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Advanced downstream processing for sustainable ethyl acetate production by fermentation

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Abstract

Ethyl acetate is a platform chemical conventionally obtained through fossil fuel routes, but more recently its production by fermentation from carbohydrates has been scaled up to a pilot scale. Yet, the complexity of downstream processing (low product concentrations in liquid broth and in off-gas, azeotrope formation, and the presence of microorganisms) may complicate industrial application. This original theoretical study is the first to develop advanced downstream processing, based on process intensification principles, for large-scale recovery (~10 kton/year) of ethyl acetate after fermentation. To minimize product losses, ethyl acetate is separated from both the liquid broth and off-gas. The final purification is performed in a highly integrated azeotropic dividing-wall column. The economic and sustainability analysis shows that using refrigeration for initial product separation from the gas phase is more cost-effective (~0.61 $/kg) and less energy-intensive (2.20–2.40 $kW_{th}h/kg$) than compression combined with high-pressure condensation using chilled water (1.09 $/kg and 9.98 $kW_{th}h/kg$).

KEYWORDS

aerobic fermentation, azeotropic dividing-wall column, downstream processing, ethyl acetate, refrigeration

1 | INTRODUCTION

Production of different bio-chemicals is rapidly gaining attention due to the depletion of fossil fuels, energy security, and firm environmental regulations. Considering this, the production of esters from renewable sources has been a recent research focus of metabolic engineering.[1] These esters can be used as platform chemicals, or directly as fuel additives, flavoring, and fragrance compounds.[2] In that context, ethyl acetate is one of the important short-chain esters that can be obtained in the fermentation process. It has various applications as a flavor compound, green solvent, and platform chemical for the synthesis of biodiesel, paints, coating additives, adhesives, solvents, herbicides, and resins.[3–5] The ethyl acetate market size has been projected to expand from 6.25 billion $ in 2024 to about 9.91 billion $ by 2031, with an annual growth rate of 6.8% during the forecast period.[3] Currently, ethyl acetate is primarily used for the production of paints and coating additives (about 47%), adhesives, sealants, and pigments, and as a solvent.[3] The ethyl acetate market price in the United States at the end of 2023 was 1.74 $/kg.[6] Some key market players are Celanese, Eastman Chemicals, INEOS Group Holdings SA, and Solvents Limited.[3]

Current industrial production of ethyl acetate uses different processes, among which Fischer esterification is the most common one (contributing about 85% to the total global production).[7] This process implies a reaction between ethanol and acetic acid in the presence of an acidic catalyst.[8] The relatively inexpensive raw materials and formation of water as a by-product are the benefits of using Fischer esterification for ethyl acetate synthesis. Alternative processes are acetaldehyde dimerization, a direct reaction between acetic acid and

ethylene, and dimerization of ethanol.[7] All the mentioned reactions depend on fossil carbon-based hydrocarbons and require relatively expensive catalysts, high temperatures and pressures.[8] An alternative to petrochemical processes is the production of bio-based ethyl acetate from bio-ethanol. For example, in 2022, CropEnergies and Johnson Matthey built the first plant in Europe that uses renewable ethanol as a petrol substitute in the production of ethyl acetate.[9] Additionally, a new option is the microbial production of ethyl acetate by fermentation of renewable sources or waste materials.[4] This process might serve specific markets and may be more sustainable when sufficiently developed. Initially, the capability of different yeasts to produce ethyl acetate has been the focus of research for the synthesis of aroma compounds.[10–12] Furthermore, significant effort has been put into developing a microbial synthesis process for the production of ethyl acetate as a bulk product.[8,13–18] The yeast *Kluyveromyces marxianus* has been selected as the best candidate for the bulk production of ethyl acetate.[8] Under aerobic conditions, this yeast can directly convert sugars into ethyl acetate while minimizing the formation of by-products. Furthermore, this yeast is capable of metabolizing lactose, which allows using whey as an inexpensive fermentation substrate.[8] Additional advantages of *K. marxianus* are good thermal tolerance and relatively high tolerance to ethyl acetate, although growth inhibition happens at about 17 g/L of ethyl acetate.[17,18] Unlike some yeast species (e.g., *Hansenula mrakii* and *Pichia anomala*),[8] microbial degradation of the formed ethyl acetate was not reported with *K. marxianus*,[19] while the ester production can be controlled by the iron level.[13,17] Lastly, the production of ethyl acetate by this yeast has been scaled up to a pilot scale (70 L stirred reactor).[17] To the best of our knowledge, similar attempts have not been reported for any other fermentation process producing ethyl acetate.

However, relatively low titer and yields are still significant drawbacks of ethyl acetate production by the fermentation.[4] For example, despite the relatively high tolerance of *K. marxianus*,[18] pilot scale experiments demonstrated that achievable concentrations of ethyl acetate in the liquid broth were less than 0.4 wt% (4 g/L).[17] Moreover, due to the aerobic nature of the fermentation process and the high volatility of ethyl acetate, some of the product will be stripped by the air-stream from the fermenter.[18] Even though higher than in the liquid phase, concentrations of ethyl acetate in the exhaust gas were still relatively low (<4 wt%).[17] Thus, advanced processes for the recovery of ethyl acetate from very dilute streams are required to increase the competitiveness of the overall bioprocess. Furthermore, continuous product recovery may mitigate the end-product inhibition effects and possibly improve the fermentation yield. To the best of our knowledge, the recovery of fermentative ethyl acetate on a large scale has not been thoroughly studied. Therefore, the main goal of this original research is to explore effective downstream processing options for the large-scale recovery of ethyl acetate from fermentation. In that respect, this study is the first one to develop several recovery processes, based on process intensification principles, for the recovery of ethyl acetate (production capacity of about 10 kton/year). The results of this study will be crucial for obtaining a fully realistic picture of the industrial feasibility of ethyl acetate synthesis by fermentation.

2 | MATERIALS AND METHODS

2.1 | Process design and simulation

Due to the high volatility of ethyl acetate, it will be present both in the exhaust gas from the fermenter and in the liquid broth. To ensure maximal product recovery, this study aims to recover ethyl acetate from both phases. The composition of the exhaust gas differs from the composition of the dry air supplied to the fermenter due to oxygen consumption, carbon dioxide (CO_2) formation by the microorganisms, and stripping of some water and ethyl acetate from the broth. The concentrations of ethyl acetate and water in the off-gas from the fermenter have been reported for the pilot scale reactor. Thereby, the maximum achievable concentration of ethyl acetate in the off-gas was assumed.[17] Additionally, the oxygen consumption, CO_2 formation, and off-gas flow rate were determined by solving a system of nonlinear equations (the nitrogen balance around the reactor,[20] Henry's law for O_2 and the ratio of the formed CO_2 and consumed O_2) in MATLAB using *fsolve* function. Thereby, it was assumed that the flow rates of inert gasses (N_2 and argon) were the same in the inlet gas and the off-gas (due to low solubility in water),[21,22] respiratory quotient was 1.15,[17] and dissolved O_2 concentration was about 30% of the equilibrium concentration. Accordingly, the composition of the humid exhaust gas is the following: 71.99 wt% N_2, 20.74 wt% O_2, 2.91 wt% ethyl acetate, 2.20 wt% CO_2, 1.32 wt% argon, and 0.84 wt% water. The ratio of the off-gas and liquid broth flow rates (1.2 on the mass basis) was determined from pilot scale data[17] (based on the known gas flow rate and an assumed operation time of 13 h for which the maximum ethyl acetate concentration was obtained in both phases).

Ethanol and acetate are common by-products in the ethyl acetate production by *K. marxianus*. Being less volatile than ethyl acetate, these compounds were reported to accumulate in the liquid fermentation broth.[17] As shown in the experiments on a pilot scale, it may be possible to minimize the formation of one of these metabolites by controlling the fermentation conditions.[17] Due to the high volatility of ethyl acetate, its evaporation from the liquid broth may be performed under conditions that will not damage the microbial viability. For example, reduced pressure evaporation may be applied to avoid high temperatures that would harm the microorganisms. Ideally, this approach would allow most of the fermentation broth, after ethyl acetate removal, to be recycled back to the fermenter, which might improve the fermentation process by avoiding loss of biomass, reducing water requirements, and increasing substrate-to-product yield.[23] However, having acetate as a by-product in the broth would significantly complicate this recycling. Removal of acetate would be complex and expensive,[24] while recycling it would lead to accumulation as the microorganisms are not able to consume it. It has been proven that *K. marxianus* can use ethanol after the depletion of sugars. However, acetate cannot be metabolized once ethanol is depleted.[13] Thus, recycling small amounts of ethanol should not negatively impact the fermentation and might even decrease the amount of substrate that is needed. In that respect, it may be more convenient to minimize the production of acetate during the fermentation, while allowing some

ethanol formation. Thus, the reported concentrations of biomass, sugars, ethyl acetate, and ethanol in the liquid broth for pilot-scale experiments were used.[17] Additionally, it was assumed that the concentration of dissolved O_2 was about 30% of the equilibrium concentration while the concentration of dissolved CO_2 was equal to its solubility. Thus, the obtained composition of the liquid fermentation broth is the following: 94.50 wt% water, 4.55 wt% non-volatile components (biomass and sugars), 0.54 wt% ethanol, 0.28 wt% ethyl acetate, 0.13 wt% CO_2, and <0.01 wt% O_2.[17] Flow rates of the gas and liquid feed to the downstream processing were back-calculated to obtain a production capacity of about 10 kton/year as sufficient for continuous industrial operation (39,800 and 33,000 kg/h, respectively).

