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A techno-economic assessment of a biocatalytic chiral amines' production process integrated with *in situ* membrane extraction

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Abstract:

The production of chiral amines through asymmetric synthesis using amine transaminase (ATA) has the potential for high yields in an efficient single-step process. Integrating *in situ* membrane extraction with this biocatalytic chiral amines production process has been demonstrated to reach higher yields by shifting the equilibrium position through product recovery. To date, however, it is unclear whether the *in situ* product recovery strategy is economically viable. This study carried out a techno-economic assessment (TEA) to understand the main drivers of the manufacturing costs and to set quantitative development targets. The chiral amine products under study are (R)-(+)- or (S)-(-)-α-methylbenzylamine (MBA) and sitagliptin. Their manufacturing costs are quantified and benchmarked to three alternative production pathways. The results yield an MBA manufacturing cost of the integrated process with membrane extraction of €17.8/mol, which is lower than the benchmark process using an ion-exchange resin (€23.4/mol). The sitagliptin manufacturing cost is estimated at €30.9/mol, which is €1.6/mol and €4.6/mol less than the benchmark processes with engineered transaminase, and the ruthenium-catalyzed process, respectively. Based on the outcomes of sensitivity analyses, development targets are set for the key parameters of membrane flux and selectivity and product concentration that influence the manufacturing cost related to the membrane.

Keywords: techno-economic assessment; membrane extraction; process development; chiral amines; in situ product recovery

1. Introduction

Chiral amines are valuable substances in chemical industries and are widely applied in stereoselective organic synthesis, as resolving agents, building blocks or chiral auxiliaries. 40% of active pharmaceutical ingredients (APIs) and 20% of agrochemicals contain chiral amine scaffolds.[1] Annual market revenues are estimated at €3 billion, which is 15% of the total revenues from sales of pharmaceutical and agrochemical industries.[2] For chiral amines used as APIs, the production scale is typically 1–1000 ton/year. Chiral amine agrochemicals are typically produced at a scale of 500 to 10,000 ton/year.[3] For these high-value products, the economically viable total manufacturing costs can be up to €500 /kg for APIs, and €100 /kg for agrochemicals.[4] Chiral amine synthesis has been a challenge due to their high density of structural information and inherent ability for hydrogen bonding. Chiral amines can be produced either by direct asymmetric synthesis from pro-chiral ketones or by kinetic resolution of racemic amines.[2,5,6] In addition to traditional synthetic chemistry, the use of enzymes, such as imine reductases, hydrolases, transaminases, and monoamine oxidases, have shown potential for asymmetric chiral amine synthesis.[7,8] Transaminases catalytic synthesis of different chiral amines, such as methylbenzylamine, sitagliptin, 2-aminopentane and 1-methoxy-2-propylamine, has been widely reported.[6,9-14] The main advantages of using biocatalysis to produce chiral amines are the excellent stereo and regioselectivity and the potential for high yield in a single synthesis step.[10,15,16] In many cases, however, the equilibrium position is unfavorable for asymmetric synthesis.[4,17] Hence, methods for shifting the reaction equilibrium are required to substantially increase reaction yield, and accordingly decrease the downstream processing cost.

To this end, *in situ* product removal (ISPR) has been the most reported strategy, e.g., adding auxiliary or tailor-made enzymes with secondary side reactions,[9,11,18-21] using organic solvents or ion-exchange resins for products removal,[11,22] precipitating products from the reaction solutions,[23] or the evaporation of volatile products.[24] Lately, Mack and Doeker et al. (2021, 2022) conducted *in situ* liquid-liquid extraction of product amine with 1-decanol as the physical extraction solvent and oleic acid as reactive extraction solvent, respectively. It achieved higher conversions of metaraminol when L-alanine was used as amine donor.[25, 26] Moreover, *in situ* product crystallization (ISPC) for an amine transaminase-catalyzed reaction was proposed to shift the reaction equilibrium towards the products.[27] A donor salt of isopropylammonium 3,3-diphenylpropionate was used in an amine transaminase reaction reported by Langermann's group (2018, 2019, 2021).[28-30] A barely soluble salt was formed with the product amine to overcome the thermodynamic limit. They further described a semi-continuous reaction approach with ISPC to obtain a product concentration of 1.2 mol/L. Similarly, an approach entailing *in situ* conversion of by-product was developed to reach full conversion. The by-product undergoes a secondary reaction with a tailor-made transaminase or amine donor. Halim et al. (2014) used the CV2025 ω-TAm with alcohol dehydrogenase and glucose dehydrogenase for the conversion of the by-product acetophenone.[31] The combination of transaminase and dehydrogenases was also reported by Truppo at al. (2010) to convert the by-product. A product concentration of 50 g/L (0.4 mol/L for MBA) was achieved.[11] Besides, bifunctional α,ω-DTA/ω-ATA enzymes are studied to accept tailor-made amine donors. The spontaneous secondary reactions after deamination shift the reaction equilibrium to a theoretical 100% yield.[32] Another option is to utilize an ion-exchange resin for chiral amine removal to

overcome product inhibition. This strategy allows the product to be easily recovered by filtration and washing of the resin. Langermann's group (2018, 2020) studied the abilities of the ion-exchange resins to recover the products of imine reductase (IRED)-catalyzed reactions and regioselective enzymatic carboxylation reactions.[33,34] High purities >99% of the products were obtained after purification by an adsorption-desorption cycle of the resins. Although these are valuable approaches of ISPR, there are still some drawbacks to overcome, such as the treatment of the extra byproducts caused by side reactions, poor selectivity of the absorbents and the decrease of the enzyme's stability.[6]

Membrane contactors, which provide interfaces between product solutions and extraction phases, have the potential to tackle the above limitations. The applications of membranes in biocatalytic processes and various other applications have been reviewed.[35-39] Nowadays, pilot-scale and full-scale applications of membrane contactors have been demonstrated in the chemical, pharmaceutical, biotechnological, environmental, and food processing areas.[40] Particularly for amine transaminase catalyzed chiral amine production, membrane extraction is applied for ISPR to shift the reaction equilibrium. Shin et al. (2001) used a hydrophobic membrane contactor to continuously remove the inhibitory ketone in an amine transaminase catalyzed reaction.[41] Rehn et al. (2014, 2015, 2016) adopted the supported liquid membrane contactors to extract the amine product constantly.[42-44] The alkaline conditions at the feed side of the membrane kept the amines mainly deprotonated, while the acid conditions at the permeate side protonated the extracted amines, avoiding back-extraction. It achieved a product concentration of 1.0 mol/L after operation for 91 hours.[44] Besides, Satyawali et al. (2016, 2017) tested the hydrophobic polypropylene (PP) membranes and ceramic nanofiltration membranes for *in situ* amine product removal.[45,46] The *n*-heptane solvent was used to achieve an enhanced solubility of the poorly water soluble substrate benzyl acetone. It allowed the product to be removed by an aqueous phase with a membrane contactor. The approach resulted in 1.4-times higher substrate conversion than the process without ISPR, owing to (i) the alleviation of product inhibition and (ii) a favorable shift in the thermodynamic equilibrium.[45]

Although the technical feasibility and advantages of integrating *in situ* membrane extraction with transaminase catalyzed chiral amines production have been demonstrated on a lab-scale,[41-46] there is currently no full-scale installation for this process. Moreover, the availability of economic assessments of such an integrated process is limited. Ho et al. (2019) validated the economic feasibility of biocatalytic continuous manufacturing of a typical chiral amine, sitagliptin.[47] Tufvesson et al. (2015) studied thermodynamic and economic constraints for the different choices of designing a biocatalytic transamination process, particularly the choice of amine donor.[4] They also set targets for the process parameters of amine donor excess, process intensity, and biocatalyst yield, but did not consider the membrane extraction for ISPR. Therefore, it is unclear whether the integrated process is economically viable. For brevity, we refer to this biocatalytic process of chiral amine synthesis integrated with membrane extraction as Case 1 henceforth.

We extend beyond the single-case by modelling alternative production pathways, which makes it possible to benchmark Case 1 against alternatives. Given that the manufacturing cost is influenced by economies of scale, which is further decided by the market for a specific product, two representative chiral amine products of (R)-(+)- or (S)-(-)-α-methylbenzylamine (MBA) and

sitagliptin are considered in this study. α-Methylbenzylamine (MBA) is called by 1-Phenylethan-1-amine or 1-Phenylethylamine (PEA) as well. MBA is a widely used chiral amine for the preparation of enantiomerically pure compounds. As a benchmark technology, we selected a transaminase catalyzed process with ISPR using an ion-exchange resin to produce MBA. This process was demonstrated to be productive and scalable, by reaching a substrate concentration of 50 g/L with amine donor alanine and by-product conversion.[11] Generally, achieving this concentration is considered a requirement for an industrial application of a biocatalyst.[48] Further in the paper, we refer to this as Case 2. The other product sitagliptin is an active ingredient in, for example, JANUVIA and JANUMET, which support the treatment of Type 2 diabetes.[49] An asymmetric synthesis process of sitagliptin using engineered transaminase is chosen as one of two benchmark technologies. Given that there is no commercially available transaminases for amination of the pro-sitagliptin ketone, Savile et al. (2010) developed an engineered transaminase for efficient sitagliptin synthesis.[12] A combination of in silico design and directed evolution was applied to create the enzyme. Their work showed that designed biocatalysis offers the possibility to prepare different optically pure amines from the corresponding ketones, even including bulky-bulky ketones.[50] The process with engineered transaminase is considered as a showcase of green chemistry and process intensification, which has been demonstrated on pilot-scale.[12,51] It is referred to as Case 3 in this study. To compare the biocatalytic process with a chemical synthesis route, an asymmetric direct reductive amination process for producing sitagliptin is chosen as the other benchmark. The researchers developed a rhodium-based catalyst for asymmetric hydrogenation of pro-sitagliptin to synthesize the chiral amine. This route is a three-step one-pot synthesis of dehydro-sitagliptin.[49] The reaction reached a high conversion rate of 98% by yielding 95% ee sitagliptin. It needed an additional crystallization to increase the optically purity to 99.9% ee, which is > 99.95% ee of Case 3 using engineered transaminase.[51] Henceforth, we refer to this process as Case 4. The detailed process flows of the four cases are illustrated in Figures 1–4 of Section 2.2. Case 1 is benchmarked to Cases 2–4 for MBA and sitagliptin production by performing a techno-economic assessment (TEA) for the four cases. Comparing the results of the TEA serves to support the process design of Case 1 by providing development targets for the membrane-based ISPR. We focus on estimating the membrane cost range, flux, and selectivity. This makes it possible to obtain a competitive manufacturing cost for the selected chiral amine product, given the selected benchmark cases.

