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Advanced process design for large-scale *de-novo* 2-phenylethanol production *via* fermentation

Tamara Janković, Tobias Fecker, Jean-Marc Daran, Adrie J.J. Straathof and Anton A. Kiss*

Abstract

BACKGROUND: 2-Phenylethanol (2PE) is a valuable aroma component that can be obtained through *de-novo* fermentation from glucose. However, its toxicity at very low concentrations (<2.5 g L^{-1}) limits the fermentation titer, rate and yield. To address these limitations, *in-situ* product removal has been explored, leading to a recent scale-up to pilot scale. Nonetheless, an industrial scale has yet to be achieved.

RESULTS: This original research pioneers conceptual development of two large-scale (2 ktonne$_{2PE}$/y) production processes for 2PE *via de-novo* fermentation from glucose. Liquid–liquid extraction with oleyl alcohol and adsorption by hydrophobic resins followed by ethanol desorption, were alternatives considered for *in-situ* 2PE removal. For either design, solvent recovery and final purification were performed using advanced distillation techniques, including a heat pump-assisted distillation and a dividing-wall column. A fermentation titer of approximately 1.5 g$_{2PE}$/L$_{broth}$ minimized production costs by achieving balance between upstream and downstream processing costs. This resulted in a cost-effective 2PE production for both designs of the recovery process (9.03–9.40 \$/kg$_{2PE}$). Sensitivity analysis revealed that glucose, oleyl alcohol, and ethanol costs strongly impact total production costs.

CONCLUSION: This novel study provides a comprehensive and scalable process framework for the large-scale production of 2PE through *de-novo* fermentation. Integrating *in-situ* product removal and energy-efficient purification strategies, it marks a significant step forward in industrial biotechnology.

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

Keywords: 2-phenylethanol; *de-novo* fermentation; *in-situ* product recovery; liquid–liquid extraction; product adsorption; distillation

INTRODUCTION

Production of various chemicals through fermentation is a promising alternative to conventional petrochemical production processes. However, product toxicity often limits achievable titers and production rates, posing challenges for scaling up to an industrial level.[1] The production of hydrophobic high-boiling products, such as 2-phenylethanol, is especially difficult due to very low concentrations attainable in the fermentation broth. Both genetic engineering to enhance microbial performance and tolerance,[2] and process development are needed to ensure a cost-effective and energy-efficient large-scale bioprocess. However, significantly more research has been focused on strain engineering than on process development. From a process development perspective, a process systems engineering approach is necessary to ensure successful commercialization.[3,4] Therefore, this research focuses on process design and simulation aspects of bioprocess development.

Among various chemicals produced *via* microbial transformation, 2-phenylethanol (2PE) has attracted significant attention due to its unique applications and relatively high market value. 2PE is valuable aromatic alcohol with a rose-like scent, widely used in fragrances and perfumes, cosmetics and personal care products, food and beverages, aroma therapy, pharmaceuticals, antiseptics, preservatives, and cleaning agents.[5,6] Global market of 2PE was estimated at over 255 million \$ in 2021 with an expected annual growth of over 5.5% between 2022 and 2028.[7] Nowadays, most of 2PE is produced through petrochemical processes. In addition to reliance on fossil carbon and harsh

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operating conditions, these methods often result in undesirable by-products that limit the applicability of 2PE.[8] As a result, biological production of 2PE has been gaining interest. Despite significant research efforts, naturally derived 2PE remains expensive (~1000 $ kg^{-1} for plant extraction and ~300 $ kg^{-1} for microbial processes)[8] compared to petrochemical production (5–20 $ kg^{-1}).[9,10] Hence, significant improvements are necessary to make the biological production of 2PE competitive with fossil fuel-based processes. In this context, this novel research advances the field of sustainable industrial fermentation by developing two cost-effective large-scale 2-phenylethanol production processes through fermentation.

PROBLEM STATEMENT

From various biotechnological approaches explored to produce 2PE, whole-cell conversion of L-phenylalanine through the Erlich pathway has garnered significant attention.[8,11,12] However, this approach involves a multi-stage process and relies on costly L-phenylalanine as a precursor. Alternatively, 2PE can be produced *de-novo* from renewable glucose through fermentation.[2,13] Due to these advantages, this study focuses on *de-novo* 2PE production through fermentation from glucose.

A major constraint in scaling up both whole-cell biotransformation of L-phenylalanine and *de-novo* fermentation from glucose is the toxicity of 2PE to microbial growth. Consequently, achievable 2PE concentrations in fermentation broth remain low, often below 2.5 g_{2PE}/L_{broth}.[13] This low product yield leads to high water throughput, necessitating large processing equipment and increasing capital costs. Additionally, 2PE's high boiling point (217.6 °C at 1 bar) complicates its separation from the dilute aqueous phase, making downstream processing highly energy-intensive and costly. To mitigate the end-product toxicity and enhance production efficiency, *in-situ* product removal (ISPR) techniques can be employed.[14] Among these, liquid–liquid extraction and adsorption by hydrophobic resins show strong potential for industrial-scale processes. In liquid–liquid extraction, a water-immiscible extractant with a high affinity for 2PE is introduced into the fermentation broth, where it continuously removes 2PE from the aqueous phase. Effectiveness of this technique has been demonstrated in several studies, with commonly used extractants including oleic acid,[15] oleyl alcohol,[13] polypropylene glycol[12] and ionic liquids.[16] DAB. bio has developed a novel integrated bioreactor concept, known as Fermentation Accelerated by Separation Technology (FAST), that enables continuous liquid–liquid extractive fermentation.[17] Recent pilot fermentation at 500 L scale have confirmed the feasibility of this approach for 2PE production.[13] Instead, in the sorption process, 2PE is adsorbed by a solid resin and subsequently desorbed using an inexpensive solvent, such as ethanol. Various hydrophobic polymers with amorphous structures have demonstrated efficiency in ISPR for 2PE production.[18-23] Both liquid–liquid extraction and product adsorption/desorption significantly increase 2PE concentrations in the solvent phase, compared to the fermentation broth. As a result, ISPR not only enhances fermentation productivity and yield but also facilitates final product purification. Given the strong potential of these ISPR techniques, this study aims to evaluate their performance for large-scale fermentation processes. To fulfil this goal, the design and simulation of both processes are required.

Along with the low 2PE concentration, thermodynamic constraints present additional challenges for downstream processing. The 2PE-rich streams will include some water, and later on in the process, the formation of a heterogeneous azeotrope of 6.49 wt% 2PE and 93.51 wt% water[24] can lead to additional liquid–liquid separation steps required to obtain a high-purity final product.