Having ethanol instead of acetate as by-product of the fermentation may allow most of the liquid broth, with microorganisms and some ethanol, to be recycled upstream after ethyl acetate separation. However, the recovery of ethyl acetate from an aqueous solution that contains ethanol is complex due to the possible formation of four azeotropes (see Table 1 and Figure 1). Therefore, an advanced downstream process is needed to effectively recover ethyl acetate after the fermentation. There are several requirements for this recovery process. Firstly, even though the amount of ethyl acetate stripped with the off-gas is much higher than the amount remaining in the liquid (streams 1 and 2 in Tables 2 and 3, respectively), efficient recovery from both phases is necessary to minimize product losses. Secondly, the initial separation of ethyl acetate from the liquid should be performed under conditions that are appropriate for the microorganisms to allow recycling of the remaining broth (moderate temperatures and absence of chemicals that may harm the microbial viability). Finally, commercial-grade purity ethyl acetate product (>99.5%)[25,26] should be obtained from the water—ethyl acetate—ethanol mixture. In the designed downstream processes, ethyl acetate was initially recovered from the liquid phase using a hybrid combination of gas stripping and heat pump-assisted vacuum evaporation, as described before.[27] Furthermore, two scenarios were developed for the initial ethyl acetate recovery from the gas phase. In one scenario, multistage compression

TABLE 1 Boiling points of pure components and formation of azeotropes at 1 bar.

Pure components		Azeotropes		
Component	**Boiling point (°C)**	**Component**	**Mass fraction**	**Temperature (°C)/type**
Ethyl acetate	77.20	Water	0.0874	70.33/homogeneous
Ethanol	78.31	Ethyl acetate	0.7864	
Water	100.02	Ethanol	0.1262	
Acetic acid	118.01	Water	0.0903	71.39/heterogeneous
		Ethyl acetate	0.9097	
		Ethanol	0.7024	71.78/homogeneous
		Ethyl acetate	0.2976	
		Water	0.0438	78.15/homogeneous
		Ethanol	0.9562	

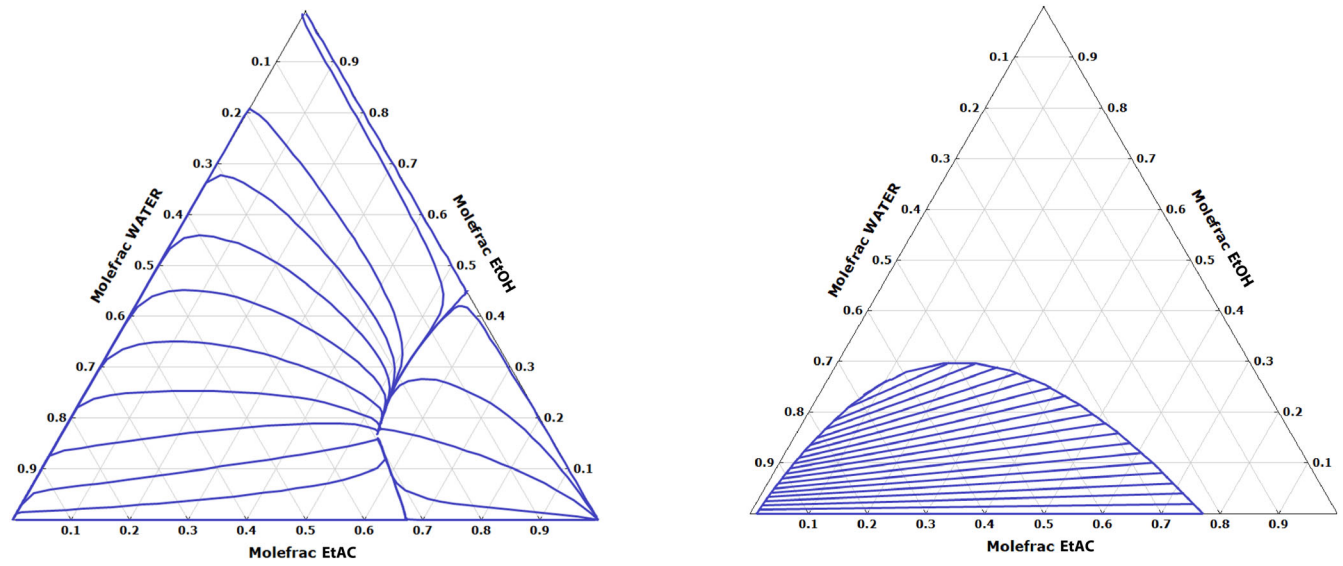

FIGURE 1 Residue curve map (left) and ternary diagram (right) of the ethyl acetate (EtAc)–ethanol (EtOH)–water system at 1 bar.

TABLE 2 Conditions and compositions of the main process streams from Figure 2 (Case 1).[a]

Stream	1	2	3	4	5	6	7	8	9	10
Temperature (°C)	11.5	32.0	30.5	25.0	20.2	21.5	25.0	52.1	25.0	44.7
Pressure (bar)	1.000	1.000	0.043	6.000	10.000	10.000	1.232	1.232	2.500	1.000
Flow rate (kg/h)	39,800	33,000	28,619	39	34,000	38,358	38,505	1249	28	2
Mass fraction										
Water	0.0084	0.9450	0.9475	0.0014	1.0000	0.0019	0.9954	0.0020	0.0066	0.0368
Ethyl acetate	0.0291	0.0028	0.0000	0.0259	0.0000	0.0000	0.0000	0.9967	0.0842	0.4691
Ethanol	0.0000	0.0054	0.0000	0.0037	0.0000	0.0000	0.0046	0.0013	0.0000	0.0157
Nitrogen	0.7199	0.0000	0.0000	0.0000	0.0000	0.7467	0.0000	0.0000	0.2905	0.1923
Oxygen	0.2074	0.0001	0.0000	0.0015	0.0000	0.2151	0.0000	0.0000	0.1453	0.0717
Carbon dioxide	0.0220	0.0013	0.0000	0.9675	0.0000	0.0226	0.0000	0.0000	0.4631	0.1981
Argon	0.0132	0.0000	0.0000	0.0000	0.0000	0.0136	0.0000	0.0000	0.0072	0.0162
Heavy components (biomass and sugars)	0.0000	0.0455	0.0525	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

[a]Conditions and compositions of all process streams in this case are presented in Supporting Information S1.

TABLE 3 Conditions and compositions of the main process streams from Figure 3 (Case 2a).[a]

Stream	1	2	3	4	5	6	7	8	9
Temperature (°C)	11.5	32.0	30.5	25.0	10.0	20.0	83.0	30.0	25.0
Pressure (bar)	1.000	1.000	0.043	6.000	1.000	1.000	1.232	1.232	3.000
Flow rate (kg/h)	39,800	33,000	28,619	39	38,396	315	4263	1155	16
Mass fraction									
Water	0.0084	0.9450	0.9475	0.0014	0.0000	1.0000	0.9583	0.0037	0.0055
Ethyl acetate	0.0291	0.0028	0.0000	0.0259	0.0025	0.0000	0.0000	0.9962	0.0712
Ethanol	0.0000	0.0054	0.0000	0.0037	0.0000	0.0000	0.0417	0.0001	0.0037
Nitrogen	0.7199	0.0000	0.0000	0.0000	0.7461	0.0000	0.0000	0.0000	0.3591
Oxygen	0.2074	0.0001	0.0000	0.0015	0.2150	0.0000	0.0000	0.0000	0.1946
Carbon dioxide	0.0220	0.0013	0.0000	0.9675	0.0228	0.0000	0.0000	0.0000	0.3329
Argon	0.0132	0.0000	0.0000	0.0000	0.0136	0.0000	0.0000	0.0000	0.0130
Heavy components (biomass and sugars)	0.0000	0.0455	0.0525	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

[a]Conditions and compositions of all process streams in this case are presented in Supplorting Information S1.

followed by high-pressure condensation with chilled water was used to separate ethyl acetate from the fermenter off-gas (Case 1). Alternatively, low-temperature condensation using refrigerants was applied in this step (Case 2). Following these initial separation steps, an azeotropic dividing-wall column (A-DWC) was designed to separate high-purity ethyl acetate from the remaining water and ethanol.