The paper is organized as follows. In the introduction, we have outlined that membrane-based ISPR for biocatalytic chiral amines production is demonstrated to be technologically feasible and promising. However, an economic outlook and resulting development targets of such an integrated process are lacking. To that end, benchmarking is required with alternatives. In the methods section, we position TEA as a decision support framework and explain how we have built the models that assess the economic feasibility for the four selected cases. The results and discussion section holds the findings that enable inferring development targets for membrane-based ISPR. The final section concludes.

2. Methodology and assumptions

2.1. Techno-economic assessment

To ensure decision making quality, the following generic steps need to be taken: (1) decide on the decision framework, (2) develop alternatives, (3) gather the required data and assess its reliability and uncertainty, (4) evaluate the alternatives using the logic set out in the framework on the selected indicators, (5) apply logical reasoning to interpret the findings and draw meaningful conclusions, (6) provide recommendations to a decision maker.[52,53]

In the present study, these steps were taken by performing a TEA. The decision-making, such as process design choices, was supported with a TEA by quantifying economic criteria resulting from a cash-flow analysis that dynamically links technical and economic parameters. The TEA methodology consists of four steps: market study, process flow diagram and mass and energy balance, economic analysis, and uncertainty analysis.[36,37,54] Step 1 identifies the market trends, related prices, and competitive processes, etc. Step 2 describes the alternatives in more detail based on their process flow diagrams. It also outlines the modelling assumptions (a) that are key to enable comparability across the evaluation of the selected alternatives and (b) that are specific to each alternative or case. In this study, two target products, MBA and sitagliptin, are considered using the production process detailed in Case 1. The latter is benchmarked to Case 2 for MBA production, and compared with Case 3 and 4 for sitagliptin production, as described in the Introduction. Further, in Step 2, the mass and energy balances of the four processes are calculated by identifying and quantifying the different input and output streams. Step 3 assesses the economic profitability of the four cases by estimating the net present value (NPV) and manufacturing cost. The NPV takes into account the discounted revenues and costs from each year of the lifetime of a project. A positive NPV indicates an economically feasible project.[54] The technological and economic parameters are integrated by linking the prices of the inputs and outputs to the mass and energy balance of the process. Step 4 entails a Monte Carlo sensitivity analysis to identify the impact of the key parameters on the manufacturing cost. The influence of the input parameters is evaluated by drawing 10,000 observations from the respective distributions and recalculating the manufacturing cost, using the Crystal Ball extension.[55] The triangular distribution (−10%; +10% from the default values) is applied to the input parameters.[54] The rank order correlation can then be used to identify which of the input parameters cause the most variation in the manufacturing cost. This is followed by local sensitivity analyses on those parameters. These steps enable us to obtain our final goal, which is to derive meaningful development targets for Case 1.

2.2. Process flow diagrams of the four cases

The four cases make use of different strategies to increase the reaction conversion rate of chiral amine synthesis. Their process flow diagrams analyzed in the TEA are shown in Figures 1–4. The process details are described in S.I. Section S1.

2.2.1. Case 1: Transaminase catalyzed synthesis of chiral amines driven by membrane extraction for ISPR

Case 1 employs membrane extraction for *in situ* product amine recovery to shift the reaction equilibrium. Two product amines – namely, MBA and sitagliptin – were analyzed for this case. Substrate ketone acetophenone, amine donor IPA, transaminase, and co-factor pyridoxal 5′-phosphate (PLP) are added to a reaction buffer with pH 9 in the reactor to synthesize the product amine. For both MBA and sitagliptin production, a membrane contactor is used for *in situ* product recovery. The product amine MBA/sitagliptin is extracted by a membrane stripping phase consisting of a buffer with pH 3.[43] A part of the amine donor, substrate ketone, and by-product ketone also permeate through the membrane to the extraction phase. An ultrafilter is placed before the membrane contactor to prevent the enzyme from entering the membrane contactor and to recycle the biocatalyst.[42,45] When the reaction at 40 °C has reached equilibrium after 120 hours, the extracted product solution is transferred to the downstream processing. A common approach of liquid–liquid extraction followed by distillation is used for the downstream processing, given that liquid–liquid extraction can be applied to recover a wide range of amines in a transamination system.[6]

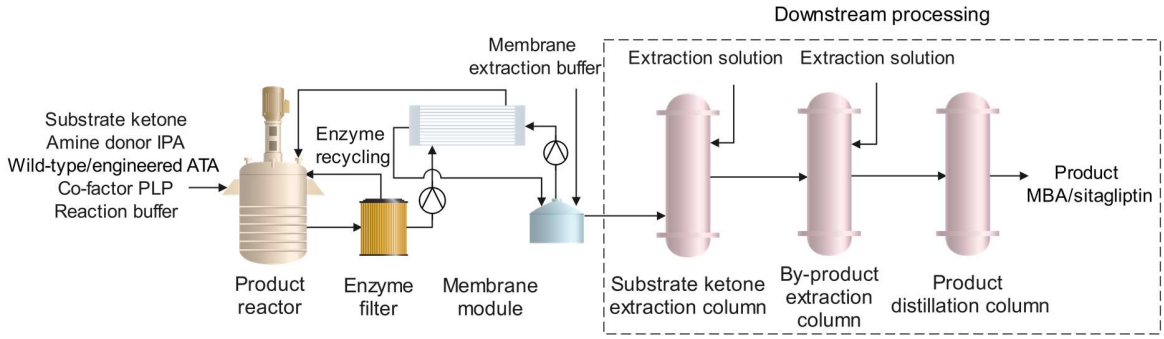


Figure 1. Process flow diagram of Case 1: Transaminase catalyzed synthesis of chiral amines driven by membrane extraction for ISPR.

2.2.2. Case 2: Transaminase catalyzed synthesis of chiral amines with ISPR using an ion-exchange resin

Case 2 depicts a route to produce MBA, utilizing an ion-exchange resin for *in situ* product amine recovery. It allows the reaction to be carried out at a high substrate concentration of 50 g/L. The production process is batch. The ion-exchange resin is used to absorb the product amine during the reaction for ISPR. A different amine donor, alanine, is employed in this process, which generates by-product pyruvate, which strongly inhibits the enzyme transaminase. Thus, an enzyme cascade consisting of the co-factor nicotinamide adenine dinucleotide (NAD^+), the enzymes glucose dehydrogenase (GDH) and lactate dehydrogenase (LDH), and glucose are added to convert by-product pyruvate into L-lactate.[11] For downstream processing, the ion-exchange resin with product amine is separated by filtration. The liquid–liquid extraction and distillation are again used for product recovery.

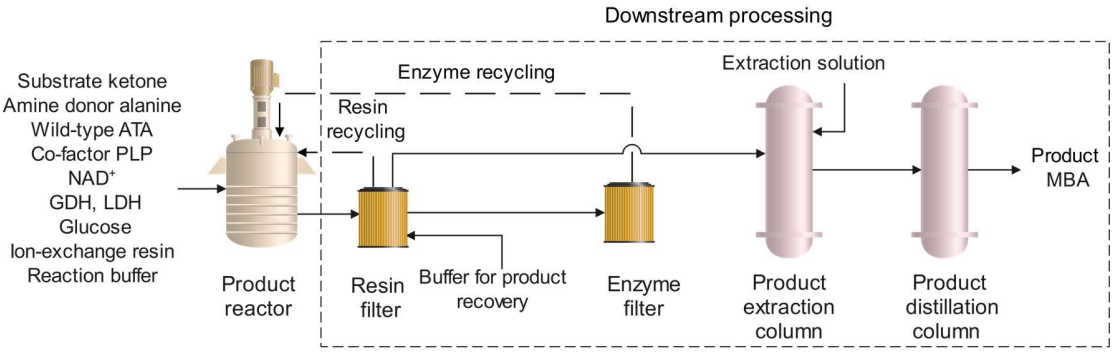


Figure 2. Process flow diagram of Case 2: Transaminase catalyzed synthesis of chiral amines with ISPR using an ion-exchange resin.

2.2.3. Case 3: Asymmetric synthesis of chiral amines with engineered transaminase

An engineered transaminase without ISPR is utilized to produce sitagliptin in Case 3. Due to the low solubility of the pro-sitagliptin ketone in an aqueous solution without ISPR, an organic cosolvent dimethyl sulfoxide (DMSO) is added to prevent precipitation during the reaction. The production process is batch. The downstream processing is assumed to consist of the same process steps as Case 1. The reaction solution goes through two extractors and a distillation column in a row to separate the substrate ketone, the by-product, and the product amine, respectively.

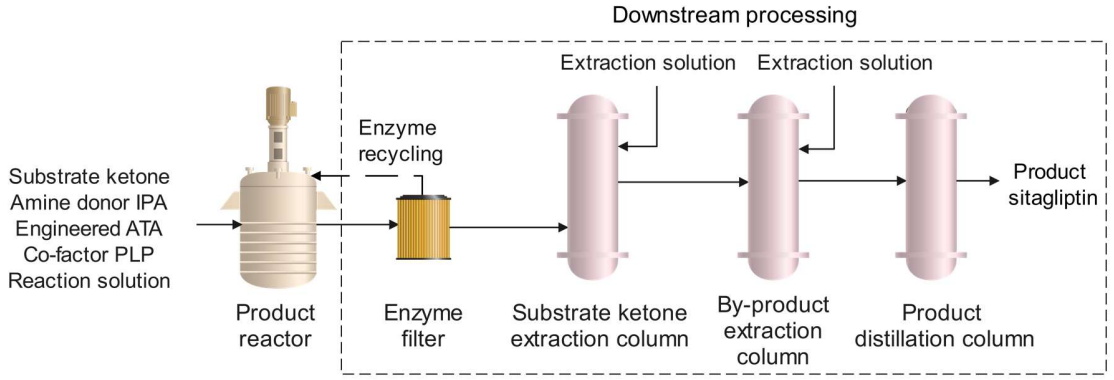


Figure 3. Process flow diagram of Case 3: Asymmetric synthesis of chiral amines with engineered transaminase.

