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Research Article

Advanced downstream process design for recovery of 2-phenylethanol and 2-phenylethyl acetate after biotransformation

Tamara Janković, Adrie JJ Straathof and **Anton A Kiss**[*]

Abstract

BACKGROUND: 2-Phenylethanol (2-PE) and 2-phenylethyl acetate (2-PEAc) are valuable aroma compounds with growing market demands. As an alternative to conventional petrochemical production, more valuable natural forms of these chemicals can be obtained by biotransformation. Low product concentrations, resulting from significant product toxicity to microorganisms, and high boiling points of products complicate recovery process. *In situ* product recovery by liquid–liquid extraction can be used to increase bioprocess yield and productivity. However, the subsequent purification of 2-PE and 2-PEAc is challenging as a consequence of the multiple phases, high-boiling temperatures of main products, occurrence of remaining substrate and byproducts, and presence of microorganisms.

RESULTS: The main goal of this original work is to improve the competitiveness of the biotechnological production of 2-PE by using *in silico* methods to develop an advanced industrial process for the final purification after centrifugation. An adaptable dividing-wall column was designed to remove 2-PE with 2-PEAc from organic phase or to esterify 2-PE to pure 2-PEAc. The production flexibility of the developed process allows adjustability to market demand. Additionally, recovery of co-produced ethanol from aqueous phase can increase the economic and environmental performance of the developed process. As confirmed by detailed techno-economic analysis, the proposed processes can cost-effectively (total recovery costs of 0.64–0.72 US $/kg$_{2\text{-PE/2-PEAc}}$) and energy-efficiently (primary energy requirements of 1.83–2.05 kW$_{th}$h/kg$_{2\text{-PE/2-PEAc}}$) recovery of 2-PE or 2-PEAc after biotransformation.

CONCLUSION: The developed process enhances economic and environmental viability of biotechnological 2-PE production by reducing costs and energy requirements, while ensuring flexibility to adapt to market demands.
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Supporting information may be found in the online version of this article.

Keywords: 2-phenylethanol; 2-phenylethyl acetate; downstream processing; distillation; dividing-wall column; reactive distillation

INTRODUCTION

Nowadays, the usage of fragrances has become widespread, with countless scents and aromas becoming an integral part of daily life. Among them, 2-phenylethanol (2-PE) stands out as a valuable aromatic alcohol with a rose scent. It has a broad range of applications in various industries, including food and beverage, cosmetics, perfumes and pharmaceuticals. It is commonly added as an organoleptic enhancer in many food products (e.g. ice creams, puddings, chewing gums, cookies, candies), and often used in aromatherapy, production of antiseptics, preservatives, cleaning agents and personal care products.[1] Lastly, 2-PE can be used for the synthesis of different valuable products (e.g. styrene, 2-phenylethyl acetate, phenylacetaldehyde, phenylacetic acid, diphenyl ether).[2] Among these components, 2-phenylethyl acetate (2-PEAc) is a valuable aroma component with a similar rose scent. In a similar way to 2-PE, 2-PEAc is often used as a flavoring agent[3,4] in the production of perfumes, cosmetics, personal care products, fragrances, cleaning products, waxes, polishes and biocidal products.[5]

Global production of 2-PE has been continuously increasing (e.g. from 7000 t in 1990 to 10 000 t in 2010).[6] Its global market has been estimated to be >US$255 million (M) in 2021, of which US$130M is the market for natural 2-PE, with an expected annual growth of >5.5% between 2022 and 2028.[7] Currently, most 2-PE is produced through three dominant petrochemical processes: Friedel–Craft reaction of ethylene oxide and benzene, catalytic reduction of styrene oxide, and oxidation of propylene with

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2-phenylethyl hydroperoxide.[6] 2-PEAc is usually produced from 2-PE by esterification with acetic acid or by transesterification with acetate esters. Conventional processes depend on fossil hydrocarbon but lead to low prices, in the order of magnitude of 20–30 US $/kg.[6] Alternatively, 2-PE also can be extracted from essential oils of different flowers (most often roses, but also jasmine, lilies, etc.). However, these processes are expensive (≈1000 US$/kg) and cannot satisfy market demand.[8] Additionally, 2-PE can be obtained as a byproduct in the production of free fatty acids from grape seed oil,[9] but the obtained quantities would be insufficient to meet market requirements. The high demand for 2-PE in food, cosmetics and pharmaceuticalshas led to growing interest in natural production methods for these compounds. Depending on the specific regulatory definitions, the final products obtained through different biotechnological processes might be classified as of natural origin.[1] Consequently, production of 2-PE by biotransformation has been a focus of research.[1,6,8,10,11] Many different pathways were studied, whereby yeast bioconversion of L-phenylalanine through the Ehrlich pathway is the dominant one.[10,12,13] Nonetheless, significant improvements are still needed to make bioprocesses economically attractive for large-scale production of this component. A major drawback that hinders scale-up of 2-PE bioconversion is product inhibition which results in low product concentrations (<1 wt%).[14] Thus, an effective downstream process is crucial for the effectiveness of the whole bioprocess. In situ product removal techniques (ISPR) have been actively studied to increase production yields. In that respect, in situ liquid–liquid extraction has been especially effective.[13] This technique implies a multiphase system in which the product is produced by microorganisms in an aqueous phase and simultaneously extracted into solvent. Consequently, the product concentration in the aqueous phase remains modest and inhibition effects can be mitigated.[6] Generally, this method has been widely used in flavor production owing to its simplicity, scalability and reasonable costs.[14] Moreover, liquid–liquid extraction has been studied as ISPR technique for the recovery of volatile fermentation products (e.g. alcohols).[15] However, this method has certain limitations, such as the need to select a solvent that is immiscible with water and nontoxic to microorganisms, as well as challenges in efficiently separating the desired product from the solvent. Alternative ISPR approaches include membrane-assisted solvent extraction, adsorption, solvent immobilization and organophilic pervaporation.[8] Membrane-assisted solvent extraction can reduce the risk of emulsion formation, but membranes can be prone to fouling from broth components and are often custom-made, making them costly for large-scale applications.[16,17] Adsorption using hydrophobic resins ensures high product purity,[18,19] but it can suffer from low adsorption capacity and potential interference from impurities in the fermentation broth. Solvent immobilization can mitigate solvent toxicity concerns but comes with increased costs and system complexity.[8] Organophilic pervaporation is another promising ISPR method but shares similar membrane-related challenges with membrane-assisted solvent extraction.[14] Considering its simplicity, scalability and potential for continuous operation, liquid–liquid extraction was chosen as the ISPR method in this work, with subsequent recovery steps designed accordingly. Solvents that have been investigated for the in situ removal of 2-PE by liquid–liquid extraction include oleic acid,[20,21] oleic alcohol, polypropylene glycol[13] and recently ionic liquids (1-hexyl-methylmor-pholinium bis(fluorosulfonyl)imide, N-octylisoquinolinium bis(fluorosulfonyl) imide, 1-butyl-1-methylpyrrolidinium bis(fluorosulfonyl)imide and

