of PEA up to 4 g L^{-1} [3]. Using genetic engineering production of PEA by manipulated *S. cerevisiae* can be enhanced and yeasts can produce up to 4.8 g L^{-1} of PEA in the batch mode [4]. However, the final product concentration is too low for a highly effective production. Therefore, the effect of product inhibition can be minimalized by an in situ product removal technique (ISPR) enhancing and intensifying the whole production process.

Over the years, many separation techniques have been studied for possible PEA removal during its bioproduction (Table 1). Extraction counts among the earliest techniques applied for PEA removal. Stark et al. [5] used oleic acid for the extraction of PEA from the fermentation medium but higher PEA productivity was reached in the work of Etschmann and Schrader [6], who used polypropylene glycol 1200 (PPG 1200) for the extraction. In both studies, the extraction solvent was added directly to the bioreactor and unwanted highly dispersed emulsions could be formed due to the intensive stirring [5]. Non-dispersive PEA extraction was studied in the work of Mihal' et al. [7], where the extraction solvent, pentane, was circulated through a hollow fiber membrane module and continual distillation had to be used for its continual regeneration due to low partition coefficient of PEA. Further increase in PEA productivity was reported using absorption used for its removal. Gao and Daugulis [8] used polymeric beads with high partition coefficient for PEA separation from the fermentation medium. The beads were placed directly in the bioreactor and in later experiments, in an external column. In both cases, the polymeric beads were in direct contact with fermentation medium containing biomass which

* Corresponding author.
E-mail addresses: ivan.cervenansky@stuba.sk (I. Červeňanský), mario.mihal@stuba.sk (M. Mihal'), jozef.markos@stuba.sk (J. Markoš).

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Nomenclature

a	specific area, $m^2\ m^{-3}$
A	area, m^2
c	concentration, $g\ L^{-1}$
d	diameter, m
D	diffusion coefficient of PEA, $m^2\ s^{-1}$
K	overall mass transfer coefficient, $m\ s^{-1}$
k	partial mass transfer coefficient, $m\ s^{-1}$
L	effective length, m
N_f	number of fibers
P	partition coefficient
r	radial coordinate, m
R	radius, m
Re	$= d_h u\rho/\mu$ Reynolds number
Sc	$= \mu/(\rho D)$ Schmidt number
Sh	Sherwood number
t	time, s
u	interstitial velocity, $m\ s^{-1}$
V	volume, L
$\dot{V}$	volumetric flow rate, $L\ s^{-1}$
z	axial coordinate, m

Greek symbols

ε	porosity
μ	viscosity, Pa s
ρ	density, $kg\ m^{-3}$
τ	tortuosity
δ	fiber wall thickness, m

Superscript

0	initial
E	equilibrium
v	vessel

Subscript

aq	aqueous
h	hydraulic
in	inner
ln	logarithmic mean
m	membrane
o	organic
out	outer
s	shell side
t	tube side
x	biomass

Abbreviations

ISPR	in situ product removal
PEA	2-phenylethanol
SLM	supported liquid membrane

might cause further technological inconveniences regarding sterility of the beads in their repeated used in the PEA production process. Another separation process studied for possible PEA removal during its production is adsorption. In the work of Mei et al. [9], after the basic adsorbent screening, the adsorbent with the highest adsorption capacity was used for PEA removal directly in the bioreactor.

The highest PEA productivity was reported by Wang et al. [10] in the system where adsorption was applied for ISPR. In their work, the production of PEA was performed by *S. cerevisiae* in a continuous bioreactor and its removal took place in an external adsorption column. To prevent the fixed bed adsorption column from clogging and pollution by biomass, a microfiltration unit was placed before the adsorption column. The microfiltration unit was the weakest part of the production system as, due to serious membrane clogging, the threshold inlet membrane pressure was reached after 63 h of cultivation and the whole biotransformation process had to be stopped.

Our previous experiences with membrane based solvent extraction has shown that by excluding the microfiltration, which was initially used in the designed ISPR system (working for 64 h) [11], it is possible to prolong the biotransformation reaching up to 80 h of production [7]. In case of adsorption used as an ISPR technique, it is not possible to entirely exclude microfiltration as a separation step and let the fermentation broth with cells flow through the adsorption column, but rather replace it with another separation approach. To prevent a membrane from serious clogging, concentration driven membrane processes are more favorable for obtaining the cell free aqueous phase than pressure driven processes like microfiltration.

Applicable concentration driven membrane processes with indistinguishable phases on both sides of the membrane are dialysis, perfusion and pertraction. Dialysis was defined by Moser [12] as a process in which solutes diffuse from a high concentration solution to a low concentration solution across a semipermeable membrane until equilibrium is reached. Nonporous membranes used in dialysis selectively allow low-molecular-weight molecules to pass while retaining those with higher molecular weight and cells [13]. If a porous membrane is used, solutes do not only diffuse from the high concentration solution to the low concentration one across the membrane, but also solutes may perfuse form one solution to another. Therefore, this process can be called perfusion and it is a combination of dialysis, as the process is mainly driven by the concentration difference, and microfiltration, as the solutes may perfuse through the porous membrane. The main advantage of perfusion compared to microfiltration is the slower rate of membrane fouling, since this process is not solely driven by the pressure difference.

Pertraction was defined by Schlosser and Kossaczký [14] as a process, where transport of solutes occurs between two liquid phases (feed and stripping solution) separated by a third liquid phase, which represents a liquid membrane. A supported liquid membrane (SLM) is created when a liquid membrane is kept in the pores of a support material by capillary forces [15]. Two

Table 1
Overview of separation techniques applied for PEA removal during its bioproduction.

Reactor regime	Production strain	Removal technique	Separation medium	Volumetric productivity [$g\ L^{-1}\ h^{-1}$]	Reference
Fed-Batch	*S. cerevisiae*	Extraction	Oleic acid	0.24	[5]
Fed-Batch	*K. marxianus*	Extraction	PPG 1200	0.33	[6]
Fed-Batch	*S. cerevisiae*	Membrane extraction	Pentane	0.27	[7]
Fed-Batch	*K. marxianus*	Absorption	Hytrel 8206	0.43	[8]
Batch	*S. cerevisiae*	Adsorption	Adsorbent D101	0.24	[9]
Continuous	*S. cerevisiae*	Adsorption	Adsorbent FD0816	0.90	[10]