2.2.4. Case 4: Chiral amines production with ruthenium-catalyzed asymmetric hydrogenation

Researchers at Merck developed a rhodium catalyzed process for sitagliptin production, taken as Case 4 in this study. The process starts from trifluorophenyl acetic acid and applies a three-step one-pot synthesis of dehydro-sitagliptin.[49] To compare the processes with an equal starting point, the process flow starts with the same pro-sitagliptin. A reactor, a filter and a dryer are employed to synthesize and recover the intermediate ketone from the substrate ketone, pro-sitagliptin. The catalyst $[(COD)RhCl]_2$ dimer with ligand ^{t}Bu JOSIPHOS are added to a methanol

solution in the reactor to generate the product sitagliptin. Absorbent Ecosorb C-94117 is used to remove the catalyst. The product is recovered by crystallization and drying, as shown in Figure 4.

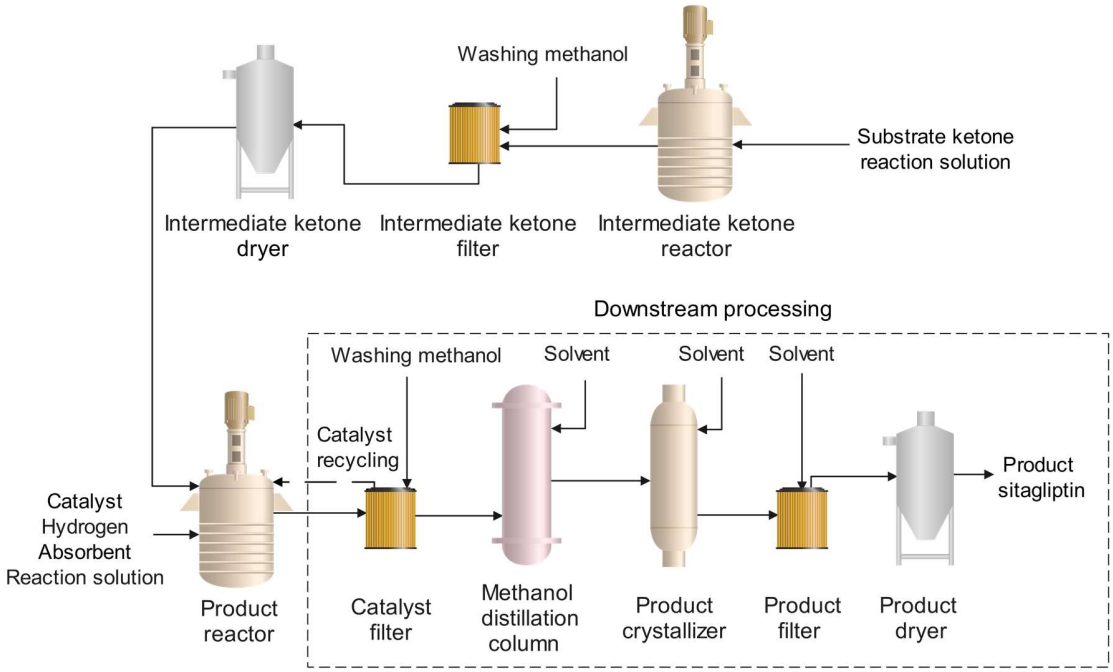


Figure 4. Process flow diagram of Case 4: Chiral amines production with ruthenium-catalyzed asymmetric hydrogenation.

2.3. TEA model assumptions

2.3.1. Process assumptions

The processes of the four cases described in Section 2.2 and S.I. Section S1. are represented in the TEA model. The process parameters that were used for the four cases in the TEA are listed in Table 1 and S.I. Table S1. To compare the four cases, the same amount of mol targeted product is produced for each case. The capital and operating costs are determined based on the same annual yield of 62,500 mol/year (see Table 1). No solvent recycling is considered in the TEA.

Table 1. The process parameters for the four cases.

Item	Case 1	Case 2[11]	Case 3[12]	Case 4[49]	Refs.
Product amine	MBA[a] Sitagliptin	MBA[a]	Sitagliptin	Sitagliptin	-
Production mode	Semi-batch	Batch	Batch	Batch	-
In situ product recovery	Membrane extraction	Ion-exchange resin	None	None	-
Annual product amine yield[b]	62,500 mol (7,563 kg for MBA; 25,438 kg for sitagliptin)				-

Table 1. (continued)

Item	Case 1		Case 2[11]	Case 3[12]	Case 4[49]	Refs.
Product amine	MBA[a]	Sitagliptin	MBA[a]	Sitagliptin	Sitagliptin	-
Product concentration (mol/L)[c]	0.5[b]		0.375	0.5	0.25	[11, 12, 49]
Catalyst	Wild-type enzyme	Engineered enzyme	Wild-type enzyme	Engineered enzyme	Rh-based catalyst	-
Amine donor excess	10	8	2.5	8	-	[11, 12, 42, 45]
Substrate ketone conversion rate (%)	89[d]	92	90	92	91	[11, 12, 49]

[a](R)-(+)- or (S)-(-)-α-methylbenzylamine (MBA).

[b]An annual product amine yield of 62,500 mol/year is set for the four cases, because a 5,000 liters reactor and one week for one process step in the pharmaceutical industry are common.[56] The usual requirement of 0.5 mol/L product concentration for an economically relevant biocatalyst process is considered.[4,11] These assumptions lead to a production of 62,500 mol/year in 25 weeks (~50% occupancy).

[c]Product concentration in the solution that is going to the downstream processing, representing the product concentration in the extractant solution for Case 1, and the product concentration in the reaction solution for Cases 2-4.

[d]The "Substrate ketone conversion rate" for Case 1 means the mole rate of product amine in the extractant solution to the substrate ketone. It is calculated by eq. 10 in Section 2.3.2.

2.3.2. Equilibrium equation of the synthesis reaction with *in situ* membrane extraction

In Case 1, membrane extraction is applied for ISPR to shift the reaction equilibrium by selectively removing the product. Increasing the membrane selectivity allows raising the conversion rate, which further decreases the manufacturing cost. To analyze the impact of the membrane selectivity on the conversion rate and the manufacturing cost, we start from the known equilibrium equation (eq 2).

For a reaction shown in eq 1, the equilibrium constant K is expressed as eq 2:

$$\mathrm{aA} + bB + \cdots \rightleftharpoons cC + dD + \cdots \quad (1)$$

$$K = \frac{[C]^c \times [D]^d \times \ldots}{[A]^a \times [B]^b \times \ldots} \quad (2)$$

K – equilibrium constant

$A,B,\ldots$ – reactants

$C,D,\ldots$ – products

$[X]$ – equilibrium concentration of X in moles

When *in situ* membrane extraction is used to recover one or more products, part of the reactants might also permeate through the membrane. When the membrane stops extracting the substances, the substance concentrations in the reactor remain stable. The equilibrium constant K_{ISM} in the reactor is formulated as eq 3:

$$K_{ISM} = \frac{[C_R]^c \times [D_R]^d \times \ldots}{[A_R]^a \times [B_R]^b \times \ldots} \quad (3)$$

K_{ISM} – equilibrium constant of the reaction with membrane extraction

A_R, B_R,… – remaining reactants in the reactor

C_R, D_R,… – remaining products in the reactor

The concentration of the total product C generated in the reaction is calculated as eq 4:

$$[C]_0 = [C_R] + [C_{EX}] \quad (4)$$

C_{EX}– extracted product C by the membrane

According to eq 1, the concentrations of the remaining substances in the reactor are expressed as eqs 5–7. In these equations we express everything as a function of product concentrations. The F_{SX} factors that are defined describe the selectivity of the membrane for the different components compared to the product:

$$[D_R] = \frac{d}{c} \times ([C_R] + [C_{EX}]) - \frac{[C_{EX}]}{F_{SD}} \quad (5)$$

$$[A_R] = [A]_0 - \frac{a}{c} \times ([C_R] + [C_{EX}]) - \frac{[C_{EX}]}{F_{SA}} \quad (6)$$

$$[B_R] = [B]_0 - \frac{b}{c} \times ([C_R] + [C_{EX}]) - \frac{[C_{EX}]}{F_{SB}} \quad (7)$$

$[A]_0$, $[B]_0$ – initial concentration of A/B in the reactor at the start of the reaction

F_{SX}– ratio of the concentration of the product C to the concentration of component X (A, B or D) in the membrane extractant solution

Finally, the overall equilibrium constant formula of the reaction with membrane extraction is obtained as eq 8:

$$K_{ISM} = \frac{[C_R]^c \times (\frac{d}{c} \times ([C_R] + [C_{EX}]) - \frac{[C_{EX}]}{F_{SD}})^d \times \ldots}{([A]_0 - \frac{a}{c} \times ([C_R] + [C_{EX}]) - \frac{[C_{EX}]}{F_{SA}})^a \times ([B]_0 - \frac{b}{c} \times ([C_R] + [C_{EX}]) - \frac{[C_{EX}]}{F_{SB}})^b \times \ldots} \quad (8)$$

For a biocatalytic transamination reaction, the reaction scheme is shown in eq 9. The equilibrium constant formula with *in situ* membrane extraction is developed as eq 10 (exponential factors a, b, c, d all equal to 1). As mentioned above, the F factors express the selectivities of the membrane.

$$substrate\ ketone + amine\ donor \rightleftharpoons product\ amine + byproduct\ ketone \quad (9)$$

$$K_{ISM} = \frac{[P_R] \times ([P_R] + [P_{EX}] - \frac{[P_{EX}]}{F_{PBP}})}{([SK]_0 - ([P_R] + [P_{EX}]) - \frac{[P_{EX}]}{F_{PSK}}) \times (e_{ADE}[SK]_0 - ([P_R] + [P_{EX}]) - \frac{[P_{EX}]}{F_{PAD}})} \quad (10)$$

P_R – remaining product amine in the reactor

P_{EX} – extracted product amine by the membrane

$[SK]_0$ – the substrate ketone concentration at the start of the reaction

e_{ADE} – amine donor excess

F_{PBP} – concentration ratio of product/by-product in the membrane extractant solution

F_{PSK} – concentration ratio of product/substrate ketone in the membrane extractant solution

F_{PAD} – concentration ratio of product/amine donor in the membrane extractant solution

Rehn et al. (2014) performed the MBA asymmetric synthesis using amine transaminase (wild type enzyme) in a reaction solution pH=9, without any ISPR action. After 24 hours, the reaction system reached equilibrium with a stable MBA concentration. In their experiment, the substrate solution (57mL) initially contained 1.14 mmol acetophenone and 28.5 mmol IPA (25-fold excess). The total conversion rate was 50%.[42] Accordingly, the substance concentrations at equilibrium are:

[product amine] = 0.01 mol/L

[by-product ketone] = 0.01 mol/L

[substrate ketone] = 0.01 mol/L

[amine donor IPA] = 0.49 mol/L

When putting these values of the substance concentrations in eq 2, the equilibrium constant K = 0.02 is obtained for MBA synthesis reaction catalyzed by amine transaminase (wild type enzyme) in a reaction solution pH=9.