N-triethyl-N-octylammonium bis(fluorosulfonyl)imide).[22] As a result of its high extraction power (partition coefficient > 30 for 2-PE), biocompatibility[23] and safety, polypropylene glycol 1200 (PPG) has been suggested as a promising solvent to effectively remove 2-PE and 2-PEAc from the aqueous phase. A fed-batch process using PPG for in situ product recovery resulted in 26.5 g L^{-1} 2-PE and 6.1 g L^{-1} 2-PEAc in the organic phase.[13] After ISPR by liquid–liquid extraction, additional downstream processing steps (solvent recovery and final product purification) are required to obtain high-purity 2-PE product from the final bioreactor content.[6] To the best of our knowledge, this has not been addressed nearly as promptly as the metabolic engineering or design of the fermentation process with ISPR.

Therefore, the main goal of this original research is to develop an advanced final purification process for industrial-scale production of 2-PE by biotransformation, using in silico methods. As downstream processing costs often contribute substantially to the overall production costs and energy usage for specialty chemicals,[24,25] the results of this study can significantly advance the development of viable industrial bioproduction of flavors and fragrances. Consequently, the proposed downstream process can reduce overall production costs for natural aroma compounds, making them more competitive with petrochemical alternatives.

MATERIALS AND METHODS

Composition of feed streams for the final recovery process was determined based on reported experimental data[13] for a 30-h fed-batch biotransformation process with ISPR using liquid–liquid extraction. Kluyveromyces marxianus CBS200 was employed to produce 2-PE and 2PEAc from L-phenylalanine (L-Phe) through biotransformation, whereas PPG was used as a solvent in the liquid–liquid extraction process.[13] The addition of the precursor (L-Phe) from the beginning, continuous addition of glucose and in situ product removal (volume ratio of solvent and aqueous phase of 1:1) resulted in high product-to-substrate molar yields of the biotransformation process: 0.82 mol$_{2-PE}$/mol$_S$ and 0.13 mol$_{2-PEAc}$/mol$_S$.[13] Following the in situ product recovery, centrifugation is needed to separate biomass, organic and aqueous phases before the final purification. The costs of this process were included in the techno-economic assessment of the solvent recovery and final purification processes. Furthermore, saturation of the organic phase with water and the aqueous phase with organic solvent was assumed (see Supporting information, section 1). Thus, the composition of the organic phase is as follows: 95.99 wt% PPG, 2.49 wt% 2-PE, 0.94 wt% water and 0. 57 wt% 2-PEAc (stream 1a or 1b in Table 1). The composition of the aqueous feed stream is as follows: 95.73 wt% water, 1.72 wt% ethanol, 0.96 wt% L-Phe, 0.96 wt% PPG, 0.48 wt% glucose, 0.13 wt% carbon dioxide (CO_2) and 0.03 wt% 2-PE (stream 1c or 1d in Table 2). Even though ethanol and CO_2 were not reported in the organic phase, if present, they would end up in the top aqueous product from the distillation column (see the '2-Phenylethanol recovery from the organic phase section' below) and not threaten product purity. Both liquid streams enter the final recovery section at 1 bar and 30 °C (biotransformation conditions) and the mass ratio of aqueous phase to organic liquid is 1.62.

Distillation was chosen as the main recovery technique because it is already a mature separation technology that can be easily operated on an industrial scale. Furthermore, various heat

Table 1. Compositions and conditions of the main process streams for 2-PE recovery from the organic phase for cases 1 and 2 [see Fig. 2(a),(b)]

Stream	1a	2a	3a	4a	5a	6a	7a	8a
Temperature (°C)	30.0	40.0	31.2	31.2	31.2	133.7	30.0	30.0
Pressure (bar)	1.000	1.000	0.053	0.053	0.053	0.100	1.000	1.000
Flowrate (kg h^{-1})	10 025	9629	160	95	65	301	249	52
Mass fractions								
PPG	0.9600	0.9995	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
2-PE	0.0249	0.0000	0.3875	0.9426	0.0042	0.8271	0.9991	0.0037
2-PEAc	0.0057	0.0005	0.0011	0.0028	0.0000	0.1729	0.0009	0.9963
Water	0.0009	0.0000	0.6113	0.0546	0.9958	0.0000	0.0000	0.0000
Stream	**1b**	**2b**	**3b**	**4b**	**5b**	**6b**	**7b**	
Temperature (°C)	30.0	40.0	34.0	34.0	34.0	30.0	30.0	
Pressure (bar)	1.000	1.000	0.053	0.053	0.053	1.000	1.000	
Flowrate (kg h^{-1})	10 025	9629	138	1	138	129	387	
Mass fractions								
PPG	0.9600	0.9995	0.0000	0.0000	0.0000	0.0000	0.0000	
2-PE	0.0249	0.0000	0.3875	0.2699	0.0034	0.0000	0.0002	
2-PEAc	0.0057	0.0005	0.0011	0.6105	0.0011	0.0000	0.9998	
Water	0.0009	0.0000	0.6113	0.0824	0.9506	0.0000	0.0000	
Acetic acid	0.0000	0.0000	0.0000	0.0000	0.0449	1.0000	0.0000	

Table 2. Compositions and conditions of the main process streams for ethanol valorization from the aqueous phase for options A and B [see Fig. 2(c),(d)]