METHODS

Thermodynamic property methods

Aspen Plus was used as a computer-aided process engineering (CAPE) tool to simulate each unit operation.[25] Due to complexity of the process, several thermodynamic property models were used: (i) ELECNRTL (extension of Non-Random Two Liquid model for application in systems with electrolytes) was used to model the fermentation process due to the presence of salts and electrolytes in the fermentation broth; (ii) UNIF-LL (UNIFAC model based on liquid–liquid equilibrium data) was used to accurately account for phase split in liquid–liquid extraction; and (iii) NRTL-HOC (Non-Random Two Liquid model with Hayden-O'Connell extension for vapor phase) was used to model adsorption and final purification using distillation.[26]

Process design

Block flow diagram of the developed processes is presented in Fig. 1. Generally, the 2PE production process consists of four steps: fermentation, *in-situ* product removal (by liquid–liquid extraction or product adsorption), solvent recovery, and final purification to

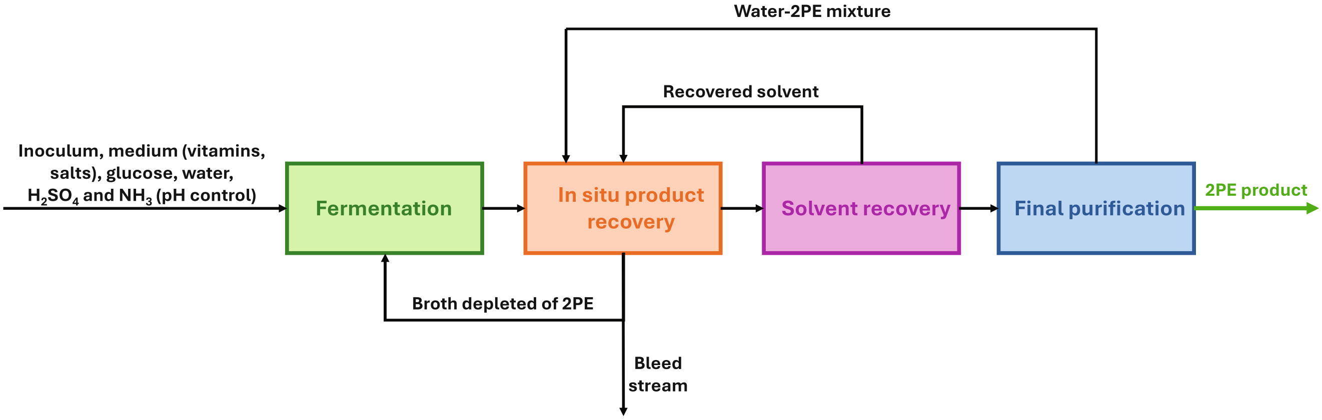


Figure 1. Block flow diagram of 2PE production process.

obtain high-purity product (removal of any remaining impurities). It is important to note that careful adjustment of operating conditions during ISPR is crucial to minimize stress on microorganisms and maintain their viability and productivity. After ISPR, most of the broth containing microorganisms is returned to the bioreactor to minimize biomass loss, reduce freshwater consumption, and enable closed-loop operation. A 10% bleed was considered for all recycle streams entering the fermenter to prevent the accumulation of salts, biomass, or fermentation by-products.

For designing industrial 2PE processes incorporating ISPR, a reliable fermentation model is essential to determine realistic values for input and output stream values for the bioreactor. This also allows for the calculation of volume-specific rates, which are crucial for estimating the required reaction volume for a specific plant capacity. Fermentation was assumed to begin in a batch phase. When a particular 2PE concentration is reached, it will be switched to fully continuous operation, including ISPR, and a steady state is maintained. ISPR is terminated at some point because of infection risk and accumulation of impurities. The 2PE remaining in the bioreactor may be recovered using end-of-pipe recovery.[27] If the steady-state can be maintained very long compared to the start-up, shut-down, and downtime phases, designing only the steady-state phase is reasonable. Hence, this work models the steady-state operation of the 2PE production process. The model for microbial kinetics and the fermentation process is given in the Supplementary Information Data S1. In short, it was assumed that 2PE inhibits product formation and growth similarly, according to the fixed ratio between the increase in biomass (X) and 2PE concentrations during batch fermentation.[2] Based on this assumption, the ratio between maximum specific production and growth rates was determined to be 0.12 $\mathrm{mol_{2PE}/mol_X}$. Furthermore, the formation of by-products was considered negligible due to continued strain development and optimization of the fermentation conditions. Hence, product-to-substrate yield (0.168 $\mathrm{mol_{2PE}/mol_S}$) was calculated based on early batch experimental data and the glucose amount that would otherwise be used for the formation of ethanol, which was a side product under suboptimal conditions. The following constant molar stoichiometry was obtained:

$$\begin{aligned} &C_6H_{12}O_6 + 2.850\,O_2 + 0.280\,NH_3 \\ &\rightarrow 1.400\,X + 3.256\,CO_2 + 4.320\,H_2O + 0.168\,C_8H_{10}O \end{aligned} \quad (1)$$

Thereby, X represents dry biomass with the formula $CH_{1\cdot 8}O_{0\cdot 5}N_{0\cdot 2}$, as commonly used in bioprocess simulations.[28]

The pH for continuous fermentation was set to ~5[13] using control by 25 wt% NH_3 and 98 wt% H_2SO_4 solutions. NH_3 is added in the fermenter as it is one of the necessary reactants in the fermentation reaction, while H_2SO_4 is used to fine-tune pH in the fermenter. Additionally, Na_3PO_4 and $MgSO_4$ were included as medium components (0.34–0.35 and 0.02–0.03 wt%, respectively), while vitamins and antifoam addition were not considered in Aspen Plus simulations. Temperature of the fermenter was specified at 30 °C as previously used in pilot experiments.[13] Glucose conversion of 99% was assumed, and the reaction heat was calculated based on the approximate combustion enthalpy for organic components of 460 $\mathrm{kJ/mol_{O2}}$.[28] Fermentation settings were determined based on the 2PE concentration in the fermentation broth, which was kept below 2.5 $\mathrm{g_{2PE}/L_{broth}}$ to prevent product inhibition. Hence, 2PE concentration was varied between 0.1 and ~2 $\mathrm{g_{2PE}/L_{broth}}$. Flowrates of all inlet streams were back-calculated to achieve a production capacity of about 2 $\mathrm{ktonne_{2PE}/y}$, regardless of the 2PE concentration in the broth. The required broth volume was calculated from the previously determined broth flowrate and growth rate. The required number of fermenters was determined assuming a maximum fermenter size of 500 m^3, with 90% working volume. To choose a fermenter configuration, aeration and mixing costs were compared for two reactor types: bubble column (BC) and stirred-tank reactor (STR).[29] A sequence of two vessels, increasing in size by a factor of 10,[29] was assumed for the seed train.