Moreover, Rehn et al. (2014) added *in situ* membrane extraction to the same MBA asymmetric synthesis. MBA remaining concentration is 0.005 mol/L in the reactor.[42] Combined with the amine donor excess of 10-fold, the concentration of the extracted product amine of 0.5 mol/L and the concentration ratios they found in the extractant solution (F_{PX} values listed in Table 2), we can derive the starting concentration of the substrate ketone = 0.56 mol/L from eq 10. Furthermore, the conversion rate of the substrate ketone is 89%.

The membrane parameters measured/derived from the research of Rehn et al. are considered as representative values for membrane extraction coupled to a transaminase reaction.[42] Therefore, these membrane parameters are used to calculate Case 1, both for MBA and sitagliptin production. Table 2 gives an overview of the membrane parameters. The membrane flux (extraction rate) of product amine is assumed to be 0.1 mol/hm^2. We conducted experiments for the MBA synthesis with a dense PDMS membrane in a contactor (it was presented at conferences.[57,58]) that supports this value. Further in this paper, the effect of variation of this value

is studied (see Figure 12) as it might be considered high compared to previous reports. The membrane parameters in Table 2 are also applied for sitagliptin production in this study due to the absence of a comparative study in the literature for sitagliptin synthesis. However, it is essential to mention that due to the hydrophobic nature of the membrane and the presence of cosolvent DMSO in the sitagliptin synthesis, the membrane may not perform entirely as predicted. It might cause wetting of the hydrophobic membrane fibres and leaching of the organic phase into the extractant phase. Yet, this could also be avoided or mitigated by adjusting the pressures on the two sides of membranes or by screening for the most suitable membranes. For example, Satyawali et al. (2017) obtained a similar high conversion rate of 99% in biocatalytic synthesis of chiral amine in organic solvent with *in situ* membrane product extraction.[45] Nevertheless, we can remark that the stability of the dense hydrophobic membranes, we used in multiple ISPR experiments with MBA (Case 1), will not be an issue, as they are widely solvent stable.[59] Therefore, for the ease of comparison and given the absence of membrane assisted sitagliptin synthesis in the literature, we have used the same values for both processes while being fully aware of the differences.

Table 2. Membrane parameters in Case 1 for both MBA and sitagliptin production.

Item	**Flux of product amine**	F_{PSK}[a]	F_{PAD}[a]	F_{PBP}[a]	**Refs.**
Unit	mol/hm^2	mol product/mol substrate ketone	mol product/mol amine donor	mol product/mol by-product ketone	-
Value	0.1	14	1	7	[57, 58, 42]

[a]F_{PX} – ratio of the concentration of the product amine to the concentration of component X (substrate ketone, amine donor or by-product ketone) in the membrane extractant solution.

However, other reaction parameters will be used for Case 1 producing sitagliptin, consistent with a higher conversion efficiency using the engineered enzyme. As mentioned already in Table 1, a higher conversion of 92% will be used in this case, and a lower donor amine excess of 8-fold. These are the same values used in Case 3 (engineered enzyme without ISPR), and that were experimentally determined by Savile et al. (2010).[12] To capture the positive effect of the ISPR on the transaminase with engineered enzyme, it was assumed that 2x less enzyme could be used in Case 1 compared with Case 3 to produce the same amount of sitagliptin. Truppo et al. (2010) reported 10 times more enzyme yield using ISPR for chiral amine synthesis.[11] The enzyme inhibition caused by reaction substances is significantly reduced. Accordingly, less enzyme loading is required. A sensitivity analysis will be performed to estimate the influence of enzyme yield on the manufacturing cost.

2.3.3. Economic input parameters

The economic feasibility of each case is assessed by calculating the NPV with S.I. eq S1. The lifetime is 10 years, with a discount rate of 10%. The manufacturing costs, which include

capital costs for process equipment and operational costs of raw materials, utilities, waste management, and labor, are estimated for the four cases in the TEA. The economic input parameters required for estimating the NPV and manufacturing cost are listed in Table 3 and S.I. Table S2. The detailed calculations for the equipment sizes and costs are described in S.I. Section S3.

Table 3. The economic input parameters for the four cases.

Item	Unit	Value	Refs.
Economic lifetime	year	10	-
Discount rate	%	10	[54]
Working hours per year (~50% utilization)	h	4,000	-
Wage rate personnel	€/h	28.5	[60]
MBA[a] selling price	€/kg	125	[4]
Sitagliptin selling price	€/kg	500	[61]

[a](R)-(+)- or (S)-(-)-α-methylbenzylamine (MBA).

3. Results and discussion

3.1. Mass and energy balance

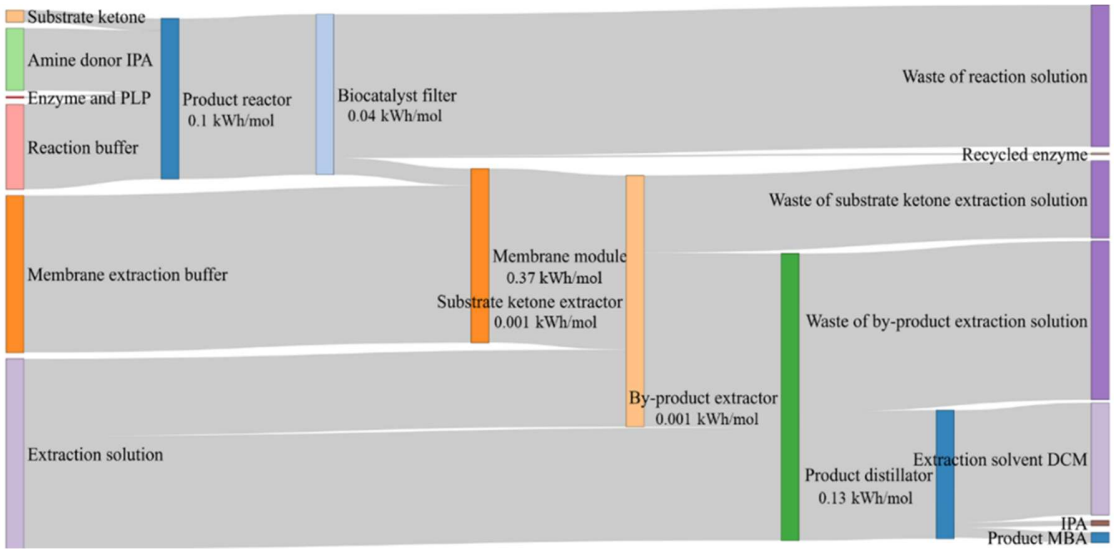
Based on the material inputs described in Section 2.2 and S.I. Section S1, and the energy consumption of each equipment calculated in S.I. Section S3, the mass and energy balance of the four cases are created, with the results shown in Table 4. The mass input and output and the energy consumption of each unit for the four cases are illustrated in Figure 5. The energy consumption of Case 1 is higher than Case 2 and 3. It is mainly attributed to the energy consumption of the membrane module (0.37 kWh/mol), given that two pumps are used to continuously circulate the reaction and extractant solutions for ISPR during the reaction. For per mol of amine product, Case 3 has the lowest material input, waste output, and energy consumption. This can be explained by the relatively high product concentration (0.5 mol/L) and the simple process without ISPR-related steps. In Case 1, the membrane extraction phase adds a material input of 1.85 kg/mol product. Case 2 has a higher material input than Case 1 because of a lower product concentration (0.375 mol/L) and an extra solvent required for washing the ion-exchange resin in the filter. Case 4 has the highest material input, waste amount, and energy consumption, resulting from the complex production process.

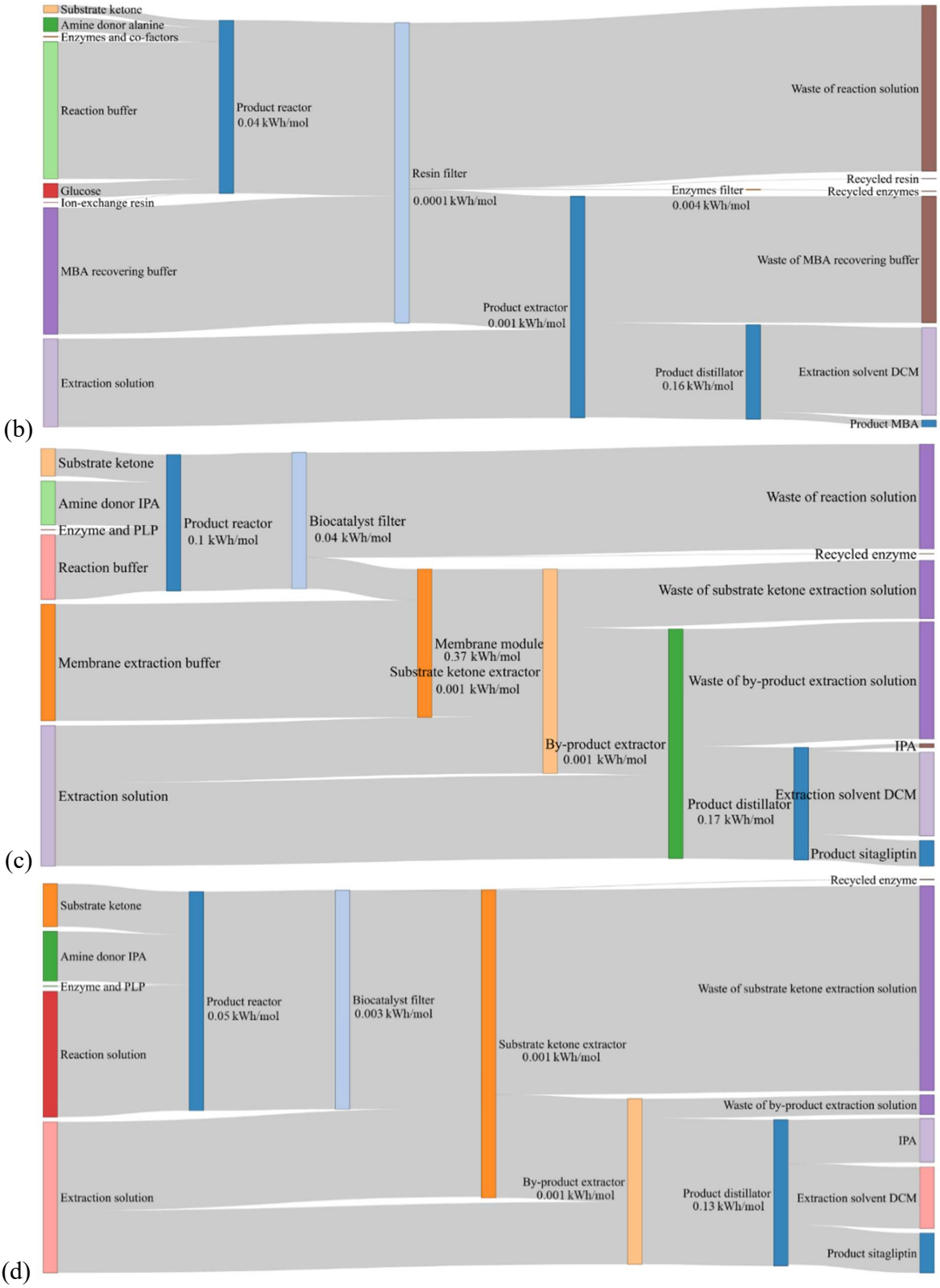
Table 4. Mass and energy balance of the four cases.