Rigorous simulations for all steps in the recovery process were designed in Aspen Plus. Even though there is no guarantee of a global minimum in optimizing non-convex mixed-integer nonlinear problems (MINLP), such as chemical processes, all decisions during process design were made to minimize energy requirements. Reducing the total energy requirements can be expected to decrease total recovery costs significantly. This is due to a substantial portion of the energy cost in the total operational expenses (OPEX), which is commonly dominant in the total annual costs (TAC) of the downstream processing after the fermentation. Several decision variables were considered in the process design, such as the total number of stages in distillation columns, position of the feed tray, reflux ratio, bottoms-to-feed ratio, liquid fraction, compression ratio, and refrigeration temperature. Additionally, constraints accounted for include high purity and recovery of ethyl acetate, as well as moderate temperatures for the initial separation of ethyl acetate from the liquid broth.

2.2 | Thermodynamic property model

Due to the multiple components present in the fermentation broth and off-gas (streams 1 and 2 in Tables 2–4), NRTL-HOC model (Non-Random Two Liquid model with Hayden-O'Connell extension) defined in Aspen Plus was chosen to properly account for the thermodynamic interactions. The NRTL model describes vapor–liquid and liquid–liquid

TABLE 4 Conditions and compositions of the main process streams from Figure 3 (Case 2b).[a]

Stream	1	2	3	4	5	6	7	8	9
Temperature (°C)	11.5	32.0	30.5	25.0	10.0	20.0	66.1	30.0	25.0
Pressure (bar)	1.000	1.000	0.043	6.000	1.000	1.000	1.232	1.232	3.000
Flow rate (kg/h)	39,800	33,000	28,619	39	38,307	314	4266	1239	18
Mass fraction									
Water	0.0084	0.9450	0.9475	0.0014	0.0000	1.0000	0.9583	0.0027	0.0056
Ethyl acetate	0.0291	0.0028	0.0000	0.0259	0.0003	0.0000	0.0000	0.9972	0.0716
Ethanol	0.0000	0.0054	0.0000	0.0037	0.0000	0.0000	0.0417	0.0000	0.0035
Nitrogen	0.7199	0.0000	0.0000	0.0000	0.7478	0.0000	0.0000	0.0000	0.3553
Oxygen	0.2074	0.0001	0.0000	0.0015	0.2155	0.0000	0.0000	0.0000	0.1909
Carbon dioxide	0.0220	0.0013	0.0000	0.9675	0.0228	0.0000	0.0000	0.0000	0.3318
Argon	0.0132	0.0000	0.0000	0.0000	0.0136	0.0000	0.0000	0.0000	0.0412
Heavy components (biomass and sugars)	0.0000	0.0455	0.0525	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

[a]Conditions and compositions of all process streams in this case are presented in Supporting Information S1.

equilibria of strongly non-ideal mixtures using binary interaction parameters. The HOC extension was used to describe the dimerization of carboxylic acids in the vapor phase. Non-condensable gasses (nitrogen, oxygen, CO_2, etc.) were designated as Henry components. However, the Non-Random Two Liquid (NRTL) liquid activity property model is reliable for fluid separations up to moderate pressures. As one of the recovery processes (Case 1) implies compression of the exhaust gas from the fermenter and high-pressure condensation with chilled water, the Predictive Soave-Redlich-Kwong (PSRK) property model from Aspen Plus was used to describe high-pressure operations. This model is based on the PSRK equation of state, which can accurately predict thermodynamic interactions up to high temperatures and pressures using UNIversal Functional Activity Coeffecient (UNIFAC) interaction parameters.[28] Furthermore, the REFerence fluid PROperties (REFPROP) model was used for the design of the refrigeration cycle (Case 2). This model was developed by the National Institute of Standards and Technology (NIST) to predict thermodynamic and transport properties of industrially important fluids such as different refrigerants and hydrocarbons.[29] More details about the used property models are provided in the Supporting Information S1.

2.3 | Economic evaluation

Economic analysis of the developed downstream processes for ethyl acetate recovery after fermentation was performed following a published NREL method.[30] According to this method, the total capital costs (CAPEX) consist of equipment purchase and installation costs, warehouse, site development, proratable expenses, field expenses, additional piping, expenses related to home office and construction, working capital, etc.[30] Equipment costs were estimated using cost correlations[31] with a Marshall and Swift cost index of 1773.4 (end of 2021). The total operating costs (OPEX) relate to the costs of utilities, operating labor,[32,33] maintenance, property insurance, etc.[30] The following utility costs were assumed: 85.60 $/MWh for electricity, 43.09 $/MWh for low-pressure steam, 0.93 $/MWh for cooling water, 11.68 $/MWh for chilled water, 50.68 and 74.12 $/MWh for refrigeration at −50 and −70°C.[31,34,35] Initial costs of refrigerants were estimated using recent market prices: 0.73 $/kg for R-170 (ethylene)[36] and 0.41 $/kg for R-290 (propane).[37] The total annual costs (TAC) include CAPEX and OPEX with a 10 years payback period (PBP) and were calculated using the following equation: TAC = CAPEX/PBP + OPEX.

2.4 | Sustainability assessment

The environmental impact of the designed process was assessed by calculating sustainability metrics: energy intensity, greenhouse gas emissions, water consumption, material intensity, pollutant, and toxic emissions.[38] Smaller values of these metrics indicate better process performance.

- *Energy intensity* is a measure of the total energy that is required to recover a kilogram of product, distinguishing between different types of used energy. Thermal and electrical energy requirements present the specific amounts of thermal and electrical energy, respectively.[38] The total primary energy requirements account for both thermal and electrical energy through the electrical-to-thermal conversion factor (using a conservative value of 2.5).[39]
- *Greenhouse gas emissions* present the specific amount of CO_2 emitted from the energy (thermal and/or electrical) usage.[38] The distinction was made between green or gray electricity (from renewable sources or fossil fuels) usage. The published correlations were used to calculate these emissions.[40,41] Additionally, any ethyl acetate remaining in the gas stream after purification was accounted for through CO_2 equivalents.[42] On the contrary, CO_2 in this gas stream was not included in the greenhouse gas emissions metrics as it has a biogenic origin and is not formed in the recovery processes.

- *Water consumption* accounts for the amount of water needed per kilogram of recovered product. Assumptions include 70% condensate recovery[40] and 7% loss of cooling water.[38]
- *Material intensity* presents the amount of waste formed per kilogram of product.
- *Pollutant and toxic materials* account for the specific amount of formed pollutant and toxic materials, respectively. As emitted CO_2 was included in the greenhouse gas emission sustainability metrics, it was not considered in this metric.[38]

3 | RESULTS AND DISCUSSION

This section presents all details about the design of the downstream processing (DSP) of ethyl acetate after the fermentation—see process flow sheets in Figures 2 and 3. In addition, results of the economic and environmental assessment are given for a fair comparison of the performance of the developed processes.