As previously mentioned, two processes were considered for 2PE purification from fermentation broth: **Process A** involves using liquid–liquid extraction as an ISPR technique. Oleyl alcohol was selected as the extractant for ISPR, as it had been demonstrated effective for pilot-scale continuous extractive fermentation.[13] The extraction process was simulated as a three-stage counter-current extraction column. Even though integrating fermentation and extraction into one equipment unit is feasible,[30] this novel unit is not available in Aspen Plus. An oleyl alcohol flowrate was determined to enable recovery of >95% of 2PE from the fermentation broth. **Process B** utilizes hydrophobic adsorption followed by desorption with ethanol as ISPR technique. Prior to the adsorption step, fermentation broth was centrifuged to remove microorganisms. As an alternative to centrifugation, filtration may be used to remove microorganisms from the fermentation broth. The biomass-rich stream was largely recycled to the fermenters to minimize biomass loss. The adsorption process was designed using experimental data as provided in the Supplementary Information Data S1. The adsorbed 2PE was desorbed using hydrous ethanol as an inexpensive solvent. In both processes, the remaining aqueous phase depleted of 2PE was largely recycled to the bioreactor. The solvent (oleyl alcohol or ethanol) rich in 2PE, was sent to the solvent recovery and final 2PE purification sections. In both processes, distillation was used as the main separation technique in these steps. Recovered solvents (oleyl alcohol and ethanol) can be recycled for reuse in ISPR. Since the amount of impurities in the recovered solvents is below 1 wt%, a conservative bleed of 1% was assumed for these streams to avoid impurity buildup. As mentioned, the processes modeled in this work present steady-state operation. More details about modelling the recovery process are outlined in the Supplementary Information Data S1.

Process evaluation

Once designed in Aspen Plus, the performance of the developed processes was evaluated by performing economic and environmental analysis. Cost-effectiveness of the developed process was determined following the established methodology of National Renewable Energy Laboratory (NREL).[31] Sustainability of the processes was estimated by calculating the key sustainability metrics.[32] More details about these methodologies are presented in the Supplementary Information Data S1.

Furthermore, applicability of the designed processes was extended by performing a sensitivity analysis on the effects of glucose price, wastewater treatment costs, oleyl alcohol and ethanol prices, and makeup fractions.

RESULTS AND DISCUSSION

Process design

Flowsheets of the developed processes are presented in Figures 2 and 3. It should be noted that additional storage tanks and buffer

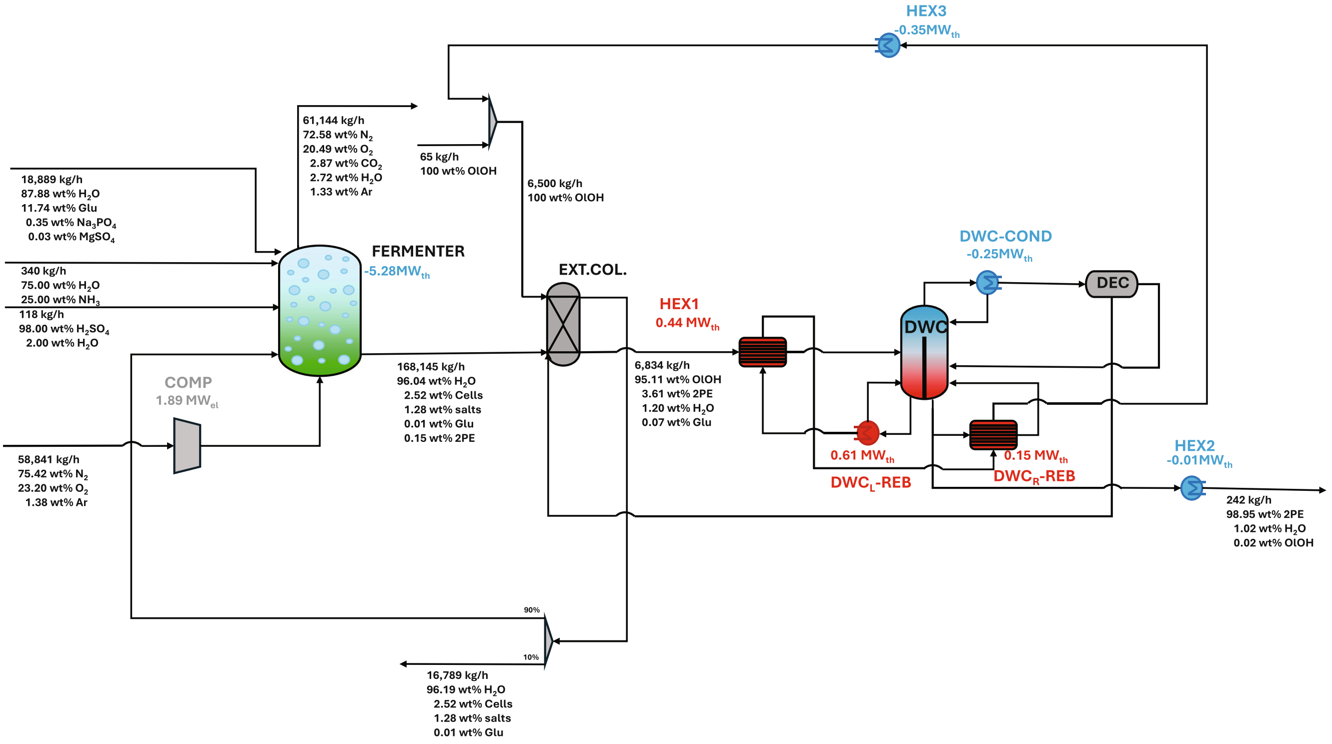


Figure 2. Process A – 2PE *de-novo* fermentation from glucose with liquid–liquid extraction as the ISPR technique and final purification by distillation.

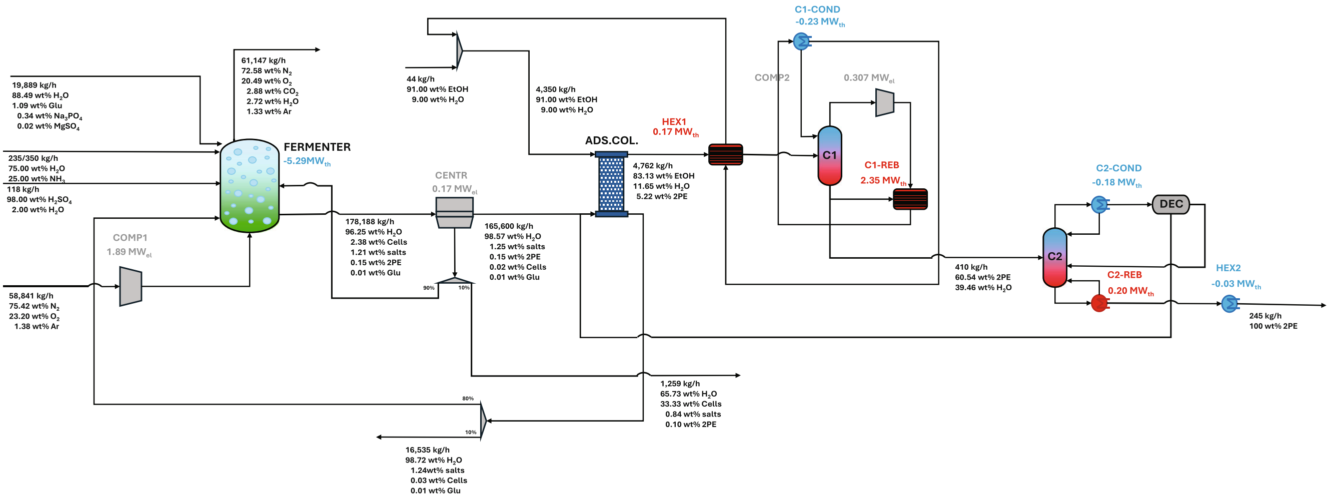


Figure 3. Process B – 2PE *de-novo* fermentation from glucose with product adsorption as the ISPR technique and final purification by distillation.

tanks would be needed to allow continuous operation of the downstream processing.