Items	Unit	Case 1		Case 2	Case 3	Case 4
		MBA[a]	Sitagliptin			
Energy consumption	kWh/mol product	0.64	0.68	0.21	0.19	1.79
Input	kg/mol product	5.982	6.274	6.685	3.797	15.837
Output of waste	kg/mol product	5.861	5.867	6.564	3.390	15.430
Output of product	kg/mol product	0.121	0.407	0.121	0.407	0.407

[a](R)-(+)- or (S)-(-)-α-methylbenzylamine (MBA).

(a)




Substrate ketone
Amine donor alanine
Enzymes and co-factors
Reaction buffer
Glucose
Ion-exchange resin
MBA recovering buffer
Extraction solution
Product reactor
0.04 kWh/mol
Resin filter
0.0001 kWh/mol
Product extractor
0.001 kWh/mol
Waste of reaction solution
Recycled resin
Enzymes filter
0.004 kWh/mol
Recycled enzymes
Waste of MBA recovering buffer
Product distillator
0.16 kWh/mol
Extraction solvent DCM
Product MBA
(b)
Substrate ketone
Amine donor IPA
Enzyme and PLP
Reaction buffer
Membrane extraction buffer
Extraction solution
Product reactor
0.1 kWh/mol
Biocatalyst filter
0.04 kWh/mol
Membrane module
0.37 kWh/mol
Substrate ketone extractor
0.001 kWh/mol
By-product extractor
0.001 kWh/mol
Waste of reaction solution
Recycled enzyme
Waste of substrate ketone extraction solution
Waste of by-product extraction solution
IPA
Extraction solvent DCM
Product distillator
0.17 kWh/mol
Product sitagliptin
(c)
Substrate ketone
Amine donor IPA
Enzyme and PLP
Reaction solution
Extraction solution
Product reactor
0.05 kWh/mol
Biocatalyst filter
0.003 kWh/mol
Substrate ketone extractor
0.001 kWh/mol
By-product extractor
0.001 kWh/mol
Recycled enzyme
Waste of substrate ketone extraction solution
Waste of by-product extraction solution
IPA
Product distillator
0.13 kWh/mol
Extraction solvent DCM
Product sitagliptin
(d)

(e)

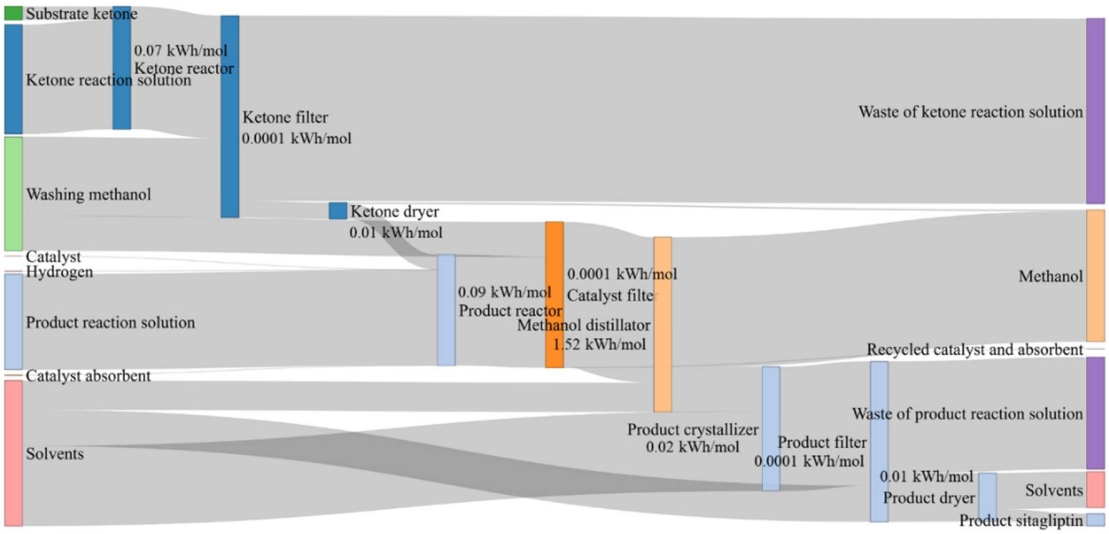


Figure 5. Mass flow and energy consumption (a) Case 1 for MBA production; (b) Case 2 for MBA production; (c) Case 1 for sitagliptin production; (d) Case 3 for sitagliptin production; (e) Case 4 for sitagliptin production.

3.2. Manufacturing costs and NPVs

The capital costs associated with the equipment costs (CapEx for synthesis and CapEx for downstream processing) and operational costs (labor, maintenance, energy, reaction solutions, catalysts, and solvents for recovery) are estimated for the four cases, as listed in S.I. Table S4. For MBA production, Cases 1 and 2 use the same reaction solution and wild-type transaminase to produce MBA. Both cases employ an ISPR strategy to shift the reaction equilibrium. The cost distributions of Cases 1 and 2 to produce MBA are illustrated in Figure 6. The results show that Case 1 has a lower manufacturing cost and a higher NPV than Case 2 (see S.I. Table S4.) For both cases, the biocatalysts and their co-factors account for the biggest share of the manufacturing costs. An amine donor alanine is employed in Case 2. Enzymes LDH and GDH, co-factor NAD^+ and glucose are added to remove the by-product pyruvate generated from alanine. Accordingly, NAD^+, GDH, LDH, and glucose bring an extra cost of €6.2/mol MBA in Case 2 given the assumed values. The cost of these enzymes is included in the item "Biocatalysts and PLP" in Figure 6. On the other hand, Case 1 employs a different amine donor, which generates the by-product acetone with smaller enzyme inhibition. Besides, part of the by-product acetone (14% is assumed in the TEA) is removed by *in situ* membrane extraction (see Table 2). The cost to remove the by-product during the reaction is avoided in Case 1.

Due to the high amine donor excesses and its high purchase prices (see Table 1 and S.I. Table S3.), the amine donors in both cases have a major share of the total manufacturing cost. The amine donor adds €2.2/mol MBA, accounting for 13% of the manufacturing cost in Case 1, and adds €3.6/mol MBA, accounting for 16% of the manufacturing cost in Case 2. The cost of amine donor IPA can be decreased by recycling the amine donor IPA from the product distillation column

at the expense of an increase in distillation cost. Approximately 10% of the total amine donor IPA permeates to the extractant solution, given that the amine donor excess is 10-fold, and the concentration ratio of the product amine to the amine donor in the membrane extractant solution is 1, as shown in Tables 1 and 2. When the amine donor IPA in the extractant solution is recovered by distillation, the amine donor cost falls slightly by €0.2/mol MBA. In addition, the waste disposal cost is also reduced by €0.04/mol MBA. The cost decrease is minor because only about 10% of the total amine donor IPA is recovered. The solvent costs for downstream processing are responsible for 13% of the manufacturing costs in Case 1 and 8% in Case 2, given the assumed values. Although Case 2 avoids the substrate ketone extractor, the CapEx of the downstream process is higher than Case 1 because an extra filter is employed to recover the absorbent resin. However, the total CapEx in Case 1 is higher than Case 2, due to the larger reactor and an extra ISPR membrane module used in Case 1. The reactor of Case 1 has a size of 5 m^3, compared to 2 m^3 for Case 2 (see S.I. Table S1) because the reaction time of Case 1 is 120 h, in contrast to the 24h of Case 2. To produce the same amount of MBA (62,500 mol/year), Case 1 requires 33 batches a year, which is five times less than the 167 batches for Case 2 (see S.I. Table S1). Accordingly, Case 1 needs a larger reactor to produce more MBA each batch than Case 2. For ISPR, the membrane module in Case 1 adds €1.9/mol MBA, covering 11% of the manufacturing cost. The ion-exchange resin costs €0.53/mol MBA when the resin is recycled 100 times given the assumed values.