Stream	1c	2c	3c	4c	5c	6c	7c				
Temperature (°C)	30	38.2	68.2	68.2	25.0	25.0	30.0				
Pressure (bar)	1.000	1.312	1.000	1.000	1.000	1.000	1.000				
Flowrate (kg h^{-1})	16 282	15 966	252	63	41	22	294				
Mass fractions											
PPG	0.0096	0.0098	0.0000	0.0000	0.0000	0.0000	0.0000				
2-PE	0.0003	0.0003	0.0000	0.0000	0.0000	0.0000	0.0000				
Water	0.9572	0.9751	0.0548	0.0355	0.0524	0.0038	0.0544				
Ethanol	0.0172	0.0000	0.9434	0.6369	0.9365	0.0749	0.9414				
L-Phe	0.0096	0.0098	0.0000	0.0000	0.0000	0.0000	0.0000				
Glucose	0.0048	0.0049	0.0000	0.0000	0.0000	0.0000	0.0000				
CO_2	0.0013	0.0000	0.0019	0.3276	0.0111	0.9214	0.0032				
Ethylene glycol	0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000				
Stream	**1d**	**2d**	**3d**	**4d**	**5d**	**6d**	**7d**	**8d**	**9d**	**10d**	**11d**
Temperature (°C)	30	38.1	68.9	68.9	25.0	25.0	62.9	60.0	43.4	156.4	30.0
Pressure (bar)	1.000	1.312	1.000	1.000	1.000	1.000	1.000	1.100	1.000	1.248	1.000
Flowrate (kg h^{-1})	16 282	15 956	261	65	44	22	305	366	279	391	25
Mass fractions											
PPG	0.0096	0.0098	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
2-PE	0.0003	0.0003	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Water	0.9572	0.9752	0.0851	0.0524	0.0760	0.0051	0.0838	0.0000	0.0005	0.0649	0.9995
Ethanol	0.0172	0.0000	0.9129	0.6316	0.9109	0.0713	0.9126	0.0000	0.9952	0.0000	0.0001
L-Phe	0.0096	0.0098	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Glucose	0.0048	0.0049	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
CO_2	0.0013	0.0000	0.0020	0.3160	0.0131	0.9237	0.0036	0.0000	0.0000	0.0000	0.0000
Ethylene glycol	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000	0.0000	0.9351	0.0004

integration and process intensification can be implemented to significantly reduce distillation energy requirements.[26] The primary focus was on 2-PE recovery from the organic phase. However, recovery of ethanol also was considered[27] owing to its significant amount in the aqueous phase (≈18 g L^{-1}).[13] Process

intensification principles were implemented to improve the energy efficiency of the developed downstream process. Rigorous simulations for every process operation in the final purification process were developed in ASPEN PLUS. NRTL-HOC (Non-Random Two Liquid model with Hayden-O'Connell extension for vapor

phase) was used to properly describe the complex interactions between components.[28] As a consequence of the substantial contribution of energy cost to the total recovery costs, the downstream process was designed to minimize energy requirements (and consequently utility costs). The performance of the developed recovery process was evaluated by analyzing economic and environmental impact following the published procedures.[29,30] Furthermore, to assess the performance of the total recovery process, centrifugation costs were roughly estimated from the market data[31] and published literature.[29] More details about the methodologies are presented in the Supporting information (sections 4 and 5).

RESULTS AND DISCUSSION

The part of 2-PE production process that was designed in this work is presented in Fig. 1 by green dashed boxes. Flowsheets of the developed processes are presented in Fig. 2, whereas the conditions and compositions of the main process streams are given in Tables 1 and 2.

2-Phenylethanol recovery from the organic phase

The boiling points (at 1 bar) of components present in the organic phase after biotransformation were 100 °C for water, 219 °C for 2-PE, 240 °C for 2-PEAc[32] and > 280 °C for PPG.[33] Thus, water, 2-PE and 2-PEAc can be evaporated from higher boiling PPG. However, both 2-PE and 2-PEAc form heterogeneous azeotropes with water (7.02 wt% 2-PE and 92.98 wt% water, and 3.42 wt% 2-PEAc and 96.58 wt% water) and liquid–liquid splitting should be coupled with distillation to obtain high-purity products. Owing to the relatively high boiling temperatures, vacuum distillation was chosen to allow steam usage for heating reboilers and avoid any decomposition risks. Moreover, process intensification through a dividing-wall column (DWC) was implemented to decrease the energy requirements (see Fig. 3; further explanation

given in the following sections). Two alternatives were considered for recovering biotransformation products from the organic phase. **Case 1** implies recovery of high-purity 2-PE and 2-PEAc as a byproduct. **Case 2** involves the esterification of 2-PE with acetic acid to high-purity 2-PEAc. Both cases allow solvent recovery and reuse in the *in situ* product recovery. The same DWC design can be used in both scenarios, with adjustments in Case 2 (e.g. addition of catalyst and a feed stream for acetic acid). The process adaptability provides valuable flexibility and enables easy adjustments in response to market demand.