3.1 | DSP design: initial separation of ethyl acetate from the liquid fermentation broth

Besides ethyl acetate and ethanol, the aqueous broth from the fermenter contains some living microorganisms. Thus, the initial separation of fermentation products should be performed under moderate conditions that will not harm the microbial viability. Consequently, most of the broth may be recycled to the fermenter to avoid biomass loss, reduce water requirements, and increase fermentation yield.[23] A recently described hybrid combination of gas stripping and vacuum evaporation[27] was used to remove volatile fermentation products from the liquid broth. An alternative option would be to use liquid–liquid extraction.[43] Nonetheless, the drawbacks of this approach are the high volatility of ethyl acetate that may potentially lead to product and solvent losses, relatively large required volumes of solvent, possibly inefficient transfer of product into solvent phase due to the high dilution of the feed stream, difficulties related to the solvent recovery, etc. On the contrary, the gas stripping combined with vacuum evaporation effectively utilizes the high volatility of ethyl acetate for its separation from the higher boiling liquid broth. In this

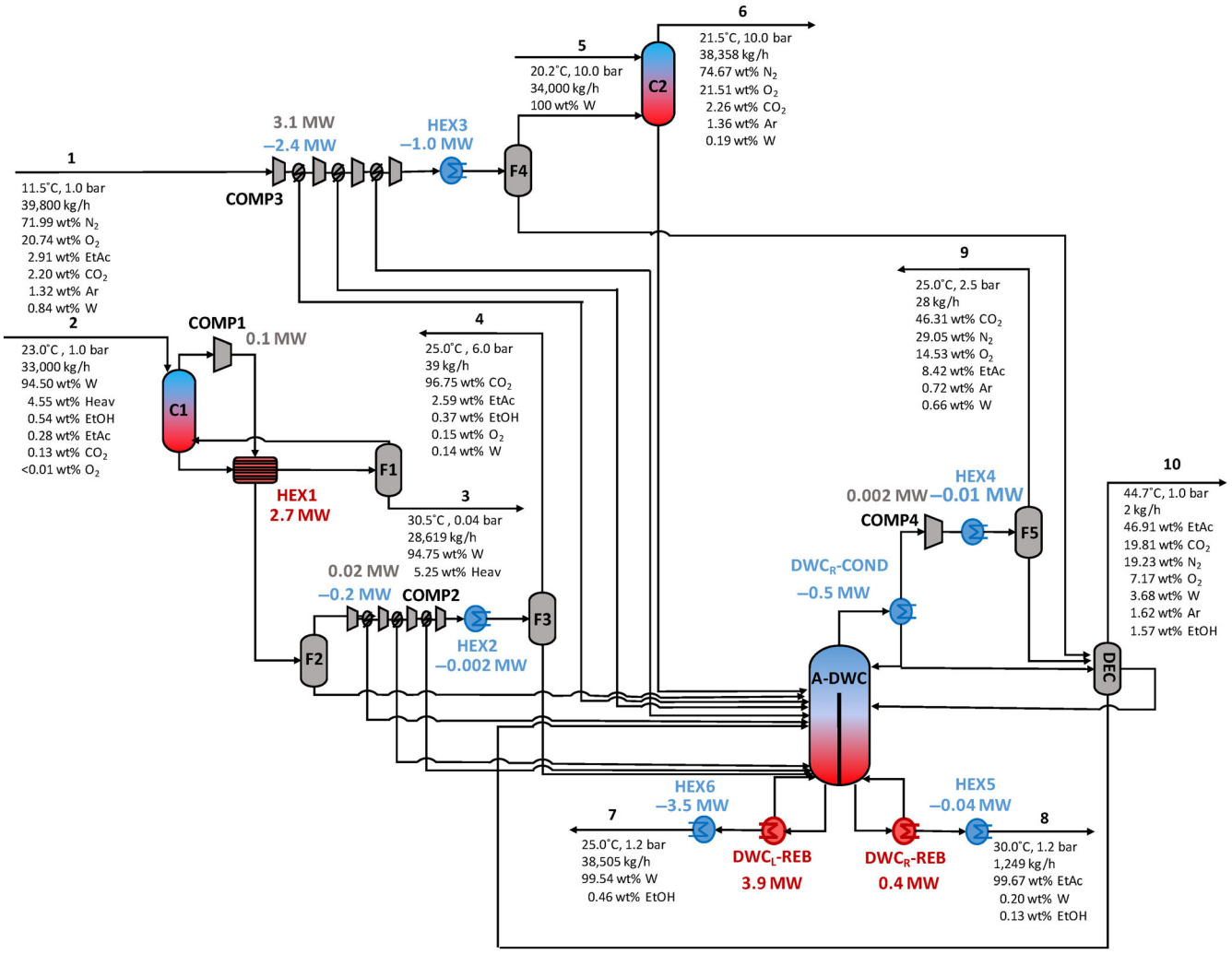

FIGURE 2 Flow sheet of the ethyl acetate recovery process—Case 1, conditions and compositions of the numbered process streams are given in Table 2. EtAc, ethyl acetate; EtOH, ethanol; Heav, heavy (non-volatile) components; W, water.

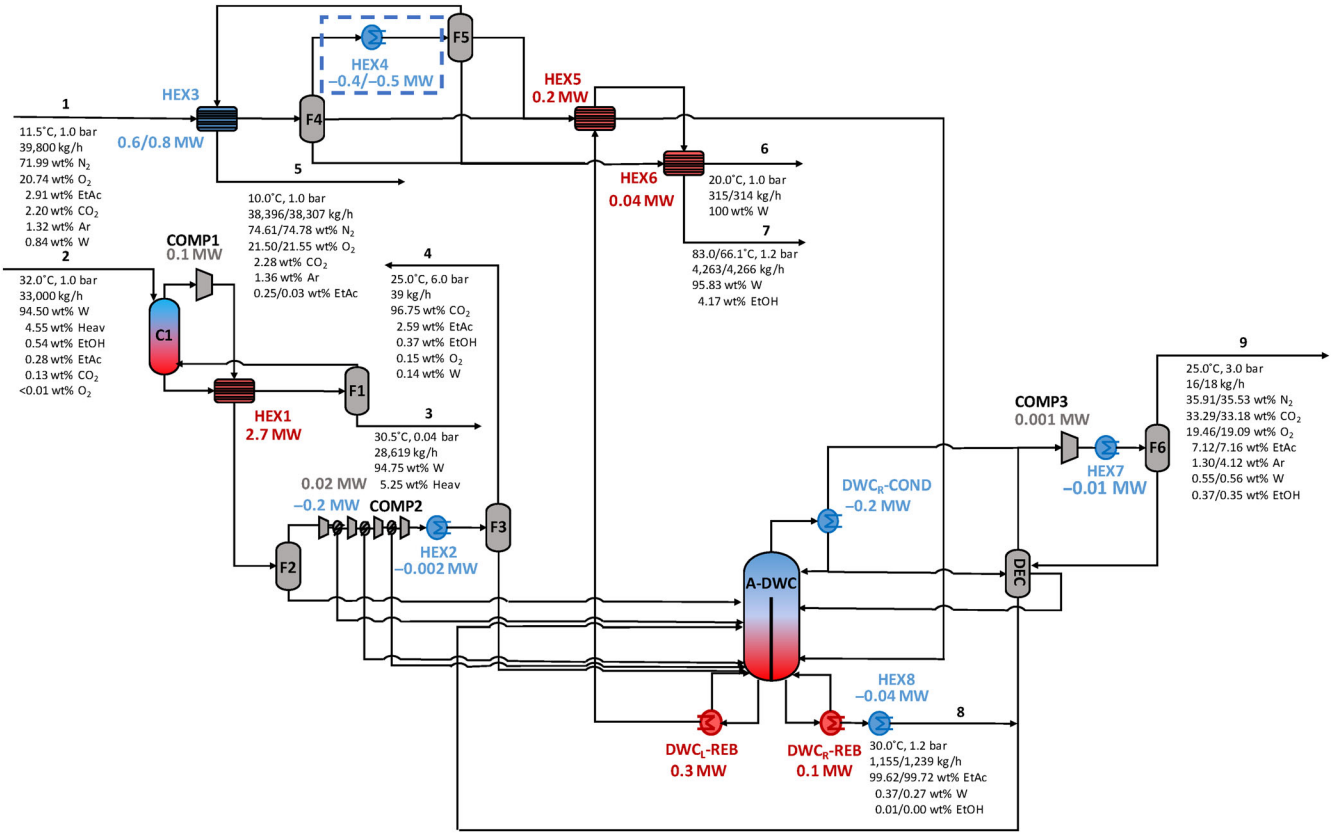

FIGURE 3 Flow sheet of the ethyl acetate recovery process—Case 2 (marked heat exchanger requires refrigeration; if there are differences in stream parameters or energy requirements, the following order applies: Case 2a/Case 2b), conditions and compositions of the numbered process streams are given in Table 3 for Case 2a and in Table 4 for Case 2b. EtAc, ethyl acetate; EtOH, ethanol; Heav, heavy (non-volatile) components; W, water.

separation technique, the liquid broth from the fermenter is fed on top of the stripping column C1, in which gas is used to separate volatile products from most of the broth. This column operates at a reduced pressure (top pressure of 0.040 bar) to ensure moderate temperatures (28.6°C at the top and 30.4°C at the bottom of the column) that will not harm the microbial viability. Due to the reduced pressure operation, structured packing type Sulzer Mellapack 250 and a pressure drop of 0.225 mbar per theoretical stage were considered for this column.[44] To provide gas for stripping in column C1, the bottom product from this column, which is the liquid depleted of ethyl acetate and ethanol, is partially evaporated under reduced pressure. The remaining liquid contains mainly water with the microorganisms and non-volatile inert components (stream 3). This stream may be recycled upstream. The formed vapor is sent to the bottom of column C1 and used to strip ethyl acetate and ethanol from the liquid fermentation broth. To reduce energy requirements of this initial separation step, a vapor recompression heat pump system is applied to the vacuum evaporation operation. The top stream from column C1, vapor rich in ethyl acetate and ethanol, is compressed (in COMP1) and used to evaporate part of the fermentation broth (in HEX1). A measure of the obtained energy savings can be evaluated by calculating the coefficient of performance (COP) of the implemented heat pump system. COP is equal to the ratio of the upgraded heat (exchanged between the liquid and the compressed vapor) and the compressor duty that is required to enable this evaporation.[45] COP values higher than 2.5, which is a conservative value of electrical-to-thermal conversion factor,[39] prove the energy efficiency of the applied heat pump systems. COP value of the described vapor recompression system is 20.5, which confirms significant energy savings. Furthermore, this system allows complete electrification of the vacuum evaporation.