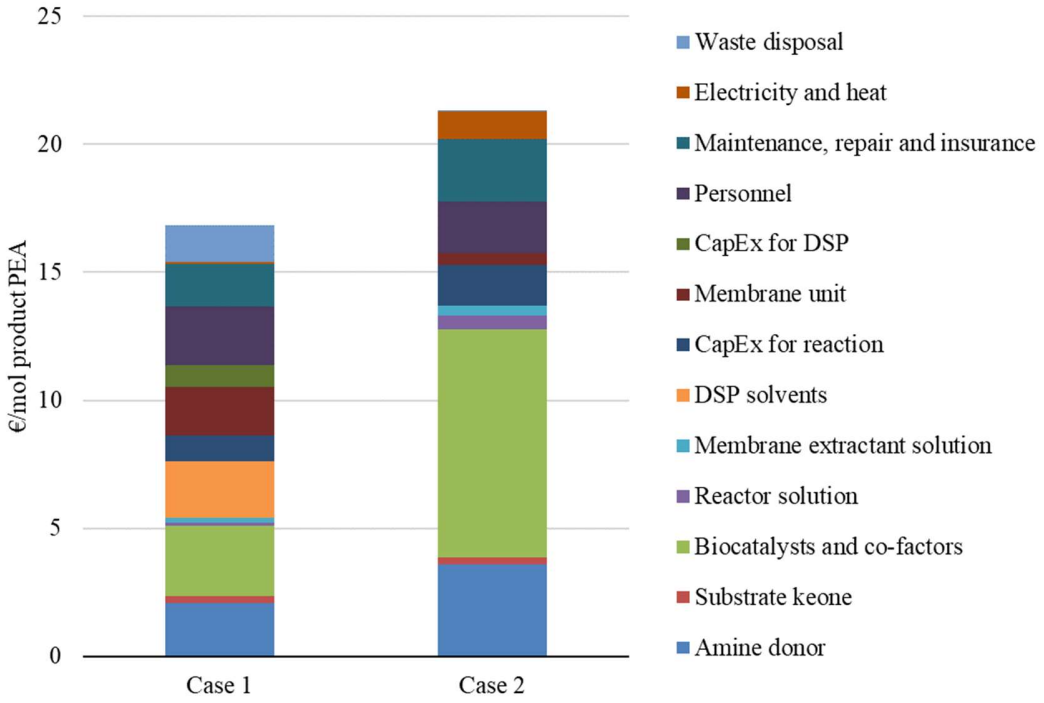


Figure 6. Breakdown of process and downstream costs (CapEx and OpEx) to produce MBA for Cases 1 and 2.

For sitagliptin production, the cost distributions and NPVs of Cases 1, 3, and 4 are illustrated in Figure 7 and S.I. Table S4. The results of the TEA show that Case 1 has the lowest

manufacturing cost and the highest NPV of the three cases. The highest manufacturing cost is reached by Case 4, due to the complex synthesis route, as described in Section 2.2. An additional reactor, filter, and dryer are needed to synthesize and recover the intermediate ketone from pro-sitagliptin, as shown in Figure 4. The manufacturing costs of all three cases are dominated by substrate ketone pro-sitagliptin, due to its high purchase price (€20/kg). The same expensive engineered transaminase and co-factor PLP are used in Cases 1 and Case 3 to synthesize sitagliptin, which have a major share of the manufacturing cost in both Case 1 (24%, causing a cost of €7.2/mol sitagliptin) and Case 2 (43%, causing a cost of €14/mol sitagliptin). The lower catalyst cost in Case 1 is attributed to a lower catalyst loading in Case 1 compared to Case 3, as described in S.I. Table S1. The catalyst $[(COD)RhCl]_2$ dimer with ligand ^{t}Bu JOSIPHOS and the catalyst absorbent Ecosorb C-94117 only cost €0.9/mol sitagliptin in Case 4, although the purchase prices of the catalyst (€6,200/kg) and the absorber (€100/kg) are high. This divergence results from the assumption that the catalyst can be recycled 10 times, with a low catalyst loading (0.001 kg catalyst/mol sitagliptin). The absorbent is recycled 100 times in the TEA. For Case 1, the membrane cost to produce sitagliptin is the same as for MBA production because the same membrane selectivities and flux are assumed for both MBA and sitagliptin synthesis, as shown in Table 2.

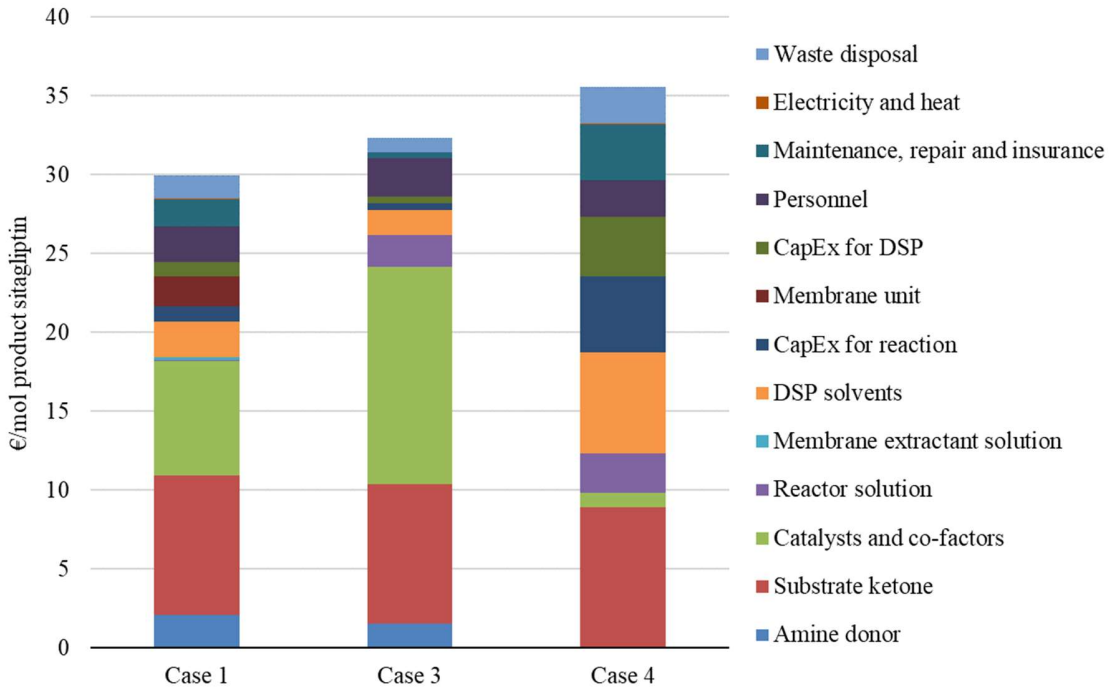


Figure 7. Breakdown of process and downstream costs (CapEx and OpEx) to produce sitagliptin for Cases 1, 3, and 4.

3.3. Monte Carlo simulation

To support the process design of Case 1, a Monte Carlo simulation is carried out to identify the parameters that have the highest influence on the manufacturing cost. The tornado plots for MBA production using the synthesis route presented as Case 1 is shown in Figure 8. As discussed above, the manufacturing cost of Case 1 is attributed mostly by the CapEx for the membrane and the OpEx for the biocatalyst, the labor, downstream processing solvents and the amine donor. They are determined by the economic and technical parameters, such as material prices, the personnel wage rate, membrane properties, and the process intensity.

Figure 8 indicates that the scale of annual production contributes the most to variation of the manufacturing cost (-23.4%). Subsequently, the product concentration in the extractant solution (-18.8%), membrane flux of product amine (-17.3%) and membrane purchase price (17.2%) explain most variation. The product concentration in the membrane extractant solution influences the solvent amounts and equipment sizes in the downstream processing. As illustrated in Figure 6, the solvents for downstream processing contribute 13% of the total manufacturing cost. The amount of solvents required in substrate ketone and product extractors is half the volume of the membrane extractant solution. The membrane cost is determined by the membrane area and the purchase price, of which the membrane area is decided by the membrane flux of product amine and the production rate (discussed in S.I. Section S3). The personnel wage rate contributes 9.2%, caused by the high wage in the EU (€28.5/h). The catalyst has the largest share of the total manufacturing cost (16%, see Figure 6). Accordingly, the biocatalyst productivity and price have contributions of -3.9% and 4.0% to the variation. Note that the parameters, such as personnel wage, purchase prices, and amine donor excess have positive impacts because the manufacturing cost increases when these parameters go up, whereas the opposite applies to the other six parameters.

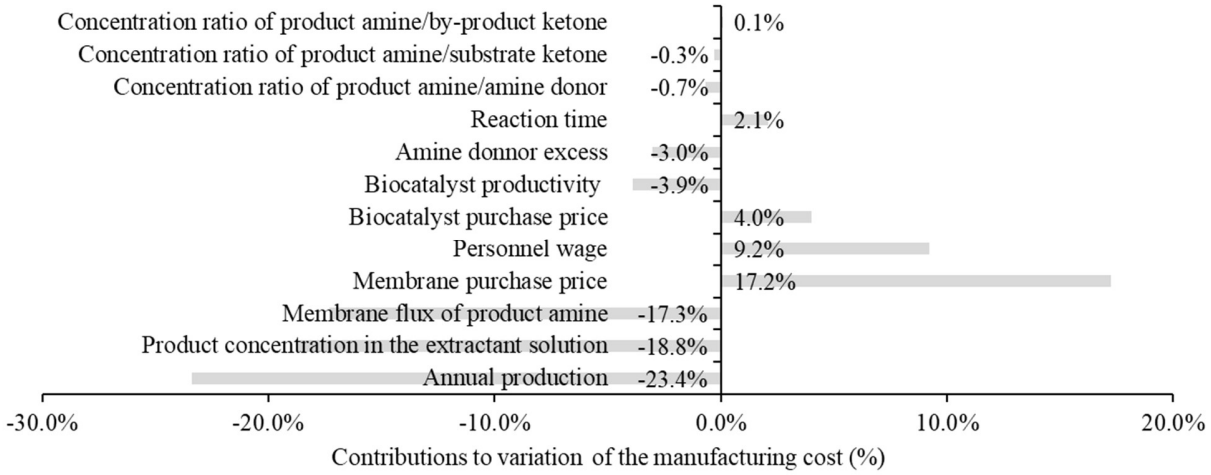


Figure 8. Monte Carlo analysis of parameter contributions to variation of the MBA manufacturing cost for Case 1.

3.4. Local sensitivity analysis

For the key parameters resulting from the Monte Carlo simulation, a local sensitivity analysis is performed, as shown in Figures 9–12 and S.I. Figures S2–4. The manufacturing cost is plotted as a function of the annual production in Figure 9. The manufacturing cost is significantly influenced by economies of scale, because manufacturing cost falls as output increases. This is consistent with the Monte Carlo analysis of Figure 8. The annual production has the biggest impact on the manufacturing cost. The manufacturing cost decreases from €50.2/mol product to €17.8/mol product, when the annual production increases from 6.25 kmol to 62.5 kmol (62.5 kmol is the default value of the annual production in the TEA model). It changes slightly, along with the annual production going up from 62.5 kmol, because the costs of the feeding materials, which are not influenced by the annual production, have a larger relative contribution to the total manufacturing cost when the annual production increases. Given that MBA is a widely used building block for pharmaceuticals and agrochemicals, the annual production of 250 kmol is feasible for the market. The cost break-down, given an annual production of 250 kmol, is illustrated in S.I. Figure S1. The biocatalyst, amine donor and the membrane still have the largest attributions to the total manufacturing cost.