Case 1: purification of 2-phenylethanol

In order to effectively separate 2-PE and 2-PEAc from PPG and water dissolved in PPG, a sequence of two distillation columns can be used. High-purity PPG can be obtained as a bottom product from the first column, whereas the second column can be used to separate the mixture of 2-PE and 2-PEAc (as the bottom product) from the remaining water (the top product). Additionally, liquid–liquid splitting of the top product in a decanter should be included to achieve concentrations of 2-PE and 2-PEAc beyond their azeotrope composition with water. Alternatively, these two columns can be merged into one thermodynamically equivalent DWC with a common overhead and dividing bottom section (see Fig. 3). This highly integrated configuration is built of one column shell, one condenser and two reboilers. Besides reducing equipment costs compared to the simple sequence of distillation columns, DWC can often improve the energy efficiency of the separation.[34] Because the DWC unit is not available off-the-shelf in Aspen Plus, it was simulated as a thermodynamically equivalent sequence of distillation columns (see Fig. 3). DWC_L and DWC_R stand for the left and right sides of DWC, respectively. The top vapor from DWC_L goes to DWC_R, whereas part of the liquid flowing down in DWC_R is redirected to DWC_L to provide sufficient liquid flow in this side of DWC. The number of stages, feed stage location, bottom-to-feed ratio, distillate-to-feed ratio, reflux
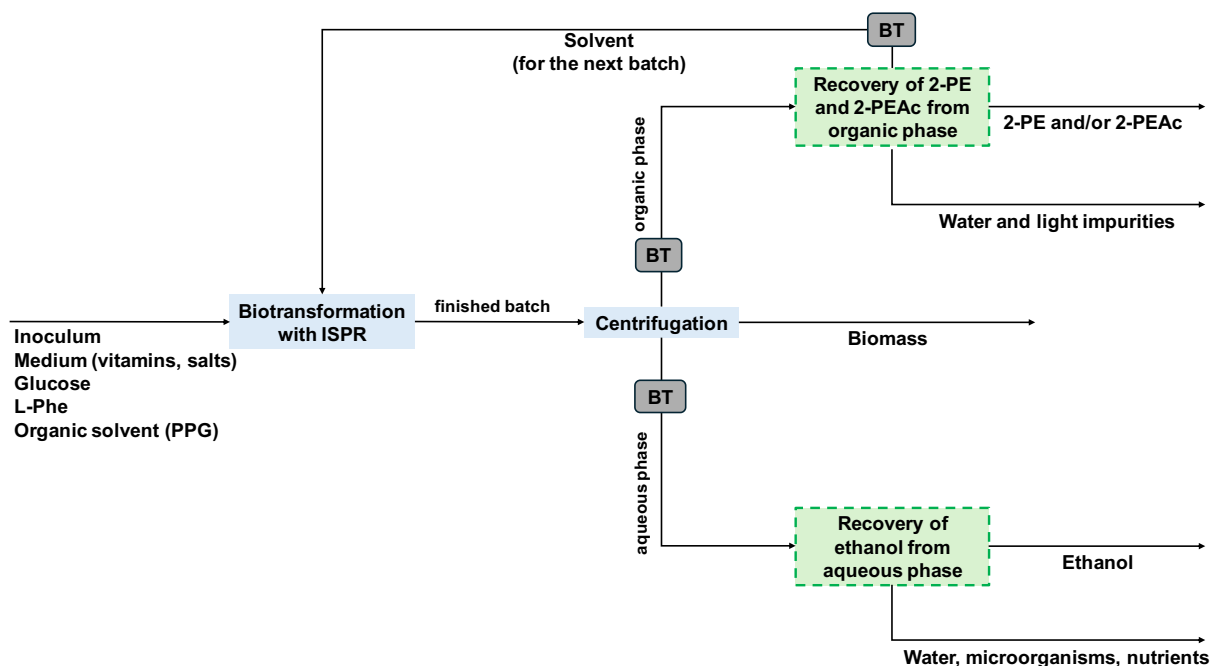

Figure 1. Block flow diagram of 2-PE production process; the focus of this study is presented by green boxes with a dashed outline; ISPR stands for *in situ* product recovery; BT stands for the buffer tanks.
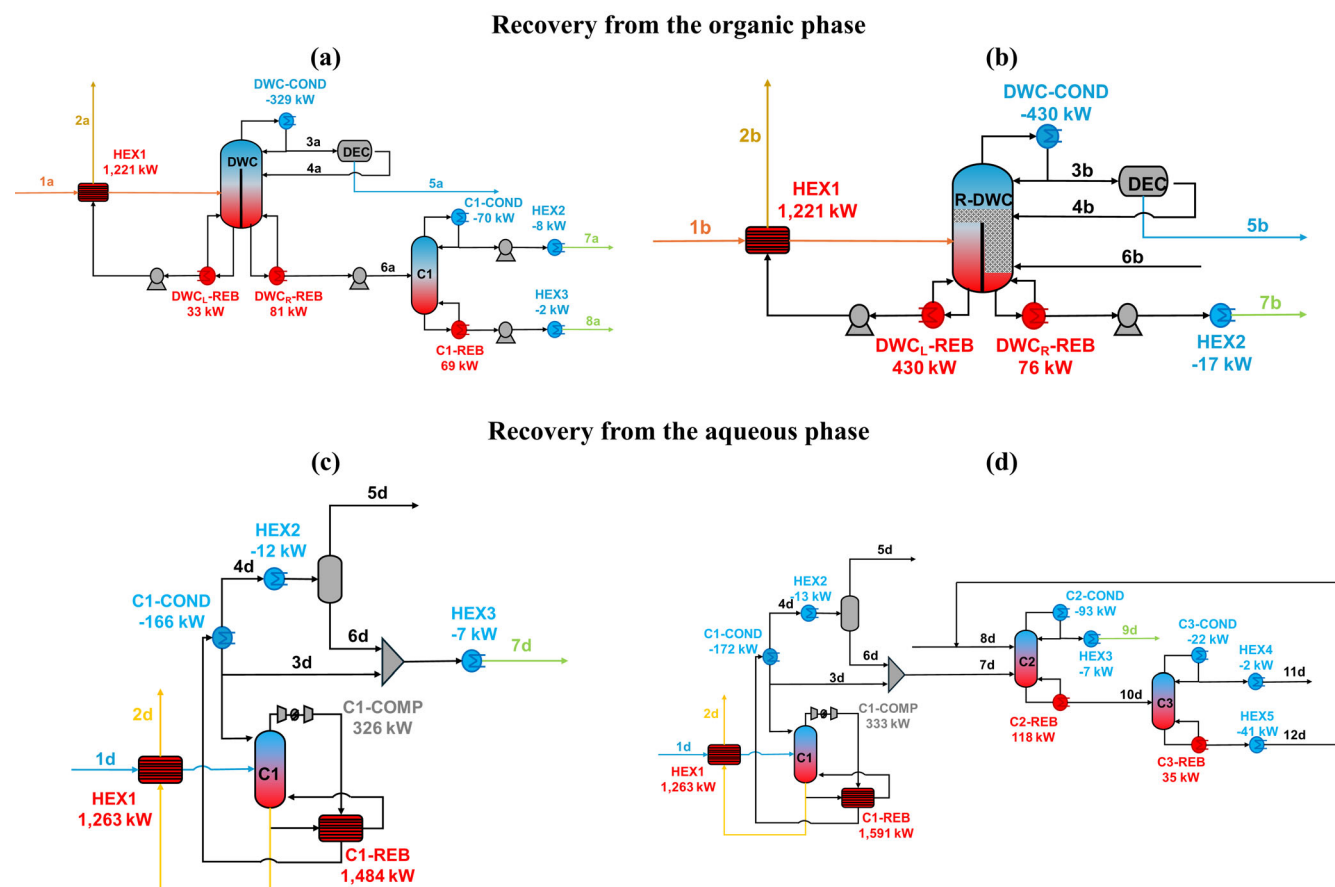
Recovery from the organic phase