After this initial step in the recovery process, about 87% of water from the fermentation broth is removed in flash vessel F1 (stream 3), while ethyl acetate and ethanol concentrations are increased by a factor of 7.5. However, this stream is still very dilute (>92 wt% water) and contains some dissolved gasses that should be removed. Initially, simple phase separation in a flashing unit F2 was used to remove most of the present gasses. As some volatile fermentation products are removed with these gasses, additional steps were applied (multistage compression (COMP2) combined with condensation using cooling water (HEX2) and phase separation in a flashing unit (F3)) to minimize product losses. The remaining liquid streams with ethyl acetate and ethanol are sent to further purification in the azeotropic dividing-wall column (A-DWC).

3.2 | DSP design: separation of ethyl acetate from the exhaust off-gas

Being a highly volatile product, ethyl acetate is mostly stripped with the off-gas. However, the concentration of the valuable fermentation

product in the off-gas is still relatively small (~2 wt%). Thus, an advanced downstream process is required to separate the fermentation product from non-condensable gasses. Two cases were considered for the recovery of ethyl acetate from the exhaust gas. In the first case (Case 1), atmospheric off-gas from the fermenter was compressed and ethyl acetate was condensed using chilled water. In the second case (Case 2), refrigeration was used to cool atmospheric exhaust gas and condense ethyl acetate. Alternative options for the initial recovery of volatile chemicals, such as ethyl acetate, from a gas stream are membrane separations, adsorption, and absorption. The performance of the membrane separation system strongly depends on the exact type of membrane used (cost, availability, susceptibility to fouling, replacement rate, etc.). As these quantitative data were not available,[46] and relatively high costs may be expected due to the large gas flow rate, membrane separations were not considered in this study. Furthermore, adsorption was not considered due to drawbacks related to a desorption step (required regeneration of adsorbent, which leads to a considerable energy cost), and large expected equipment and adsorbent costs due to the large gas flow rate.[47] Finally, when using absorption to recover low-concentrated volatile components from gas phase, large amounts of absorbing liquid are likely to be needed. Thus, large equipment units are needed for further processing, which may result in high capital and operating costs. Moreover, depending on the specific application of ethyl acetate and related standards regarding product purity, absorbent liquid may also need to satisfy safety standards (e.g., for applications in the food industry). Thus, absorption was also not examined for the initial separation of ethyl acetate from the gas phase.[47,48] As pilot-scale experiments reported that ethanol accumulated in the liquid phase and was not present in the off-gas,[17] it was not included in the gas feed stream to the downstream processing. However, if some ethanol is also present in the off-gas, it will be separated with ethyl acetate due to a higher boiling point (see Table 1).

3.2.1 | Case 1—compression and high-pressure condensation using chilled water

Due to the high volatility of ethyl acetate and the presence of non-condensable gasses (nitrogen, oxygen, CO_2, and argon), low-temperature cooling (using refrigeration) is required to completely separate ethyl acetate from the atmospheric exhaust gas. Alternatively, if off-gas from the fermenter is compressed, cooling at moderate temperatures (using chilled water) may be sufficient to condense ethyl acetate. In this case, off-gas from the fermenter is firstly compressed (in a multistage compressor COMP3) to the defined pressure. High-pressure gas is then cooled using chilled water (HEX3), while condensed ethyl acetate and water are separated from the remaining gasses in a simple flash unit (F4). However, some ethyl acetate will likely remain in the gas phase and additional stripping with water will be needed to minimize product loss. This can be performed in a stripping column C2 where gas is fed at the bottom while water is fed at the top. The bottom product from this column is mainly water with stripped ethyl acetate, while the top product is a vapor stream with the remaining non-condensable gasses (stream 6). As this column operates at increased pressure, simple sieve trays with a pressure drop of 8 mbar per stage were used for its internals.[49]

With a defined cooling temperature of 10°C (which may be achieved using chilled water at 5°C), the relationship between the outlet pressure of the compressor COMP3 (and consequently compressor duty) and flow rate of the water needed for stripping (in column C2) was examined (see Figure 4), with the constraint of recovering over 99.9% of ethyl acetate from the off-gas. With the increase in gas pressure, the amount of ethyl acetate that remains in the gas phase after cooling decreases. Consequently, the amount of water needed to strip the remaining product from the gas also decreases. Since this aqueous stream with stripped ethyl acetate needs to be further processed, lower water flow rates will result in lower thermal energy requirements for the following distillation steps. On the contrary, gas compression to higher pressures will lead to

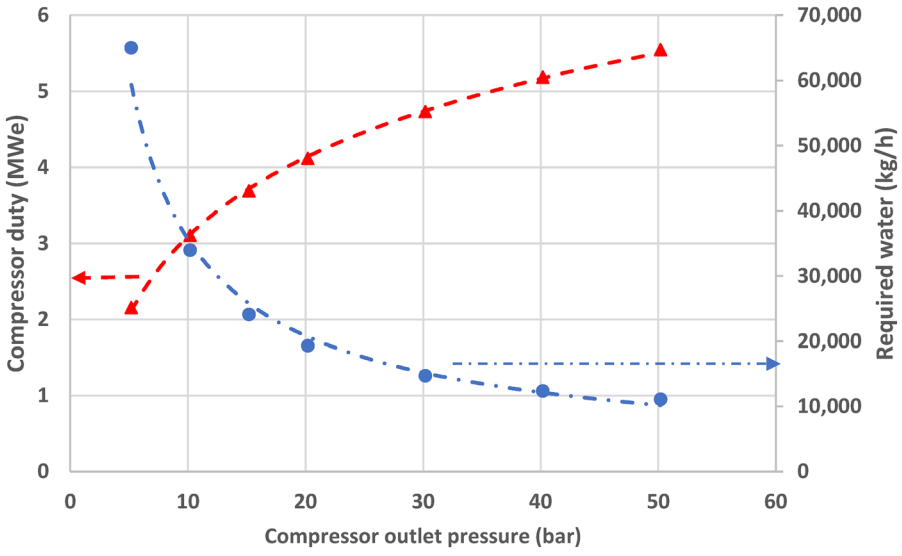

FIGURE 4 Determination of the compressor outlet pressure in Case 1.

larger compressor duties. Thus, there is a trade-off between the electrical energy needed to power the compressor and the thermal energy needed in the following distillation processes. Total primary energy requirements that account for both electrical and thermal energy were considered to determine the outlet pressure of the compressor and the water flow rate. The total energy requirements of the whole recovery process were compared for two compressor outlet pressures (10 and 40 bar). These pressures were chosen because the amount of water required to strip ethyl acetate from the remaining gas stream drastically increases for outlet compressor pressures lower than 10 bar but does not decrease significantly for gas pressures higher than 40 bar. When the off-gas from the fermenter is compressed to 40 rather than 10 bar, the electrical energy requirements increase by 64% while the thermal energy requirements decrease by 43%. Consequently, the total energy requirements are approximately 24% higher if the gas is compressed to 40 bar. This is mainly due to the higher electrical energy requirements in the multistage compressor. Due to the large flow rate of the exhaust gas from the fermenter, compression is very energy-intensive. Thus, compressing off-gas to moderate pressures and using larger amounts of water leads to a less energy-intensive recovery process. Consequently, a compressor outlet pressure of 10 bar was chosen. Lower pressures would require a much larger water flow rate (e.g., almost two times larger water flow rate is needed if outlet pressure from the compressor is 5 bar), which would lead to higher CAPEX and were therefore not considered.