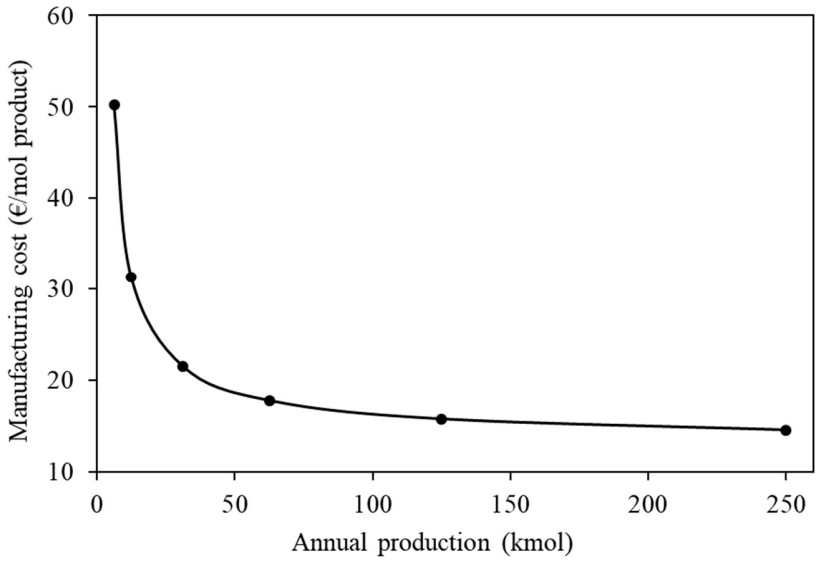


Figure 9. The manufacturing cost of Case 1 as a function of the annual production.

The model's sensitivity to the product concentration in the extractant phase on the manufacturing cost are plotted in Figure 10. The product concentration determines equipment sizes, the solvent amounts and the ease of downstream separation and recovery. Figure 10 shows that the manufacturing costs reduce quickly until the product concentrations increase to 0.5 mol/L. This is consistent with the usual requirement of 0.5 mol/L product concentration for an economically relevant biocatalyst process.[11,48,62] The total manufacturing cost decreases slower when the product concentration growing from 0.5 to 2 mol/L. It's because the costs influenced by the product

concentration, such as the costs of equipment and solvents, take a smaller share of the total manufacturing cost when the product concentration gets bigger. Rehn et al. (2016) managed to increase the product concentrations to above 1 mol/L in the extractant phase by continuous controlling the reactor pH and regenerating the supported liquid membrane unit regularly.[45] In our TEA model, the manufacturing cost decreases from €17.8/mol to €15.6/mol product, when the product concentration in the extractant phase increases from 0.5 mol/L to 1 mol/L. The cost reduction is mainly attributed to a decrease of the costs related to the extractant solution, waste disposal, downstream processing solvents and equipment. Especially, the downstream processing cost lowers with 35%.

It is worth pointing out that substrate ketones are slightly soluble in water (for example, acetophenone: 0.05 mol/L at 25 °C; 0.1 mol/L at 80 °C). In this study, the semi-batch is employed for chiral amine production. The substrate ketone is fed intermittently to the reactor in order to reach a final product concentrate of 0.5 mol/L. When the production is operated in a batch mode without feeding the substrate ketone as the reaction proceeds, the maximal product concentration will reach only 0.05 mol/L to 0.1 mol/L (the reaction temperature is 40 °C). Accordingly, the manufacturing cost will rise to €26–42/mol product, as shown in Figure 10. Another way is to add organic cosolvents (for example, dimethyl sulfoxide, n-heptane) to improve the solubility of poorly water soluble substrates.[45] However, in that case, the membrane fluxes of reaction substances will change due to the different substance solubility in a biphasic system of the reaction solution.

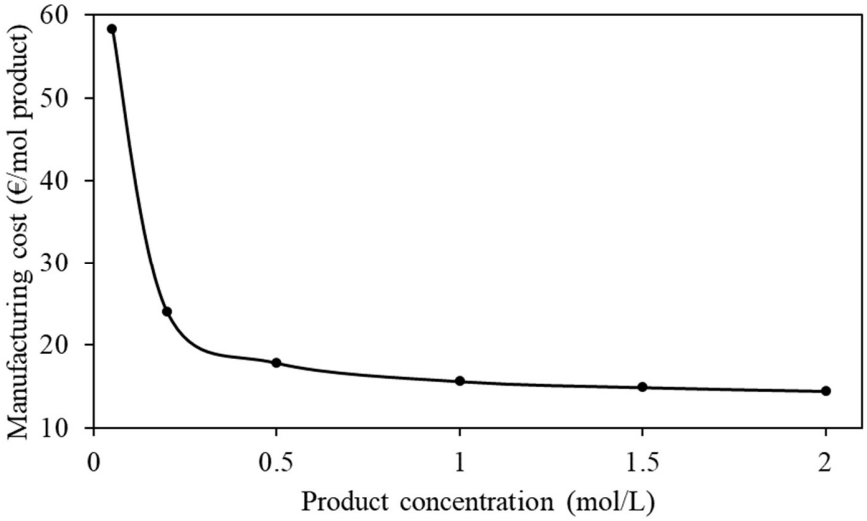


Figure 10. The manufacturing cost of Case 1 as a function of the product concentration in the membrane extractant phase.

IPA is selected as the amine donor in Case 1. Accordingly, the by-product acetone is removed by adding NaOH and DCM to the DSP, as shown in Figure 1. Amine donor excess is a common strategy for shifting the thermodynamic equilibrium of the transaminase reaction. eq 10 is built to formulate the impact of amine donor excess on the conversion rate. Accordingly, Figure 11 illustrates the conversion rate of substrate ketone and the manufacturing cost as functions of the

amine donor excess. The manufacturing cost increases slightly from €16.6 to 17/mol product, when the IPA excess rises from 1 to 5 times, with the substrate ketone conversion rate increasing from 43% to 84%. The manufacturing cost increases to €25/mol product, when the IPA excess rises to 50-fold, with the substrate ketone conversion rate growing to 92%. The cost curve is shaped by the combination of the negative impact of the amine donor cost and the positive impact of amine donor excess on the conversion rate of the substrate ketone. On the one hand, the amine donor cost per mol product increases when the amine donor excess rises. On the other hand, the substrate ketone cost per mol product reduces due to the increased conversion rate of the substrate ketone. In the base case, the amine donor has a major share of the total manufacturing cost (13%), adding a cost of €2.1/mol MBA, when the amine donor excess is 10-fold (see Figure 6).

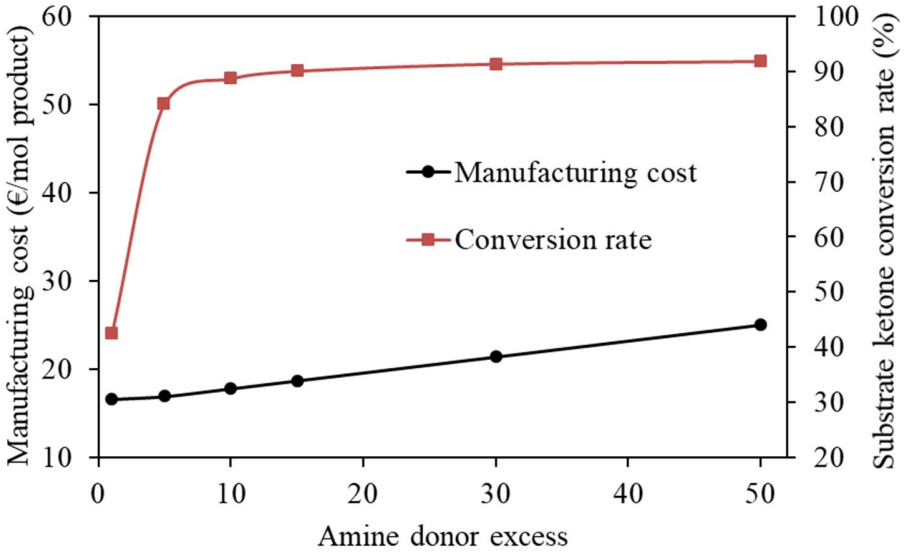


Figure 11. Manufacturing cost and substrate ketone conversion rate of Case 1 as functions of the amine donor excess.

A sensitivity analysis of the membrane flux of the product amine is performed for Case 1. The base case of membrane selectivities (concentration ratios in the extractant solution) of product amine/reaction substances is illustrated in Figure 12 with the red curve. The manufacturing cost decreases significantly when the membrane flux of the product amine rises from 0.01 to 0.1 mol/hm^2. Afterwards, the cost plot goes flat. As discussed in Section 3.2, the *in situ* extraction membrane has a major contribution to the total manufacturing cost (11%), by adding a cost of €1.9/mol product MBA, when the membrane flux of MBA is 0.1 mol/hm^2. The membrane flux of MBA achieved about 0.05 mol/hm^2 at pH 9 with a hollow fiber PP membrane contactor in the study of Rehn et al. (2016).[44] They found that the membrane flux of MBA increased with an increase of reaction pH. In recent years, some engineered ATAs for increased stability can stand a high pH above10.[63] This would benefit the *in situ* membrane extraction as it results in a higher membrane flux of the product amine. The membrane cost is obtained by multiplying the required membrane area and the purchase price. The membrane area is determined by dividing the production rate by the membrane flux of the product amine (elaborated in S.I. Section S3). When

the membrane flux of the product amine rises, the membrane cost and the contribution to the total manufacturing cost decrease, as shown in Figure 12.

The impact of the membrane selectivities (concentration ratios in the extractant solution) of product amine/reaction substances on the conversion rate for ISPR is formulated by eq 10, and is also shown Figure 12. When the concentration ratio of product amine/amine donor decreases from 1 to 0.1, the substrate ketone conversion rate declines from 89% to 77%. This decline is caused by the fact that 10 times more amine donor is removed from the reactor. Accordingly, the manufacturing cost rises from the red curve to the blue curve in Figure 12. The costs of both product amine synthesis and downstream processing increase due to more amine donor going to the downstream processing. When the concentration ratio of product amine/substrate ketone decreases to 0.1, the manufacturing cost goes up to the yellow curve with a conversion rate of 49%. The manufacturing cost does not reduce much when the concentration ratio of product amine/by-product ketone changes from 7 to 1, as shown in the black dotted curve. Because the conversion rate is increased slightly from 89% to 92%. Meanwhile, seven times more by-product ketone needs to be separated from the downstream processing.