Recovery from the aqueous phase

Figure 2. Flowsheets of the recovery processes: (a) Case 1 for organic phase, (b) Case 2 for organic phase, (c) Option A for aqueous phase and (d) Option B for aqueous phase. Compositions and conditions of the numbered process streams are presented in Tables 1 and 2.

ratio and liquid split were varied to minimize energy requirements while allowing separation of high-purity products. More details about the design of DWC are presented in the Supporting information (sections 2 and 3). In short, DWC has 20 stages in total (in ASPEN PLUS, condenser and reboiler are the 1st and last stage, respectively), whereby the wall is placed at the bottom 15 stages (14 stages excluding reboiler). The operating pressure of 0.053 bar (in the condenser) was selected to ensure that steam can be used in reboilers and cooling water in condenser. Structured packing type Mellapak 250X was chosen to minimize pressure drop (0.225 mbar per theoretical stage) in the column.[35]

Finally, pure PPG (> 99.9 wt%) is recovered as the bottom product from DWC_L and can be reused for *in situ* product recovery. The top product from DWC_R is sent to the decanter for phase-splitting. The formed light phase, which can be used upstream, contains 99.6 wt% water and 0.4 wt% 2-PE; however, the heavy phase from the decanter is sent back to DWC_R. The bottom product of DWC_R is a mixture of 2-PE and 2-PEAc (82.7 and 17.3 wt%, respectively). An additional purification step in distillation column C1 [see Fig. 2(a)] is needed to recover high-purity 2-PE (99.9 wt%) and 2-PEAc (99.6 wt%) as top and bottom products. Additionally, as PPG needs to be cooled before being reused, it can preheat the feed stream to DWC [HEX1 in Fig. 2(a)]. This heat integration reduces the energy requirements of DWC by >74%. Compared to the simple sequence of distillation columns, DWC implementation reduced energy requirements by ≈28% (both options with heat integration). In total, ≈1.388 kW$_{th}$h/kg$_{2-PE/2-PEAc}$ is needed to

recover high-purity 2-PE (99.9 wt%) and 2-PEAc (99.6 wt%). Considering the biotransformation yields, and the product recoveries (99.9% for 2-PE and 90.8% for 2-PEAc), the total molar substrate-to-product yields would be 81.8% for 2-PE and 13.6% for 2-PEAc. Additionally, >99.9% of PPG can be reused for *in situ* product recovery.

Case 2: esterification of 2-phenylethanol to 2-phenylethyl acetate

An alternative to recovering pure 2-PE is to transform it into 2-PEAc by esterification with acetic acid, according to reaction: 2-PE + HAc ⇌ 2-PEAc + H$_2$O. Amberlyst 15 can effectively catalyze this reaction.[36,37] Kinetic data for the esterification and hydrolysis reactions were obtained from literature[37] and adapted to the power law model used in ASPEN PLUS. The pre-exponential factors were expressed per kg of catalyst, whereas molarity was used as basis for rate expressions. Liquid phase was defined as the reactive phase. Kinetic parameters are provided in Table 3.

The previously developed DWC was transformed into a reactive (R)-DWC (see Fig. 3). By integrating separation and reaction into a single equipment unit, reactive distillation offers major advantages over conventional technologies.[38] Similar to Case 1, PPG is recovered as the bottom product from R-DWC$_L$. However, in this case, the esterification reaction is performed in R-DWC$_R$. Position of acetic acid feed stage, reactive zone and catalyst amount were varied according to the literature recommendations[39] to ensure high purity 2-PEAc product and minimize impurities in water

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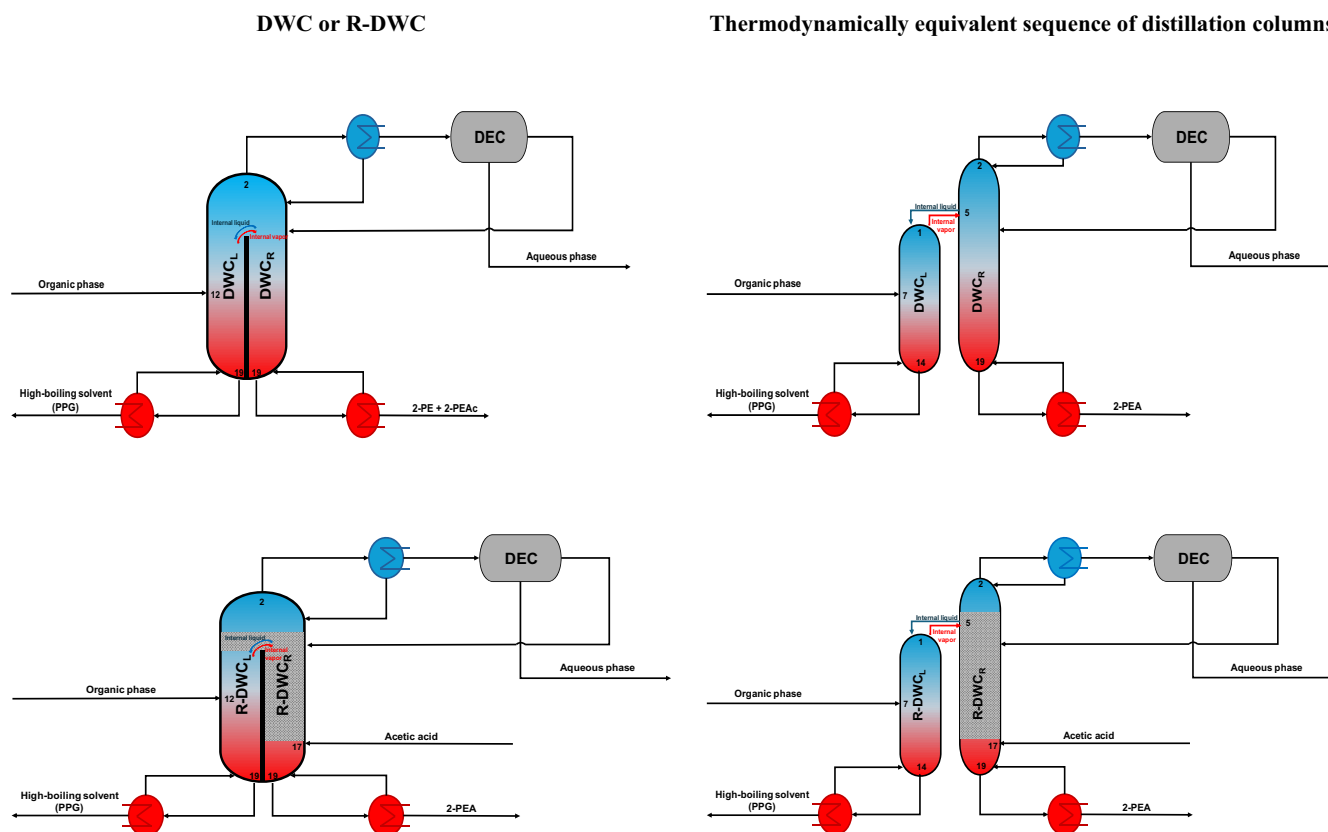
Figure 3. Design of dividing-wall column (DWC) and reactive dividing-wall column (R-DWC) and the equivalent sequences of distillation columns.