Finally, over 99.9% of ethyl acetate is recovered from the exhaust gas. The aqueous streams containing ethyl acetate should be sent to the final purification in A-DWC. The remaining non-condensable gasses were obtained in the top product stream (stream 6) from stripping column C2 (74.67 wt% nitrogen, 21.51 wt% oxygen, 2.26 wt% CO_2, 1.36 wt% argon, and 0.19 wt% water). However, even with moderate outlet compressor pressure, the total energy requirements of the initial separation of ethyl acetate from the off-gas are relatively high (about 3.1 MW_e is needed to recover 1157 kg/h of ethyl acetate).

3.2.2 | Case 2—low-temperature condensation using refrigerants

Since compression of large gas flows is energy-intensive, an alternative recovery process implying condensation of ethyl acetate directly from the atmospheric off-gas was designed. Due to the high volatility of ethyl acetate, very low temperatures are needed for sufficient product recovery. Thus, an auto-cascade refrigeration cycle may be used. In this system, a zeotropic mixture of two refrigerants with different boiling points (e.g., R-170 and R-290) is used to achieve low temperatures through multiple stages of evaporation and condensation within a single cycle (see Figure 5).[50]

The exhaust gas from the fermenter is cooled in two steps. In the first step, cold gas from the final cooling step is used instead of cooling utility in HEX3. Parts of the ethyl acetate and water have been removed from the gas phase after this step (in flashing unit F4). The remaining gas is sent to the final cooling step in which the refrigeration cycle is used to provide sufficiently low temperatures (in HEX 4). The residual ethyl acetate and water may be separated from non-condensable gasses in this step. The described two-step cooling drastically reduces refrigeration usage. Specifically, using the cooled gas from the second step in the first cooling step may decrease the total cooling duty by 58%–65%.

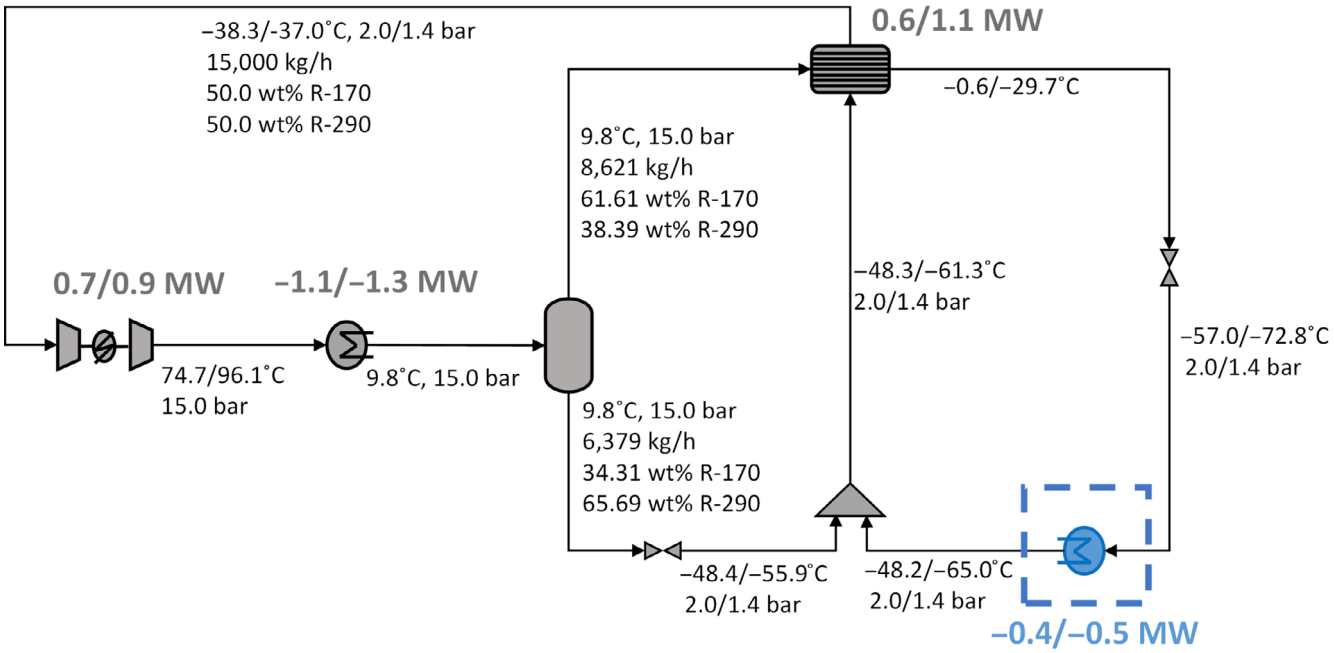

FIGURE 5 Flow sheet of the refrigeration cycle (ethyl acetate is condensed in the blue marked heat exchanger; if there are differences in stream parameters or energy requirements, the following order applies: Case 2a/Case 2b).

Initially, a moderate refrigeration at −50°C was used for the final cooling step (Case 2a). However, this temperature is not sufficiently low to completely recover ethyl acetate from the gas. About 8.3% of ethyl acetate from the fermenter off-gas is lost in the gas stream remaining from the final cooling. A possible option would be to strip the remaining ethyl acetate from the gas using water. However, due to relatively low concentrations of ethyl acetate in the remaining gas (about 0.25 wt%) and the large gas flow rate, large amounts of water would be required to ensure sufficient product recovery. This would further increase CAPEX and thermal energy needs in the final purification step. For example, to ensure that ethyl acetate losses in the remaining gas are not higher than 5%, about 80,000 kg/h of water would be needed in the stripping column. Additionally, the liquid product from this column is very dilute (about 0.1 wt% ethyl acetate). Thus, this option was not considered. Alternatively, even lower-temperature refrigeration at −70°C may be used to recover over 99.0% of ethyl acetate from the exhaust gas (Case 2b). Even though the product recovery is higher in the case of lower-temperature condensation, the refrigeration cycle becomes more expensive. Thus, to compare the performance of these recovery processes, a final purification of ethyl acetate was designed for both refrigeration temperatures (Cases 2a and 2b). In both cases, ethyl acetate-rich streams (>97 wt% ethyl acetate), recovered after the refrigeration, are heated in HEX 5 (using the aqueous bottom product of A-DWC) and sent to the final purification in A-DWC. After being used in the first cooling step (as a cooling utility in HEX5), the remaining gas that consists of nitrogen (74.61–74.78 wt%), oxygen (21.50–51.55 wt%), CO_2 (2.28 wt%), and ethyl acetate (0.03–0.25 wt%) is heated to ~10°C. The total energy use for the initial separation of ethyl acetate from the fermenter off-gas are 0.7 MW_e (Case 2a) and 0.9 MW_e (Case 2b), with recoveries of 92.7% and 99.0%.

3.3 | DSP design: final purification of ethyl acetate

After the initial recovery steps, ethyl acetate, ethanol and some water are separated from the liquid broth and the off-gas from the fermenter. This separation is challenging due to the potential formation of four azeotropes (see Table 1). Many separation techniques have been studied to work around thermodynamic limitations, whereby distillation-based operations are commonly proposed for large-scale processes. For example, pressure swing distillation uses pressure changes to influence vapor–liquid equilibrium of azeotropic mixture and move distillation boundaries.[51] Pervaporation-assisted distillation enhances conventional distillation by using selective membranes to effectively remove desired components.[52] Extractive distillation implies the addition of an extractive reagent that changes the relative volatility of the mixture. Thereby, the potential of ionic liquids to replace conventional solvents for ethanol–ethyl acetate separation is intensively investigated.[53,54] Furthermore, heterogeneous-azeotropic distillation has been proposed as a novel distillation technique that effectively couples distillation column with liquid–liquid separator.[55] Water (which is already present in the mixture) may be used as an extractive agent to facilitate the separation of ethyl acetate and ethanol. The benefits of this technique are the absence of additional chemicals that may lead to complications related to recovery, handling, toxicity, or disposal; moderate capital investment for a large-scale process and reduction in energy requirements of separation due to effective usage of liquid–liquid–vapor separation.[56] Thus, it was used as the main recovery technique in the final purification after separating valuable products from the fermentation broth and off-gas.