Fermentation

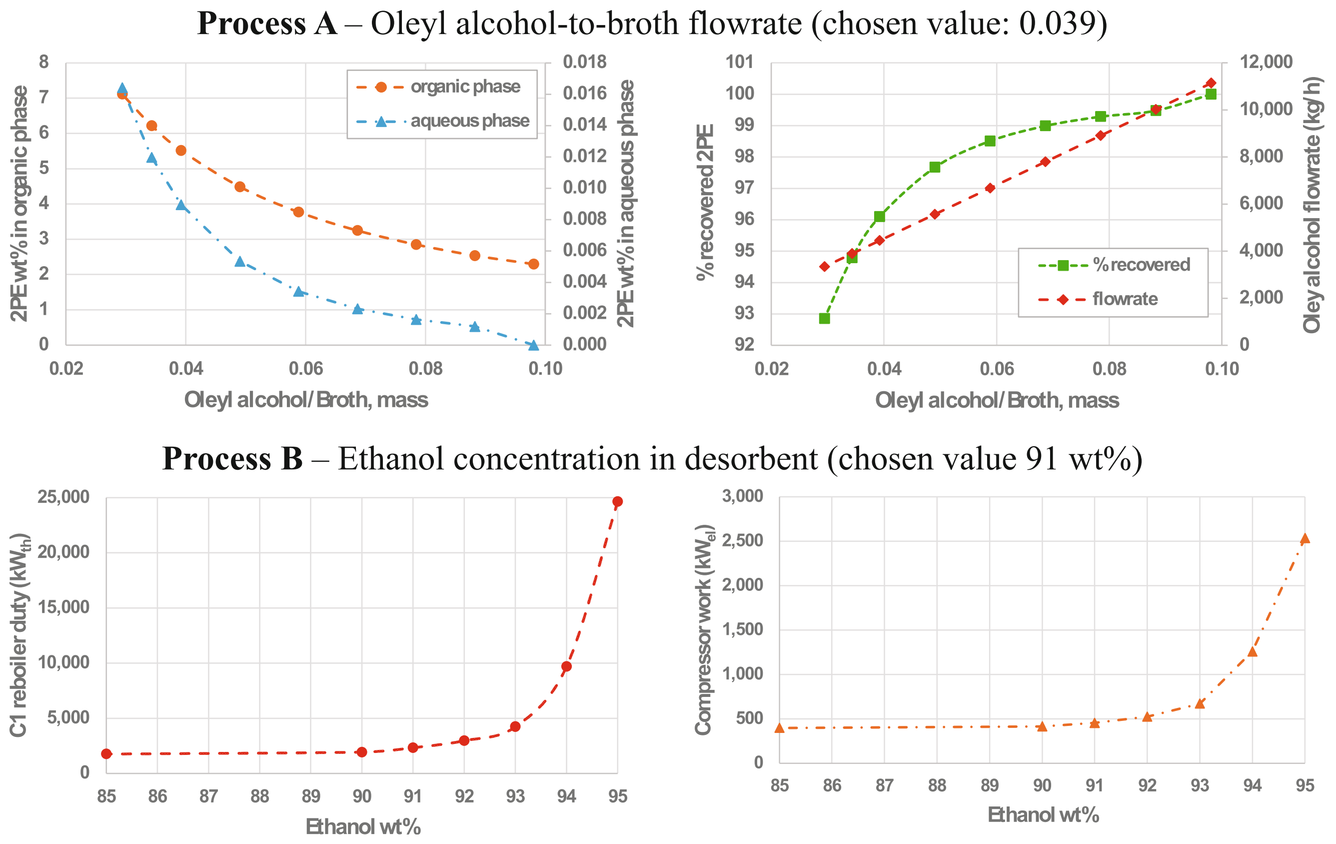
To ensure sufficient mixing, a superficial gas velocity (corrected for pressure) of 0.2 m/s was assumed for a BC. This resulted in a gassed power input of 1.96 kW $m^{-3}{}_{broth}$, or an electrical energy requirement of 396 kW$_{el}$ for gas compression (assumed isentropic operation) if the fermenter is BC. In a STR, an impeller power input of 1.79 kW $m^{-3}{}_{broth}$ is required to achieve the same total power input while maintaining sufficient reactor aeration, resulting in a specific power of approximately 0.17 kW $m^{-3}{}_{broth}$. In total, 836 kW$_{el}$ are required if the fermenter is STR. Since the capital costs of BC and STR are relatively similar[33] and STR requires significantly more electrical energy input, the BC fermenter configuration was chosen, and appropriate capital and operating costs were included in the economic analysis. More details about this comparison are available in the Supplementary Information Data S1.

Downstream processing

Process A – ISPR using liquid–liquid extraction. When determining the oleyl alcohol flowrate, a trade-off must be considered: using more solvent increases 2PE recovery but results in a lower 2PE concentration in the solvent, complicating subsequent separation.