Table 3. Kinetic parameters of the esterification and hydrolysis reactions

Equilibrium reaction	Reaction type	Pre-exponential factor $((m^3)^2/(kmol\ kg_{cat}\ s))$	Activation energy $(J\ mol^{-1})$
2-PE + HAc $\rightleftharpoons$	Esterification	0.147566	32 722.68
2-PEAc +H_2O	Hydrolysis	0.000331	49 292.68

distillate stream. Finally, acetic acid is fed to the 17th stage of R-DWC$_R$, whereas catalyst is placed from the 3rd–16th stage of R-DWC$_R$. As concluded previously, the reaction is kinetically controlled and large amounts of catalysts (maximum allowable catalyst amount per stage) are needed to ensure sufficient conversion (>99%).[37] Hence, 30 kg Amberlyst 15 is placed on the 3rd–5th stages and 20 kg on the 6th–16th stages, equal to 13% and 27% of the available volume per stage, respectively. Finally, high-purity 2-PEAc (> 99.9 wt%) is obtained as the bottom product from R-DWC$_R$. As in Case 1, the top product from R-DWC$_R$ is sent to the decanter. The heavy phase is returned to R-DWC$_R$, whereas the light phase contains water (95.1 wt%) and acetic acid (4.5 wt%) with small amounts of remaining 2-PE (0.3 wt%) and 2-PEAc (0.1 wt%). As a worst-case scenario, it was assumed that this stream would be sent to the wastewater treatment and related costs are included. As in Case 1, high-temperature PPG bottom product was used to preheat the feed stream to R-DWC [HEX1 in Fig. 2(b)] resulting in >70% reduction in energy requirements. Thus, ≈1.320 kW$_{th}$h/kg$_{2-PEAc}$ is needed to esterify 2-PE and

recover high-purity 2-PEAc (>99.9 wt%). Considering the previously mentioned biotransformation yields, and the total molar substate-to-product (2-PEAc) yield would be 96.7%. Furthermore, >99.9% of PPG (99.9 wt%) can be reused in the biotransformation process.

Ethanol recovery from the aqueous phase

In addition to 2-PE recovery from the organic phase, ethanol recovery from the aqueous phase was considered. Ethanol is a bulk chemical with a market price significantly (>100-fold) smaller than those of 2-PE and 2-PEAc. Additionally, the production capacity of ethanol in this process is relatively small (≈2.3 kt year^{-1}). After centrifugation, the aqueous phase is commonly sent for wastewater treatment. Hence, if ethanol is not recovered, additional wastewater treatment costs would need to be paid for this stream. Furthermore, as this phase contains some valuable nutrients, ethanol separation allows its reuse in the bioreactor. However, this requires additional testing before implementation. Thus, sending the aqueous phase after ethanol separation to the wastewater treatment was considered as the worst-case scenario.

Ethanol forms a light homogeneous azeotrope with water (95.57 wt% ethanol and 4.43 wt% water, 78.15 °C at 1 bar). Hence, a simple distillation can separate only the azeotropic mixture, whereas a more complex dehydration step is required to obtain a high-purity product. To compare these alternatives, two options were considered for the ethanol recovery: **Option A** – separation of hydrous (95 vol%) and **Option B** – separation of anhydrous ethanol (99.5 wt%). In both cases, initial separation of ethanol (with some water) from most of the aqueous phase is performed in distillation column C1 [Fig. 2(c),(d)]. Bwaottom product from this

column contains most of the water (97.5 wt%), L-Phe (1.0 wt%), PPG (1.0 wt%) and glucose (0.5 wt%). This stream can heat the feed stream to column C1 [HEX1 in Fig. 2(c),(d)] to reduce the reboiler duty by 44–46%. Use of a partial vapor–liquid condenser was assumed for this column to remove the remaining CO_2. As some ethanol is separated with vapor distillate, cooling and simple phase splitting was designed to minimize ethanol loss. Condensed ethanol is mixed with liquid distillate from column C1 and makes the final hydrous ethanol product stream in Option A [stream 7c in Fig. 2(c) – 95.3 vol% ethanol, 4.4 vol% water and 0.3 vol% CO_2]. However, the top product from column C1 in Option B contains ≈91 wt% ethanol [stream 7d in Fig. 2(d)] as this concentration has been proven to minimize total energy requirements for the preconcentration and final purification steps in ethanol recovery.[40] Final purification was performed by extractive distillation with ethylene glycol [column C2 in Fig. 2(d)] with a solvent-to-feed mass ratio of 1.2.[27] High-purity ethanol product (99.5 wt%) is obtained as the distillate of column C2, whereas the ethylene glycol–water mixture is sent to the solvent recovery [column C4 in Fig. 2(d)]. Pure water (99.8 wt%) is separated as top product of this column, whereas ethylene glycol (> 99.9 wt%) is recovered as bottom product and can be reused in the extractive distillation column C2. Distillation columns C1, C2 and C3 operate at atmospheric pressure and sieve trays with a pressure drop of 8 mbar per tray were assumed.[41] More details about the design of distillation columns are presented in the Supporting information (section 2). Because the temperature difference between the top and the bottom of column C1 is not significant (≈20 °C), a mechanical vapor recompression (MVR) can be applied to reduce energy requirements. This heat pump system implies compressing the top vapor from the distillation column and using hot compressed vapor to evaporate the bottom liquid (instead of heating utility in the reboiler).[42] Implementing the MVR system reduces the energy requirements of column C1 by >78%. Another alternative is not to separate ethanol and to send the whole aqueous phase to the wastewater treatment. This option is referred to as **Option C** and was used in the economic analysis.