When the membrane selectively extracts the product amine without substrate ketone permeation (F_{PSK}=infinity), as shown by the grey curve in Figure 12 below, it alleviates the need for downstream processing of the substrate ketone removal. The manufacturing cost decreases to €16/mol product, when the membrane flux of MBA, and selectivities of product amine/amine donor and by-product ketone are 0.1 mol/hm^2, 1 and 7, respectively (see the grey curve in Figure 12). Accordingly, the cost of downstream processing decreases to €1.5/mol product from € 3.1/mol product.

The membrane extraction without selectivity is analyzed in the TEA as well. It means that the production process does not have any ISPR to drive the reaction equilibrium. According to eq 10, the extracted product concentration $[P_{EX}]$ is zero. When equilibrium constant K is 0.02, and the substrate ketone's solubility (it is the same with the concentration, when keeping feeding the substrate ketone during the reaction, as a semi-batch mode) is 0.1 mol/L with an amine donor excess of 10, a conversion rate of 35% is determined by eq 10. Accordingly, the product concentration decreases to 0.035 mol/L. This low process intensity and conversion rate lead to a manufacturing cost of €136/mol product, which is much higher than the base case (€17.8/mol product).

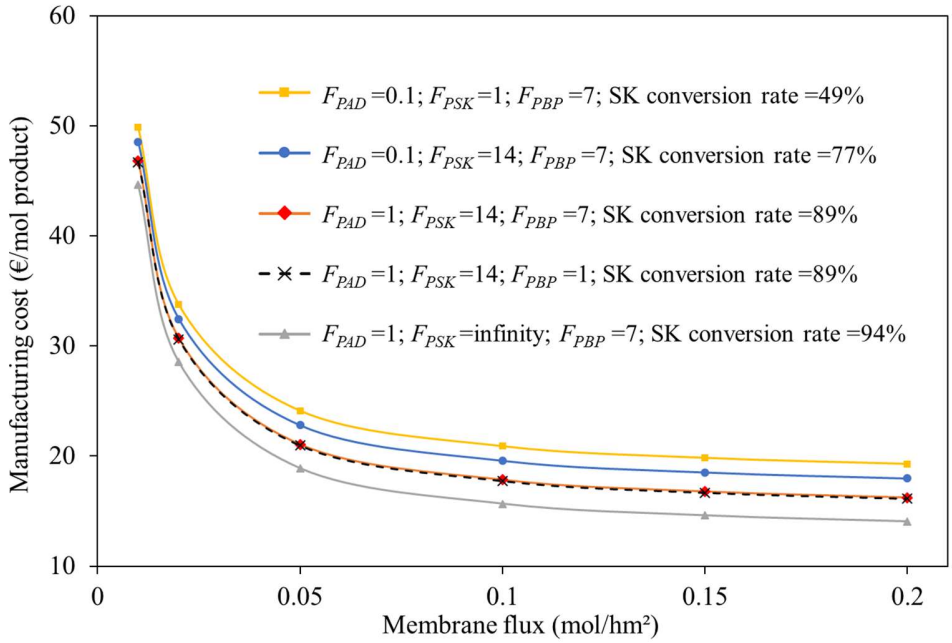


Figure 12. Manufacturing costs of Case 1 as functions of membrane flux of product amine, the concentration ratios of product amine/amine donor (F_{PAD}), product amine/substrate ketone (F_{PSK}) and product amine/by-product ketone (F_{PBP}) in the extractant solution; substrate ketone (SK).

3.5. Membrane development targets

Other than the three benchmarks of Cases 2–4, Case 1 reaches higher yields driven by membrane extraction for ISPR. Membrane parameters have an essential impact on the manufacturing cost, as shown in Figures 8 and 12. To indicate the membrane parameters' ranges for which the chiral amines production processes supported by ISPR are economically feasible and competitive, membrane development targets are set depending on the manufacturing costs. The manufacturing costs depend heavily on the target products, which decide the complexity of the reaction routes and production scales. Three target manufacturing costs of €10, 15, and 50/mol product (€100, 150, 500/kg product, assuming the product molecular weight is 100 g/mol) are set to present manufacturing different chiral amine products, which accordingly have different market prices and applications. The target manufacturing cost of €10/mol product is representative for agrochemicals, having routes involving one or multiple synthesis steps. The €15/mol cost is for the amine products, which are the building blocks applied for both agrochemicals and APIs. The €50/mol product cost is representative for APIs, having complex synthesis steps in the competing processes.

To reach the manufacturing costs of €10, 15, 50/mol product, the product concentration in the extractant solution, membrane flux of product amine, and membrane selectivities of product amine/amine donor are adjusted, as shown in S.I. Figures S5–7. These three parameters are chosen based on the Monte Carlo analysis in Figure 8. They are membrane-related parameters that make

the largest contribution to the manufacturing cost. The development targets for these three membrane parameters to meet the goal manufacturing costs are indicated in Table 5. Each parameter needs to fulfil the target value for an expected manufacturing cost. The other process parameters remain at the default values listed in Table 1 and S.I. Table S1. Pathways that make reaching such numbers more feasible are discussed below.

As can be inferred from Table 5, it will be challenging to achieve the target cost of €10/mol, where the product concentration in the extractant solution needs to increase to 12 mol/L. Rehn et al. (2014) reached a product concentration in the extractant solution of 0.5 mol/L with the extractant solution pH 3.[42] Due to the low solubility of most product amines in water solution, adding organic cosolvents can be considered (e.g., dimethyl sulfoxide, n-heptane) to enhance the product solubility. A membrane flux for the product amine of 3 mol/hm^2 is required for reaching the target cost of €10/mol. In the experimental work we did related to this case (paper to be submitted), we found that increasing cross-flow rates can result in higher fluxes. Besides, adjusting the pH difference between the membrane's two phases makes it possible to enhance the product concentration in the extractant solution and the membrane flux of the product amine. To partition the product amine from the reaction solution, the reaction solution pH needs to be set close to the pK_a value of the product amine; it deprotonates the product amine and pushes it to permeate through the hydrophobic membrane. At that pH, a higher amount of amine donor and substrate ketone molecules are charged and remain in the reaction solution. On the other hand, the membrane extractant solution is adjusted to acidic, with the pH below the pK_a value of the product amine. The product amine is protonated and trapped in the extractant solution, it enriches the product amine to reach a higher concentration in the extractant solution than in the reaction solution. In this particular case of MBA, the reaction solution pH needs to be set at around 9 to deprotonate the product amine and maintain the transaminase activity. Higher pH causes severe inactivation. The extractant solution pH <3 is considered to fully protonate the product amine.[42,43,45]

Table 5. Development targets for the membrane parameters of a biocatalytic chiral amine production process with ISPR using membrane extraction

Target cost	**Product concentration in extractant solution**	**Membrane flux of product amine**	**Concentration ratio of product amine/amine donor in extractant solution (F_{PAD})**
€/mol	mol/L	mol/hm^2	-
10	≥12	≥3	≥2.5
15	≥0.8	≥0.2	≥1.0
17.8[a]	0.5	0.1	1.0
50	≥0.3	≥0.01	≥0.3

[a]The manufacturing cost resulting from the default values used Case 1.

A concentration ratio of product amine/amine donor of 1 was obtained for the membrane used by Rehn et al. (2014).[42] A different amine donor (such as zwitter-ionic L-alanine), which is

charged at a high pH value of the reaction solution, can be chosen to prevent coextraction.[43] This is advised because partial coextraction of amine donor IPA is unavoidable, due to the similar pK_a values of IPA (10.73) and product amines (for example, 9.6 for MBA). The substrate ketone is slightly extracted with a supported liquid membrane, which can be avoided by using a tight membrane.[42,43,45] The coextraction of the by-product ketone is also expected, but is favorable for chiral amine synthesis. When the target costs are higher than the manufacturing cost of the default baseline (€17.8/mol), a lower product concentration, membrane flux, and concentration ratio of product amine/amine donor are allowed, making the process easily feasible.

4. Conclusions

To assess the economic viability of the biocatalytic synthesis of chiral amines integrated with *in situ* membrane extraction (Case 1), a techno-economic assessment (TEA) was performed. Two representative chiral amines, being MBA and sitagliptin, are selected as product amines and are modelled to be manufactured using this route. To establish whether this route is competitive, the resulting manufacturing costs are compared to those of three alternative processes, being the transaminase catalyzed synthesis of MBA with ISPR using an ion-exchange resin (Case 2), the asymmetric synthesis of sitagliptin with engineered transaminase (Case 3), and sitagliptin production with ruthenium-catalyzed asymmetric hydrogenation (Case 4). We find that, at the baseline values, Case 1 has a lower cost to produce MBA (€17.8/mol MBA) than Case 2 (€23.4/mol MBA). The ISPR membrane module in Case 1 costs €1.9/mol MBA with a membrane flux of 0.1 mol/hm^2 and a membrane purchase price of €1,500/m^2. The ion-exchange resin for ISPR adds a cost of €0.53/mol MBA with recycling 100 times in Case 2. For sitagliptin production, the manufacturing cost in Case 1 is €30.9/mol sitagliptin, which is smaller than Case 3 (€31.5/mol sitagliptin) and Case 4 (€35.5/mol sitagliptin). The performance of the membrane is crucial in reaching this cost advantage. Consequently, membrane development targets were set that make it possible to reach a manufacturing cost of €10, 15, 50/mol product. We find that it is challenging to achieve the target cost of €10/mol for agrochemical production. The product concentration in extractant solution, the membrane flux of product amine and the concentration ratio of product amine/amine donor need to increase to 12 mol/L, 3 mol/hm^2, and 2.5, respectively, when the membrane price is €1,500/m^2. It is expected to be easier to reach the target cost of €15/mol with lower parameter values of 0.8 mol/L, 0.2 mol/hm^2 and 1.0 for product concentration in extractant solution, membrane flux of product amine and concentration ratio of product amine/amine donor. We did not find any membrane processes reported being able to reach these values. However, measures such as adding organic cosolvents, increasing membrane flow rate, and adjusting the pH difference between the membrane's two phases make it possible to improve the feasibility.

Supporting Information

Process description of the four cases; TEA model assumptions of process and economic parameters; capital costs of the process equipment; manufacturing costs and NPVs; sensitivity analysis of

process parameters including reaction time, catalyst yield and membrane price; figures of membrane development targets.

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