Additionally, higher solvent flowrates lead to larger equipment sizes and increased capital costs, as well as higher operating costs in downstream steps due to greater reboiler energy requirements. Oleyl alcohol-to-broth flowrate ratio of 0.039 (mass basis) was selected to allow recovery of >95% of 2PE from the fermentation broth (Fig. 4). As a result, after the extraction process, aqueous phase was completely depleted of 2PE (<0.01 wt%), while the concentration of oleyl alcohol in the aqueous phase remained insignificant (<0.01 wt%). Conversely, the concentration of 2PE in the organic phase was more than 24 times higher than in the fermentation broth. This stream was sent to the solvent recovery and final 2PE purification sections. A two-step purification was required to obtain highly pure 2PE from its mixture with oleyl alcohol and water. Oleyl alcohol could be recovered at the bottom of a first distillation column, while a second distillation column could separate 2PE at the bottom, from the water-2PE mixture at the top. Additionally, liquid–liquid separation in a decanter was needed due to the formation of a heterogeneous azeotrope between water and 2PE. The two columns were effectively integrated into one azeotropic dividing-wall column (A-DWC) with a common overhead section and a divided bottom section (Fig. 5). This energy-efficient unit reduces capital costs as it minimizes the number of column shells, heat exchangers, and the required plant area. As A-DWC unit is not available in Aspen Plus, it was simulated as an equivalent sequence of thermally integrated distillation columns (Fig. 5), where A-DWC$_L$ and A-DWC$_R$ represent the left and right parts of A-DWC, respectively. Oleyl alcohol (>99.8 wt%) was recovered at the bottom of A-DWC$_L$ and was reused in the extraction step. High-purity 2PE (~99.0 wt%) was recovered at the bottom of A-DWC$_R$, while a nearly azeotropic water-2PE mixture was obtained as the top product. This mixture was sent to the decanter for liquid–liquid phase splitting. To minimize 2PE loss, the 2PE-rich phase was returned to A-DWC, while the water-rich phase was recycled to the extraction column. Since oleyl alcohol needs to be cooled before being reused in the extraction column, it was used to preheat the feed stream to A-DWC, reducing the reboiler duty of A-DWC$_L$ by ~38%. Additionally, oleyl alcohol can also replace heating utility in A-DWC$_R$'s reboiler. Thus, the two mentioned heat integrations reduced the total heating duty of A-DWC by 45–49%.

Process 2 – ISPR using adsorption by hydrophobic resin. Macronet MN200 resin which efficiently adsorbs 2PE from a multicomponent mixture was assumed for process modeling (adsorption capacity of 210 g_{2PE}/kg_{resin}). However, alternative resins with similar properties have already been explored.[18-23] As previously mentioned, adsorbed 2PE was desorbed using hydrous ethanol as a cost-effective solvent. The obtained mixture of ethanol, 2PE, and water was sent to the solvent recovery and final 2PE purification sections. Being the lightest boiling component, ethanol (with some water) was recovered as the top product from column C1 (Figure 3). The remaining 2PE and water, separated at the bottom of column C2, are sent to column C2. A high-purity 2PE (~100 wt %) was obtained as the bottom product from C2. The nearly azeotropic 2PE-water mixture obtained as the top product of column C2 was sent to a decanter for liquid–liquid phase splitting. To minimize product loss, the 2PE-rich phase was returned to column C2, while the aqueous phase was recycled to the adsorption step. The optimal ethanol concentration for desorption was determined to minimize the energy requirements of column C1 (Fig. 4). As ethanol concentration approaches the azeotropic point, its separation


Process A – Oleyl alcohol-to-broth flowrate (chosen value: 0.039)

Process B – Ethanol concentration in desorbent (chosen value 91 wt%)

Figure 4. Determination of oleyl alcohol flowrate and ethanol concentration.

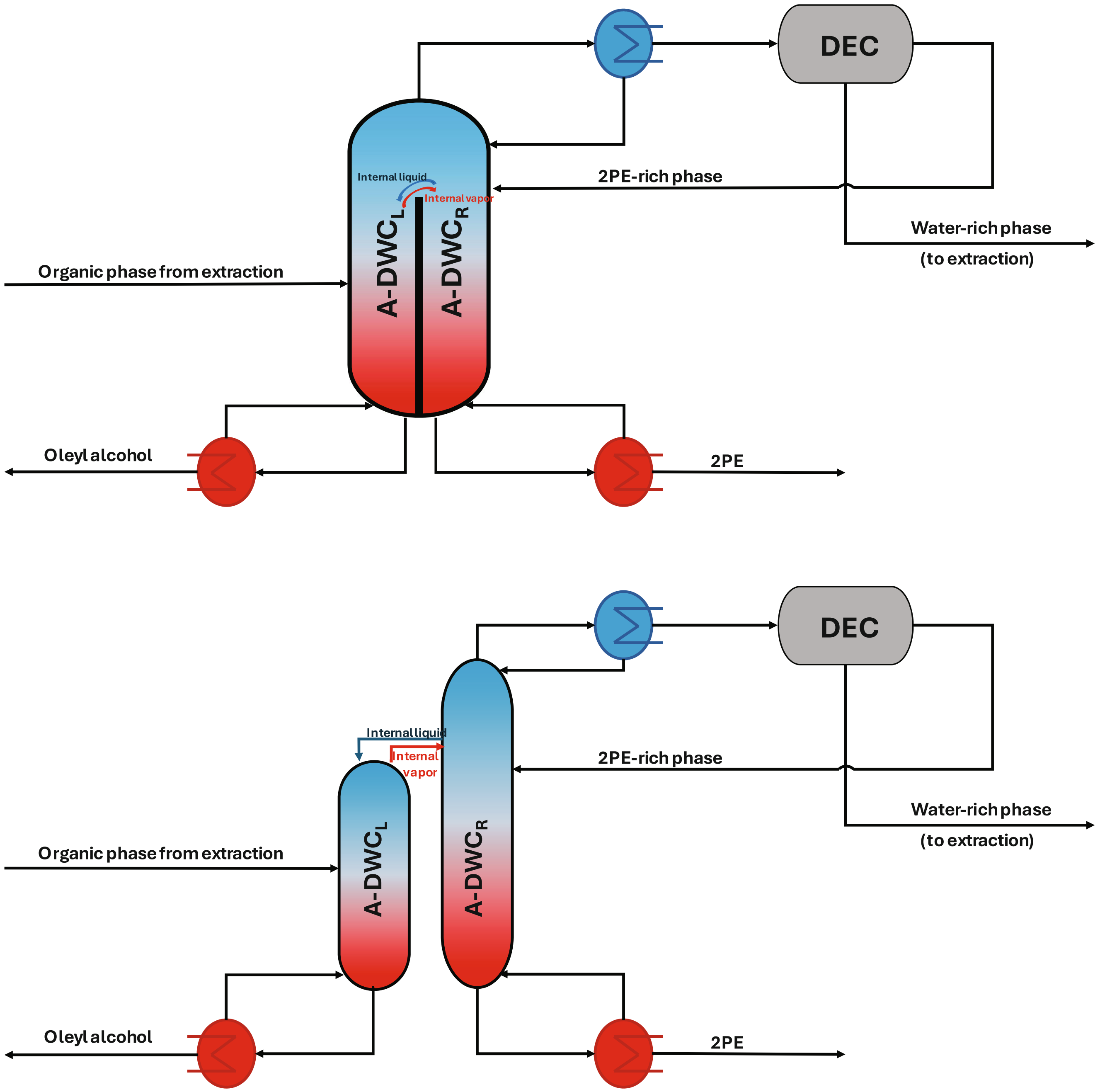


Figure 5. Azeotropic dividing-wall column (top) and equivalent sequence of distillation columns (bottom).