Economic analysis

The total costs for separating ethanol from the aqueous phase are 0.604 US$/kg$_{EtOH}$ when hydrous ethanol is recovered (Option A) or 0.851 US$/kg$_{EtOH}$ when anhydrous ethanol is recovered (Option B). Comparing these costs with the market prices of hydrous and anhydrous ethanol (0.64 US$/kg and 0.80 US$/kg, respectively),[43] shows that only recovering hydrous ethanol from the aqueous stream (Option A) is marginally profitable. Further dehydration is more expensive owing to the required capital and operating costs for two additional process steps (extractive distillation and solvent recovery) and the relatively small process scale for producing bulk chemical such as ethanol. Because recovering hydrous ethanol may be only marginally profitable, further comparison of the total recovery costs in cases 1 and 2 was performed assuming that the whole aqueous stream is sent to the wastewater treatment (the worst-case scenario) and related costs were included. Results of the economic analysis are presented in Table 4 and Fig. 4. For simplicity, specific costs are expressed per kg of recovered 2-PE and/or 2-PEAc. More details about the economic analysis are presented in the Supporting information (section 4).

Thus, the total recovery costs when recovering 2-PE as the main product and 2-PEAc as a by-product (0.643 US$/kg$_{2-PE/2-PEAc}$) are slightly lower compared to when esterification is performed and

Table 4. Key performance indicators in terms of economics and sustainability when recovering 2-PE and 2-PEAc (Case 1) or 2-PEAc (Case 2) from the organic phase while sending the aqueous phase to the wastewater treatment[a]

	Case 1	Case 2
Products		
2-PE (ktonne year^{-1})	1.99	/
2-PEAc (ktonne year^{-1})	0.42	3.09
Economic indicators		
CAPEX (×1000 US$)	2557	2003
OPEX (×1000 US$year^{-1})	1295	2049
TAC (×1000 US$year^{-1})	1551	2250
TAC (US$/kg$_{2-PE/2-PEAc}$)	0.643	0.728
Sustainability metrics		
Thermal energy requirements (kW$_{th}$h/ kg$_{2-PE/2-PEAc}$)	1.374	1.310
Electrical energy requirements (kW$_e$h/ kg$_{2-PE/2-PEAc}$)	0.271	0.211
Primary energy requirements (kW$_{th}$h/ kg$_{2-PE/2-PEAc}$)	2.052	1.838
Water consumption (m^3_w/kg$_{2-PE/2-PEAc}$)	0.237	0.271
Water loss (m^3_w/kg$_{2-PE/2-PEAc}$)	0.018	0.020
CO_2 emissions, green electricity (kg$_{CO2}$/ kg$_{2-PE/2-PEAc}$)	0.287	0.242
CO_2 emissions, grey electricity (kg$_{CO2}$/ kg$_{2-PE/2-PEAc}$)	0.411	0.338
Material intensity (kg$_{waste}$/kg$_{2-PE/2-PEAc}$)	0	0
Wastewater intensity (m$^3_{ww}$/kg$_{2-PE/2-PEAc}$)	0.054	0.042
Pollutants and toxic materials (kg$_{pt}$/kg$_{2-PE/2-PEAc}$)	0	0

[a] Cost of centrifuge is included.

2-PEAc is recovered (0.728 US$/kg$_{2-PE/2-PEAc}$). However, the total recovery costs make only a minor fraction of the market price of petrochemically produced 2-PE and 2-PEAc (≈22 and 28 US$/kg, respectively)[6] in both cases. Hence, choice of the main product should depend on the current market prices and demand for these chemicals.

Sustainability assessment

Key sustainability metrics of the total recovery process in cases 1 and 2 when the whole aqueous stream is sent to the wastewater treatment are summarized in Table 4. Specific values presented in this section are expressed per kg of recovered 2-PE and/or 2-PEAc. More details about the sustainability assessment are presented in the Supporting information (section 5).