Due to the highly complex mixture, several steps are required to obtain a high-purity ethyl acetate product. Initially, water may be used as an entrainer to break ethanol–ethyl acetate azeotrope[55,56] and remove most of the ethanol. Theoretically, in the first distillation column, most of the water with ethanol may be obtained as the bottom product, while ethyl acetate with some water may be obtained at the top. Furthermore, since ethyl acetate and water form a heterogeneous azeotrope, an additional treatment using distillation coupled with phase splitting in a decanter (DEC) is needed. High-purity ethyl acetate product may be obtained at the bottom of the second distillation column, while a nearly azeotropic mixture may be obtained at the top. This stream is sent to the DEC from which the ethyl acetate-rich phase is returned to the second distillation column while the aqueous phase may be recycled to the first distillation column. Hence, a sequence of two distillation columns with a decanter is needed to recover the high-purity ethyl acetate. Alternatively, these columns may be integrated into one A-DWC with a common overhead and a divided bottom section (see Figure 6).[31,57] As dividing-wall column equipment unit is not available in Aspen Plus, it was simulated as a thermodynamically equivalent sequence of distillation columns (see Figure 6). The left and right sides of A-DWC are presented with A-DWC_L and A-DWC_R, respectively. Due to relatively small temperature differences between different sides of the inner wall, thermal insulation will not be required to allow thermal efficiency of A-DWC. Moreover, a suitable control strategy has been proven to effectively handle heat transfer across the wall of DWC.[58] In this system, most of the water with ethanol is obtained as the bottom product from A-DWC_L. If worth it, the ethanol from this stream may be recovered using a heat pump-assisted preconcentration step followed by final purification using extractive distillation with ethylene glycol, as previously suggested.[59] However, ethanol recovery is kept out-of-scope of the current article since the costs and revenues of this process would obscure the costs and revenues of the ethyl acetate recovery. The top vapor from this column is sent to the A-DWC_R, while part of the liquid flowing down in A-DWC_R is directed to the first floor of A-DWC_L to ensure sufficient liquid flow in this part of A-DWC. High-purity ethyl acetate product is recovered at the bottom of A-DWC_R while a nearly azeotropic ethyl acetate–water mixture is obtained at the top of A-DWC_R and sent to the DEC. The ethyl acetate-rich phase from DEC is returned to A-DWC_R, while the water-rich phase is returned to A-DWC_L. Thus, the dividing-wall column merges two conventional distillation columns into only one unit while reducing the number of heat exchangers (A-DWC has two reboilers (DWC_L-REB and DWC_R-REB) and only one condenser (DWC-COND)). Even though this is a highly integrated system, it has been proven that dividing-wall columns can be effectively controlled.[60] The number of

stages, position of the feed stage, bottom product rate, reflux ratio, and liquid split were varied to ensure high recovery of high-purity ethyl acetate product while minimizing the total energy requirements for the final purification. Due to the atmospheric operation, sieve trays with a pressure drop of 8 mbar were chosen for A-DWC's internals.[49] A-DWC has 30 stages in total, with the condenser as first stage and

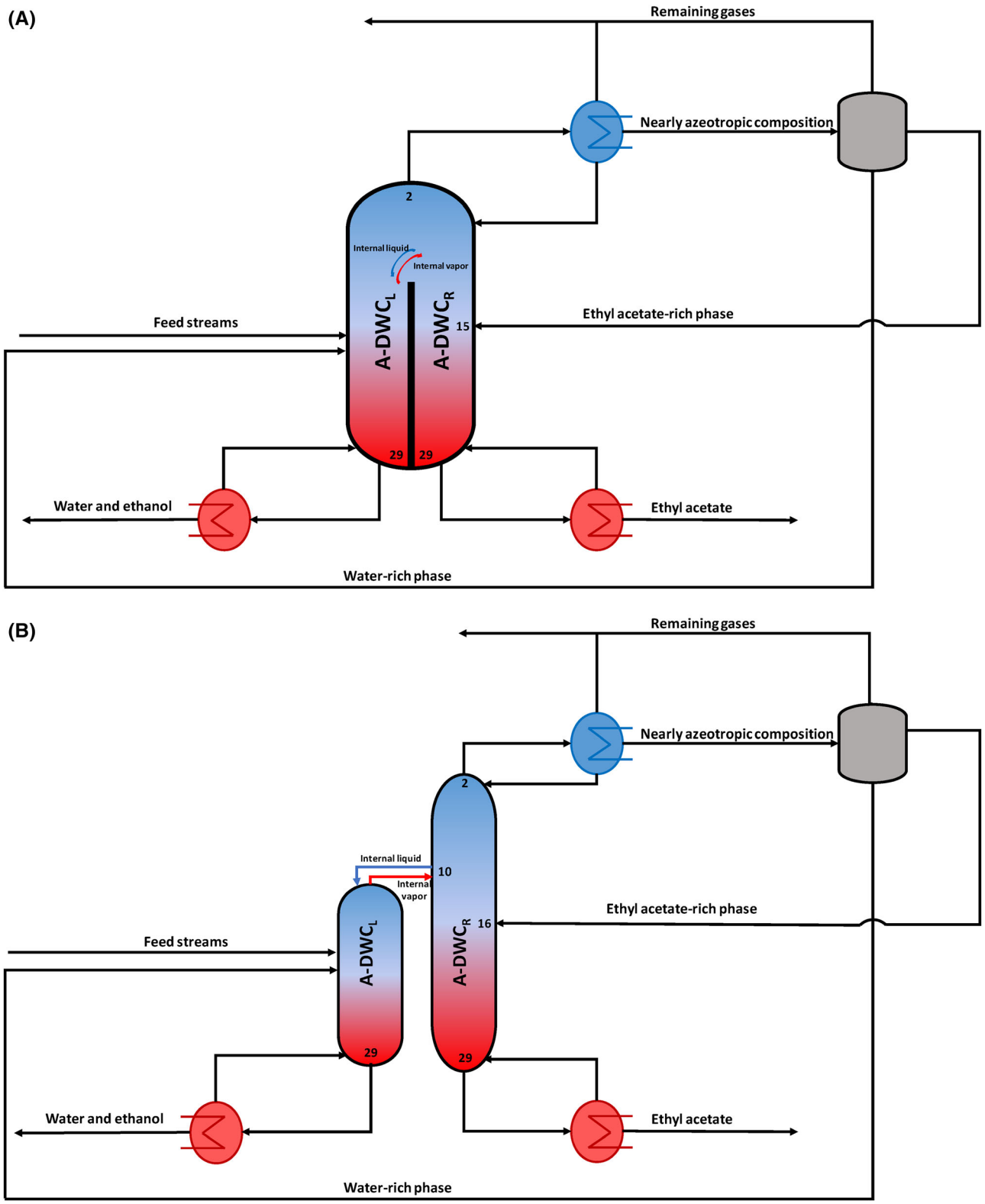

FIGURE 6 Azeotropic dividing-wall column (A-DWC) design (A) and the equivalent sequence of distillation columns (B), the numbers in the columns indicate stage numbers. A-DWC$_L$, left side of A-DWC; A-DWC$_R$, right side of A-DWC.

the reboiler as last stage according to the convention in Aspen Plus, while the wall is placed at the bottom 20 stages (19 excluding reboilers). After the initial separation of ethyl acetate, ethanol, and some water (either using high-pressure condensation with chilled water in Case 1 or low-temperature atmospheric condensation with refrigerants in Cases 2a and 2b), multiple streams need to be fed to A-DWC$_L$. The exact location of the feed stages depends on the technique applied for the initial recovery of ethyl acetate from the gas stream, due to differences in the compositions and flow rates of the feed streams. Generally, feed streams with high water concentration are fed on the top stages, as water is used as an extractive agent. Contrarily, feed streams with lower water and higher ethyl acetate concentrations are fed on the bottom stages. Consequently, ethanol is removed with most of the water as the bottom stream from A-DWC while a nearly azeotropic mixture of ethyl acetate and water is obtained in the top vapor stream. This vapor stream is fed to the 10th stage of A-DWC$_R$ (also the 10th stage of A-DWC, see Figure 6), while part of the liquid from this stage is directed to A-DWC$_L$. The distillate from A-DWC$_R$ is sent to DEC for phase splitting. Organic and aqueous phases from the DEC are returned to the 12th stage of A-DWC$_R$ (also the 12th stage of A-DWC, see Figure 6) and the 6th stage of A-DWC$_L$ (the 16th stage of A-DWC, see Figure 6), respectively. The high-purity ethyl acetate product is recovered as the bottom stream from A-DWC$_R$. Lastly, ethyl acetate recovered from the fermenter off-gas in Cases 2a and 2b is fed to the 25th stage of A-DWC$_R$. This stream may be preheated using the bottom product stream from A-DWC$_L$ to reduce thermal energy requirements in A-DWC$_R$ by over 60%. More details about the design of A-DWC are presented in the Supporting Information S1.