on the top of column C1 becomes more energy-intensive, requiring a higher reboiler duty due to the higher required reflux ratio. Given the non-significant temperature difference at the top and the bottom of column C1, a mechanical vapor recompression (MVR) heat pump system could be implemented to decrease energy requirements. This heat pump system implies compressing vapor from the top of the column and using it as a heating source in the reboiler. Consequently, the electrical energy required to power the compressor can replace much higher thermal energy.[34] Additionally, as the top vapor from column C1 needs to be further cooled before being returned to the column, it may be used to preheat the feed stream to this column. This may reduce the reboiler duty of column C1 by about 7–8%. However, as ethanol concentration approaches the azeotropic point, a higher compressor power in the MVR system is required (Fig. 4). Finally, an ethanol concentration of 91 wt% in the desorbent was chosen as higher concentrations significantly increase energy requirements. Simultaneously, lower concentrations only marginally reduce energy requirements while increasing the required equipment size. The obtained energy savings with implementation of the MVR system to column C1 can be measured through the coefficient of performance (COP). COP is equal to the ratio of the upgraded heat (exchanged between the compressed vapor and bottom liquid) and the required compressor power. Since the COP of the designed system is 5.0–9.7, which is significantly higher than the conservative value of the electrical-to-thermal conversion factor (about 2.5), it may be concluded that the implemented MVR system brings significant energy savings compared to simple distillation without a heat pump. Furthermore, this allows (green) electrification of the ethanol recovery step, while thermal energy is required only in the final 2PE purification.

Optimal 2PE concentration in the fermentation broth

The impact of 2PE concentration in the fermentation broth on unit production costs (UPC) was analyzed using the derived stoichiometric model of the fermentation reaction. Results presented in Fig. 6 indicate that UPC increases significantly only when 2PE concentrations fall below 0.5 g_{2PE}/L_{broth}. Conversely, for concentrations above 1.0 g_{2PE}/L_{broth}, the changes in UPC are not that significant. A key finding is that 2PE concentration of approximately 1.5 g_{2PE}/L_{broth} in the fermentation broth results in the minimum unit production costs for both processes. Notably, this does not necessarily align with the highest product formation rate. Using the calculated maximum specific growth rate (μ^{max}) of 0.17 h^{-1}, the highest product formation rate is observed at a 2PE concentration of 1.0 g_{2PE}/L_{broth}, decreasing for both lower and higher concentrations. A 2PE concentration of 1.0 g_{2PE}/L_{broth} results in a slightly higher UPC than 1.5 g_{2PE}/L_{broth} because of the increased cost of downstream processing. Lower 2PE concentrations necessitate larger processing throughput to achieve the same production capacity, resulting in larger equipment units (higher CAPEX) and a more energy-intensive recovery process (higher OPEX). On the contrary, despite reducing the costs of the recovery process costs, 2PE concentration higher than 1.5 g_{2PE}/L_{broth} increases the fermentation costs, leading to a rise in the total production costs. These insights are crucial for optimizing industrial-scale natural 2PE production. They demonstrate that it is not necessary to push 2PE concentration to extreme limits, as an optimal balance between upstream and downstream processing exists. Maintaining a 2PE concentration of around 1.5 g_{2PE}/L_{broth} offers a cost-effective solution by minimizing overall production expenses while ensuring competitive process efficiency. Detailed analysis of the economic and environmental performance is provided for this concentration of 2PE in the fermentation broth of 1.5g_{2PE}/L_{broth}. More details about process performance with different 2PE concentrations are available in the Supplementary Information Data S1.

Economic analysis

A comparison of the main economic indicators for 2PE production process with 1.5 g_{2PE}/L_{broth} is given in Table 1 and Fig. 7.

In general, the largest contributors to equipment costs are fermenters and seed trains (46–53%), compressors (about 25–26%) and flash units (about 10–11%). The extraction or adsorption column also represent a significant portion of the total equipment costs in both processes (about 5–81%). Furthermore, the centrifuge cost is not a negligible contributor when using adsorption for ISPR (about 4%). The costs of distillation columns are low (about 1–2%). Also, heat exchangers and pumps contribute relatively little (accounting for 2–4% and 1–2% of the total equipment costs, respectively). Overall, the total equipment costs are higher if adsorption is used for ISPR (process B), primarily due to the additional compressor and centrifuge. As a result, the total capital costs (CAPEX) for process B are 15% higher than those for process A.

The total operating costs (OPEX) for process B are also slightly higher compared to process A (by 4%). This difference is mainly due to higher resin replacement costs (assuming an annual replacement) and increased electricity due to the additional compressor in the MVR system. In contrast, the costs of solvent make up and high-pressure steam are higher in process A. However, by far the largest contribution to OPEX in both cases is feedstock (glucose, NH_3, Na_2PO_4, $MgSO_4$, H_2SO_4, water) cost, which accounts for approximately 57–59% of the total operating expenses. This is because substantial amounts of glucose are required relative to the product yield. Other operating costs, such as energy requirements, are minimized, reflecting an energy-efficient process design.

When considering both CAPEX and OPEX with a payback period of 10 years, total annual costs (TAC), are slightly lower (by 6%) when using liquid–liquid extraction (process A) than when using adsorption (process B). Nonetheless, unit production costs in both processes (9.03–9.40 $/$kg_{2PE}$) are in the range of the price of petrochemically produced 2PE (5–20 $ kg^{-1})[9,10] and are significantly lower than the costs associated with naturally produced 2PE (~1000 $ kg^{-1} for plant extraction and ~300 $ kg^{-1} for microbial

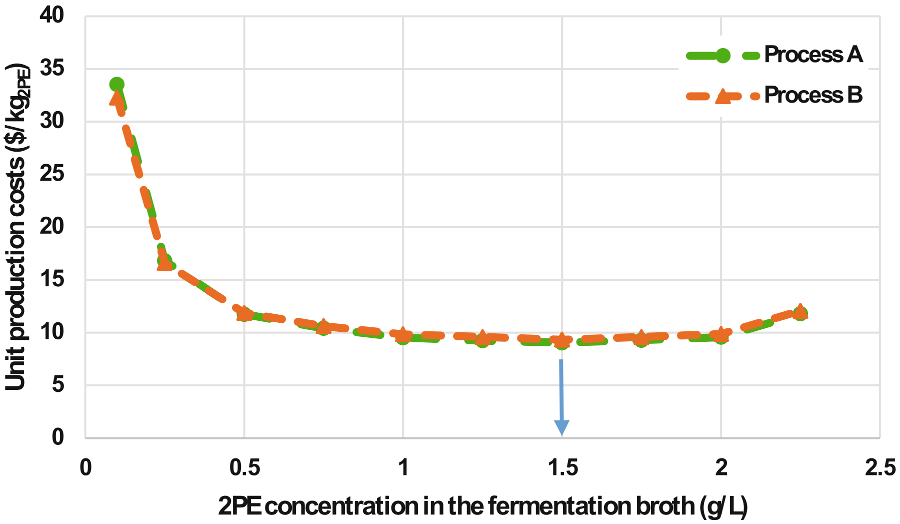


Figure 6. Effects of 2PE concentration in the fermentation broth on the total production costs (minimal value for 1.5 g_{2PE}/L_{broth}).

processes).[8] This suggests that both designed processes represent a major step forward in advancing industrial biotechnology of 2PE production. The choice of optimal ISPR design depends strongly on plant location and available utilities. ISPR by liquid–liquid extraction with oleyl alcohol allows continuous operation, while adsorption-based ISPR followed by ethanol desorption requires cyclic processing. Oleyl alcohol is more expensive, and its recovery demands higher thermal energy, whereas usage of ethanol involves lower solvent costs but higher electrical energy requirements for solvent recovery. Furthermore, considering that the market price of L-phenylalanine (~10–30 \$/kg$_{\text{L-Phe}}$)[35] is very similar to that of 2PE, and given the cost-effectiveness demonstrated in this study, large-scale fermentative production of 2PE from glucose is more attractive than bioconversion of L-phenylalanine to 2PE.