- *Energy requirements*: Total energy requirements are slightly lower when 2-PEAc is recovered (1.838 kW$_{th}$h/kg$_{2-PE/2-PEAc}$) compared to when 2-PE and 2-PEA are recovered separately (2.052 kW$_{th}$h/kg$_{2-PE/2-PEAc}$). This is mainly a consequence of higher product flowrate. Thereby, thermal energy requirements contribute 67–71% to the total energy requirements.
- *Water consumption*: Water requirements and water loss are slightly lower if 2-PE is valorized (0.237 and 0.018 m^3_w/kg$_{2-PE/2-PEAc}$, respectively) compared to when 2-PEAc is recovered (0.271 and 0.020 m^3_w/kg$_{2-PE/2-PEAc}$).
- *Greenhouse gas emissions*: CO_2 emissions are slightly higher when 2-PE is recovered (0.411 kg$_{CO2}$/kg$_{2-PE/2-PEAc}$ if using grey
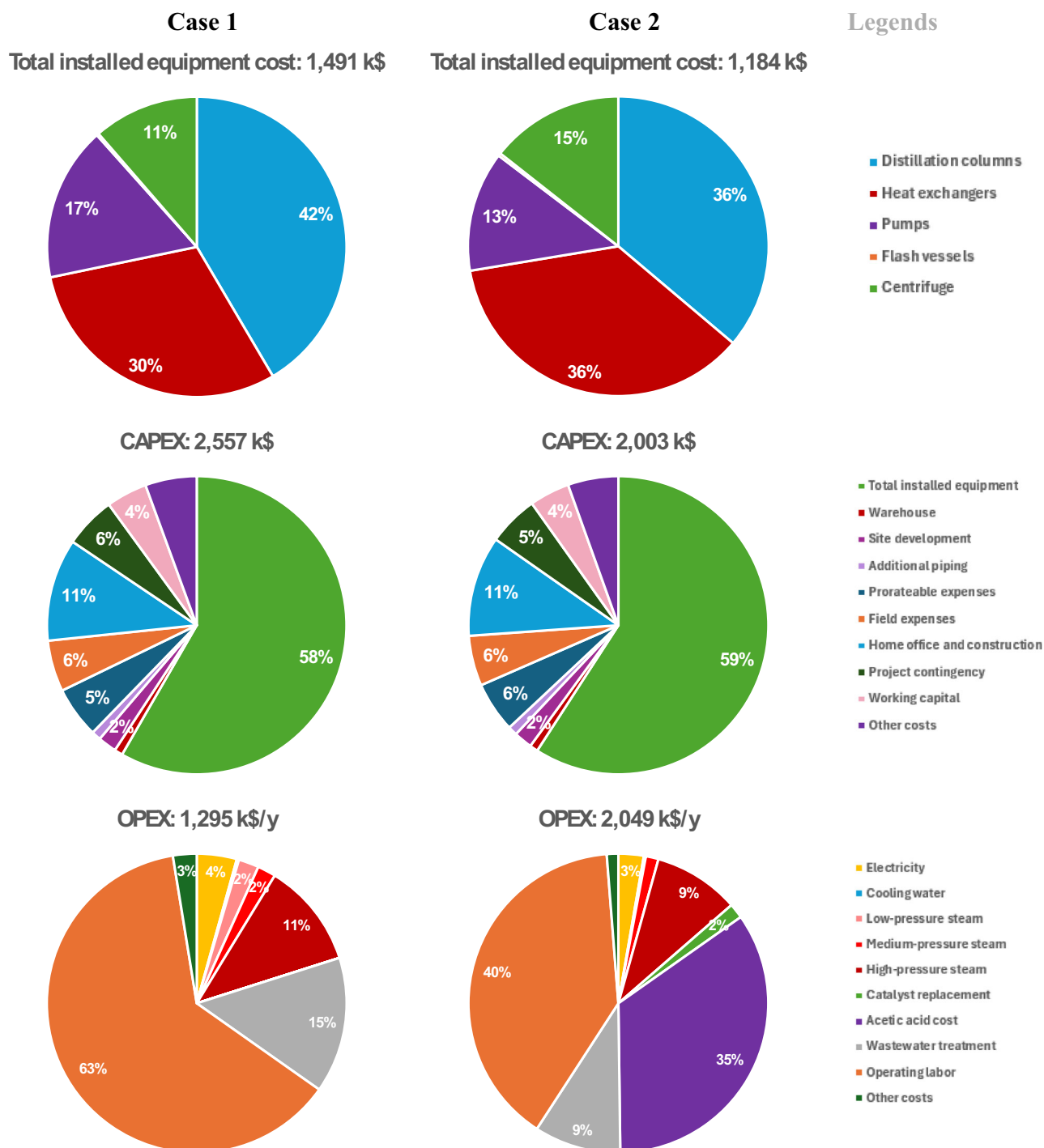
Figure 4. Economic indicators of the total downstream process when recovering 2-PE and 2-PEAc (Case 1) or only 2-PEAc (Case 2) from the organic phase while sending the aqueous phase to the wastewater treatment (centrifuge costs are included).

electricity or 0.287 $kg_{CO2}/kg_{2-PE/2-PEAc}$ if using green electricity) compared to when 2-PEAc is recovered (0.338 $kg_{CO2}/kg_{2-PE/2-PEAc}$ if using grey electricity or 0.242 $kg_{CO2}/kg_{2-PE/2-PEAc}$ if using green electricity).

- *Material intensity*: Because waste is not formed in the developed processes, values of these metrics are equal to zero. The wastewater streams were accounted for in the wastewater intensity metrics and not included here.

- *Wastewater intensity*: The wastewater intensity is mainly the result of treating the aqueous phase after centrifugation. This metric is slightly lower when 2-PEAC is recovered due to a larger product flowrate (0.042 compared to 0.054 $m^3_{ww}/kg_{2-PE/2-PEAc}$).

- *Pollutant and toxic materials*: Because pollutant and toxic materials are not formed in the developed recovery processes, values of these metrics are zero.

Additional options

Note that this bioprocess[13] converts L-Phe to 2-PE while using glucose as a substrate for microbial growth. An alternative option would be *de novo* fermentation from glucose, which is less expensive than L-Phe.[44] Furthermore, lignocellulosic biomass has been studied as a carbon source for 2PE fermentation.[45] Nonetheless, concentrations of main products in the feed streams to downstream processing after *de novo* fermentation with *in situ* product removal are likely to be similar to the assumed ones. Therefore, the proposed process design is easily adaptable to various upstream production methods. Additionally, it can be modified to accommodate use of different high-boiling solvents in the ISPR process (e.g. oleyl alcohol). As ethanol recovery is hardly profitable, there is an incentive to minimize ethanol production during fermentation. That will not only save on glucose for fermentation, but also 4000 US\$ year^{-1} on aqueous waste treatment costs. Higher concentrations of 2-PE and 2-PEAc in the organic phase would improve downstream processing performance (e.g. energy requirements would decrease by $\approx$14% if product concentrations doubled). Nonetheless, this would complicate and increase costs of the biotransformation process. Thus, an analysis covering both upstream and downstream processes is required to obtain the overall picture.[46] The results of our work present a step forward and can give an indication of the cost-effectiveness and energy-efficiency of the recovery process.

CONCLUSIONS

The novel adaptive downstream process design proposed here can be effectively used for industrial recovery of 2-phenylethanol after biotransformation with *in situ* product removal by liquid–liquid extraction and centrifugation. At the core of the downstream process is an integrated vacuum DWC that can effectively remove the high-boiling organic solvent (e.g. PPG) and separate 2-PE and 2-PEAc product mixture from water, or perform an esterification reaction to obtain the pure 2-PEAc. The developed final purification process can recover high-purity 2-PE or 2-PEAc products in a cost-effective way (total recovery costs of 0.64–0.72 US\$/kg$_{2\text{-PE}/2\text{-PEAc}}$). Advanced process intensification, heat pump and heat integration systems resulted in an energy-efficient recovery process (primary energy requirements of 1.83–2.05 kW$_{th}$h/kg$_{2\text{-PE}/2\text{-PEAc}}$). Furthermore, the adaptability of the developed process to recover either 2-PE or 2-PEAc allows modifications in response to market demand. An additional profit can be obtained by recovering hydrous ethanol from the aqueous phase.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

The data supporting the results of this study are available in the Supporting information accompanying this publication. This file contains details about the feed stream composition, design of distillation columns, and economic and sustainability analysis (including separate analyses for recovery from the organic and aqueous phases).

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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