The impact of the payback period on the production costs is given in the Supplementary Information Data S1. Even with a payback period of two or only 1 year, unit production costs remain below 16 or 24 \$/kg$_{2PE}$, respectively.

Table 1. Comparison of the main key performance indicators of *de-novo* 2PE production with 1.5 g$_{2PE}$/L$_{broth}$

	Process A	Process B
2PE concentration in the broth (wt%)	0.15	0.15
Total process yield (mol$_{2PE}$/mol$_{Glu}$)	0.160	0.165
Economic indicators		
CAPEX (k\$)	27 251	31 207
OPEX (k\$/y)	14 704	15 323
OPEX (\$/kg$_{2PE}$)	7.62	7.81
TAC (k\$/y)	17 429	18 444
TAC (\$/kg$_{2PE}$)	9.03	9.40
Sustainability indicators		
Thermal energy requirements (kW$_{th}$h/kg$_{2PE}$)	2.508	0.652
Electrical energy requirements (kW$_{el}$h/kg$_{2PE}$)	8.722	10.493
Primary energy requirements (kW$_{th}$h/kg$_{2PE}$)	24.312	26.884
CO$_2$ emissions, green electricity (kg$_{CO2}$/kg$_{2PE}$)	0.462	4.912
CO$_2$ emissions, grey electricity (kg$_{CO2}$/kg$_{2PE}$)	4.440	0.127
Utility water requirements (m^3_W/kg$_{2PE}$)	4.664	4.064
Utility water losses (m^3_W/kg$_{2PE}$)	0.329	0.285
Wastewater intensity (m$^3_{WW}$/kg$_{2PE}$)	0.069	0.072

Effects of glucose price, solvent price and wastewater treatment costs

The effects of glucose price, wastewater treatment costs, solvent price, and makeup fraction on the total 2PE production costs were analyzed using 1.5 g$_{2PE}$/L$_{broth}$ as representative concentration. The results are summarized in Fig. 8.

- Glucose price: As major contributor to OPEX, the cost of glucose has the most significant contribution to the total 2PE production costs. Any increase or decrease in glucose price results in a proportional change in the production costs for both processes.
- Wastewater treatment costs: Compared to the glucose price, variations in wastewater treatment costs have a much lower effect on the total production costs. Nonetheless, this is slightly

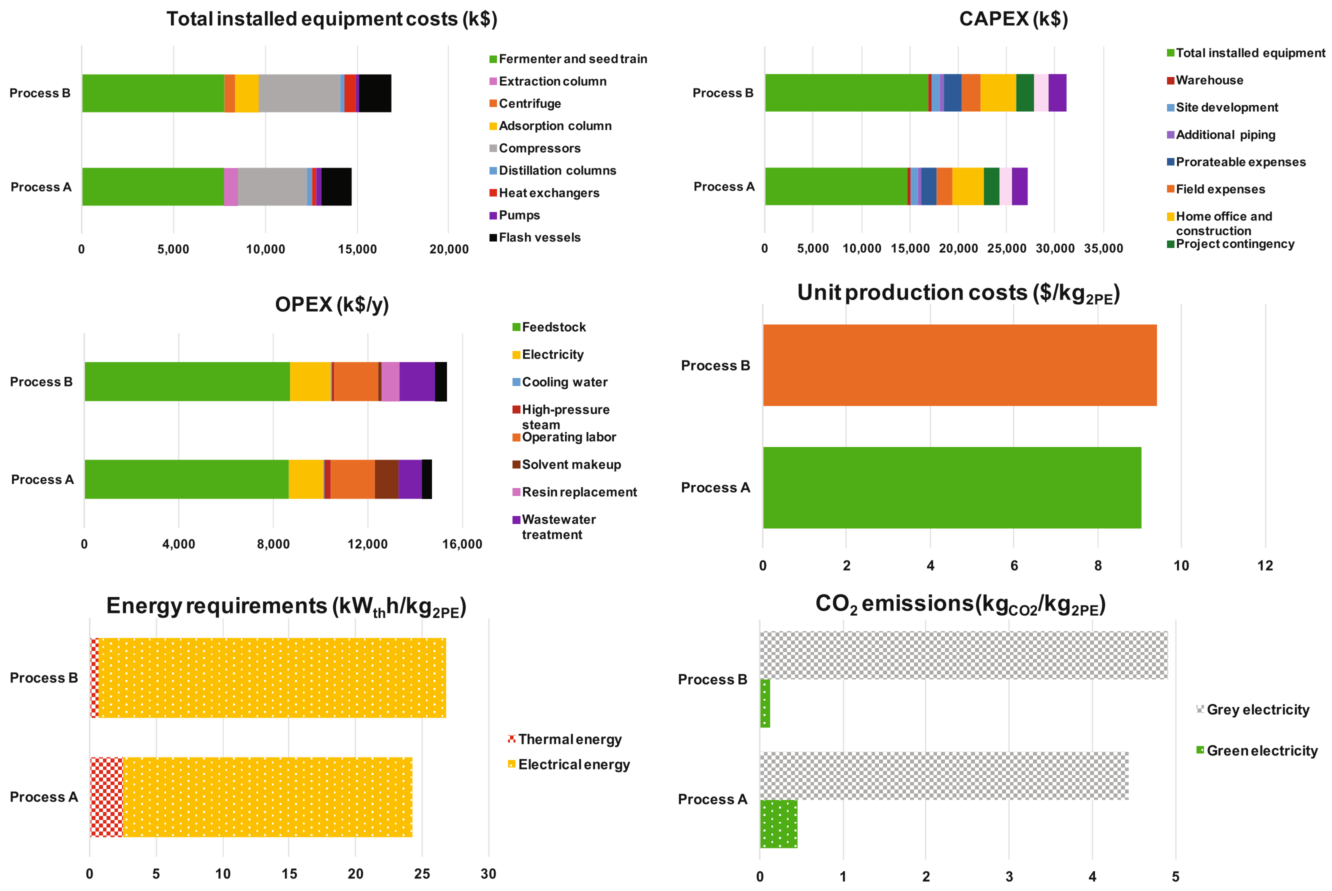


Figure 7. Comparison of the key performance indicators for the two designed 2PE production processes.

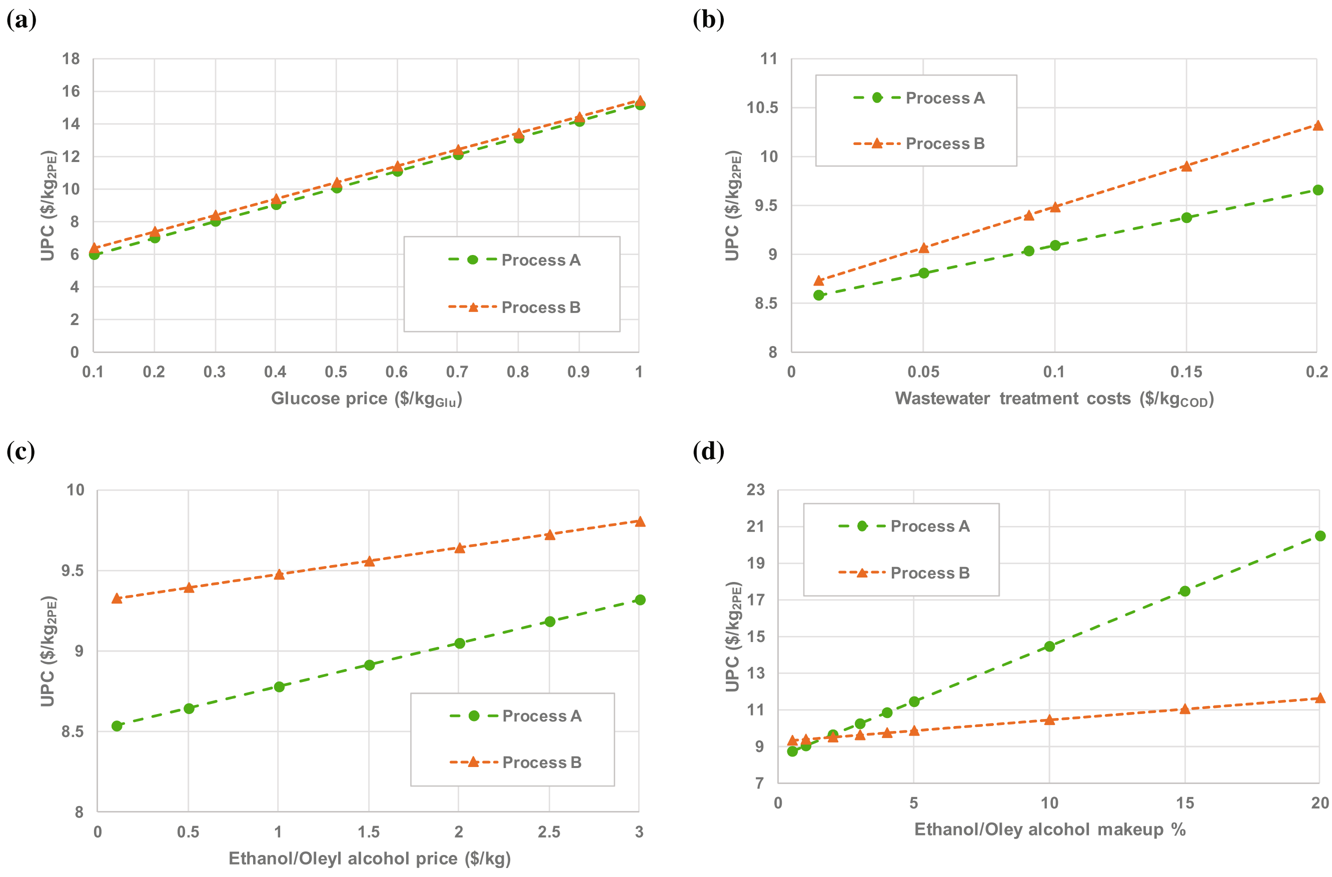


Figure 8. Effects of glucose price (a), wastewater treatment costs (b), ethanol or oleyl alcohol costs (c) and makeup fraction (d).

more pronounced in process B due to its higher wastewater amount.

- Solvent prices: The cost-effectiveness of the process is highly sensitive to fluctuations in oleyl alcohol and ethanol prices. An increase or a decrease in these prices directly affects 2PE production costs.
- Makeup fraction of solvent: A makeup of 1% was assumed for both oleyl alcohol or ethanol. However, variations in the makeup fraction may influence the process's economics by changing both solvent makeup costs and waste treatment expenses. The makeup fraction of oleyl alcohol has a greater influence on 2PE production costs than ethanol, due to its higher price.

Sustainability assessment

The main sustainability metrics for the developed processes are summarized in Table 1. A graphic representation is presented in Fig. 7.

- *Energy requirements*: Generally, thermal energy requirements are higher in process A due to the reboiler duty of A-DWC. In contrast, electrical energy requirements are higher in process B due to the compressor in the MVR system. As a result, primary energy requirements are slightly higher in process B.
- *Greenhouse gas emissions*: If electricity is sourced from fossil fuels (grey electricity), process B results in higher CO_2 emissions due to its greater primary energy requirements. However, if renewable electricity (green electricity) is used, process A produces more CO_2 emissions due to its higher reliance on thermal energy.
- *Utility water requirements and loss*: Utility water requirements are related to the cooling of the fermenters and thermal energy requirements. Utility water requirements and water loss are higher in process A due to higher thermal energy requirements.
- *Wastewater intensity*: Process B generates slightly more wastewater compared to process A. This difference is primarily due to water losses in the bleed stream after centrifugation.

CONCLUSION

This original study is the first to develop two large-scale (~2 ktonne$_{2PE}$/y) processes for fermentative production of *de-novo* 2PE from glucose. This study compares *in-situ* product removal by liquid–liquid extraction and adsorption by hydrophobic resins, followed by final purification using advanced distillation techniques. A key finding of this work is the identification of an optimal 2PE concentration of approximately 1.5 g_{2PE}/L_{broth}, where unit production costs are minimized due to the balance between upstream and downstream processing. This resulted in highly cost-competitive 2PE production (9.03–9.40 $/kg$_{2PE}$), regardless of the method used for *in-situ* product recovery. Higher 2PE concentration leads to higher fermentation costs, while lower concentrations significantly increase both upstream and downstream expenses. Furthermore, sensitivity analysis revealed that glucose, oleyl alcohol and ethanol prices have a significant impact on the total production costs. These findings are crucial for developing a competitive industrial-scale process, demonstrating that maximizing 2PE concentration is not always necessary, but rather finding an optimal balance is key to reducing overall production costs.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the Supplementary Information file.

SUPPORTING INFORMATION

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

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