Furthermore, due to the residual gasses after the initial purification steps, a partial vapor–liquid condenser (DWC$_R$-COND) was defined for A-DWC$_R$ and a possibility of vapor separation in DEC was considered. To minimize ethyl acetate losses, these vapor streams are compressed (in COMP3) and cooled to 25°C using cooling water (in HEX7). As condensed ethyl acetate may be recycled back to DEC, product loss in the remaining vapor is less than 0.2%. Finally, the high-purity ethyl acetate product is recovered in all three cases (product purity ≥99.5 wt%). Product recoveries in Cases 1 and 2b are high (100% and 99%, respectively). On the contrary, product recovery in Case 2a is about 92%, mainly due to the ethyl acetate that was not recovered from the off-gas after moderate refrigeration at −50°C.

3.4 | Economic evaluation

The economic performance indicators of the developed downstream processes (Cases 1, 2a, and 2b) are summarized in Table 5 and presented in Figure 7. The total equipment installation costs are the highest in Case 1, when ethyl acetate is recovered from the gas phase by compression and high-pressure condensation using cooling water (8190 k$). This is mainly due to the costs of the compressors (about 72% of the total installed equipment costs), whereby the cost of a multistage compressor for compressing the off-gas from the fermenter is the biggest contributor (about 92% of the total costs of compressors, or about 66% of the total installed equipment costs). On the contrary, the total equipment installation costs are lower when low-temperature condensation by refrigeration is used to recover ethyl acetate from the fermenter off-gas (4797 k$ in Case 2a and 5316 k$ in Case 2b). The

TABLE 5 Key performance indicators of the designed ethyl acetate recovery processes (dry air supply to the fermenter).

	Case 1	Case 2a	Case 2b
Ethyl acetate recovery (%)	100.0	92.1	98.9
Economic indicators			
CAPEX (k$)	15,137	8823	9784
OPEX (k$/year)	9328	4732	5093
OPEX ($/kg)	0.934	0.511	0.514
TAC (k$/year)	10,842	5615	6071
TAC ($/kg)	1.085	0.607	0.613
Sustainability metrics			
Thermal energy requirements ($kW_{th}h/kg$)	3.410	0.323	0.298
Electrical energy requirements (kW_eh/kg)	2.627	0.752	0.840
Primary energy requirements ($kW_{th}h/kg$)	9.976	2.204	2.399
CO_2 emissions (kg_{CO_2}/kg)[a]	0.494/1.692	0.284/0.627	0.070/0.453
Water consumption (m^3_w/kg)	0.570	0.123	0.056
Water loss (m^3_w/kg)	0.042	0.009	0.004
Material intensity (kg_{waste}/kg)	0	0	0
Pollutant emissions ($kg_{pollutant}/kg$)	0	0	0
Toxic emissions ($kg_{toxic\ material}/kg$)	0	0	0

Abbreviations: CAPEX, capital costs; OPEX, operational expenses; TAC, total annual costs.
[a]Green/gray electricity usage.

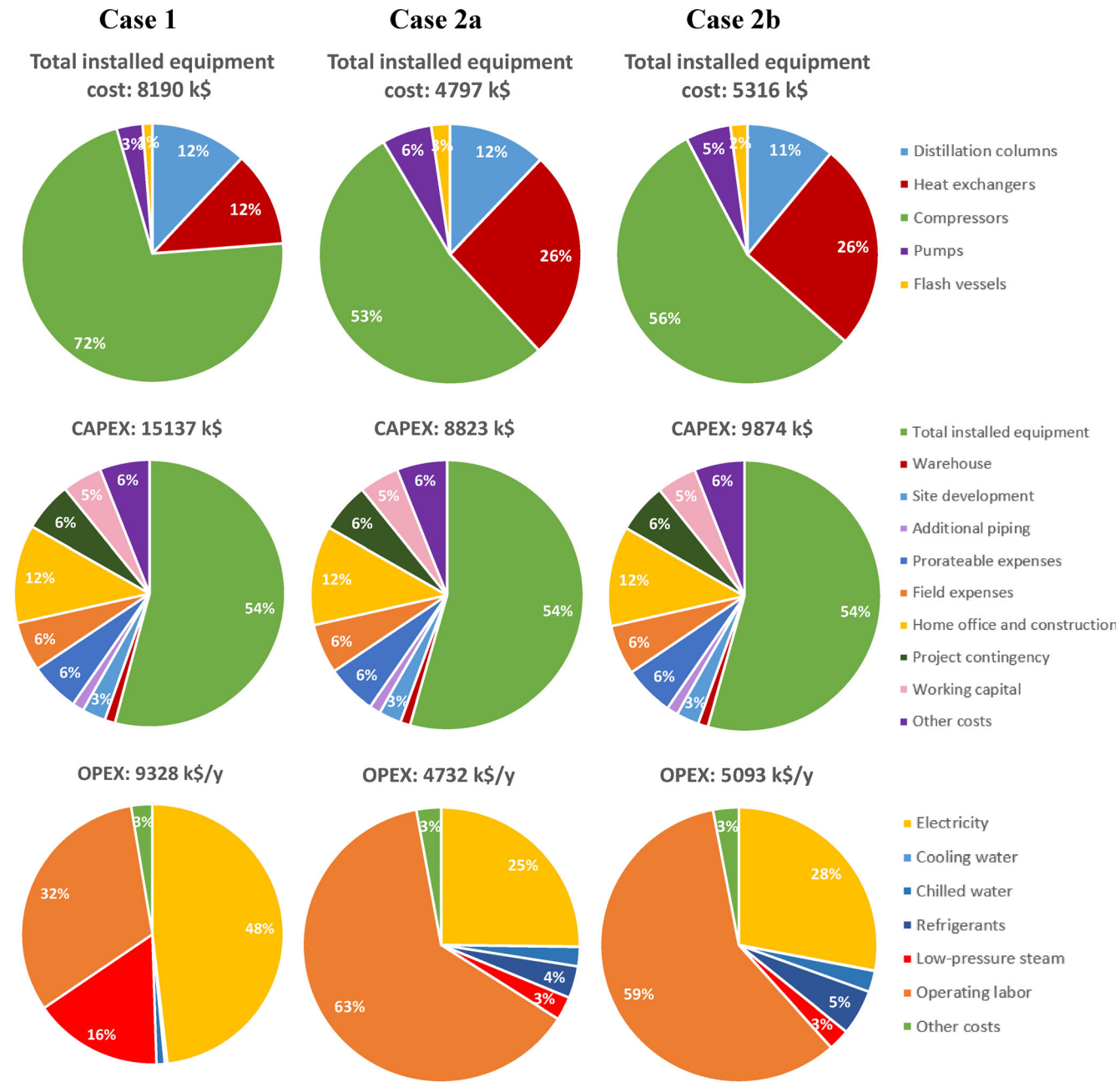

FIGURE 7 Comparison of economic indicators of ethyl acetate recovery. CAPEX, capital costs; OPEX, operational expenses.

cost of equipment in the refrigeration cycle makes up approximately 50% and 54% of the total equipment cost in Cases 2a and 2b, respectively. The higher cost of refrigeration equipment in Case 2b is mainly due to the more expensive compressor required to ensure lower condensation temperature. Considering different equipment types, the biggest contributors are the costs of compressors (72%, 53%, and 56% of the total equipment costs in Cases 1, 2a, and 2b, respectively), heat exchangers (12%, 26%, and 26% of the total equipment costs in Cases 1, 2a, and 2b, respectively) and distillation columns (12%, 12%, and 11% of the total equipment costs in Cases 1, 2a, and 2b, respectively), while costs of pumps (3%, 6%, and 5% of the total equipment costs in Cases 1, 2a, and 2b, respectively) and flash vessels are significantly lower (1%, 2%, and 2% of the total equipment costs in Cases 1, 2a, and 2b, respectively). Furthermore, the calculated CAPEX are much higher in Case 1 (15,137 k$) compared to Cases 2a (8823 k$) and 2b (9784 k$). This is mainly due to the higher equipment cost, which make up about 54% of CAPEX.

The determined OPEX are 9328, 4732, and 5093 k$/year in Cases 1, 2a, and 2b. Expressed per kilogram of recovered ethyl acetate, these values are 0.934, 0.511, and 0.514 $/kg, respectively. In Case 1, the biggest contributor to OPEX is the cost of utilities (about 65% of OPEX) and labor (about 32% of OPEX). The largest part of utilities' costs is the cost of electricity (about 74%), whereby the cost of electricity to power the multistage compressor for the initial

compression of the fermenter off-gas is the most dominant one (about 95% of the total electricity cost). On the contrary, utilities' costs are smaller when refrigeration is used in the initial recovery of ethyl acetate from the gas phase (about 34% and 38% in Cases 2a and 2b, respectively). Consequently, the contribution of labor costs in OPEX is higher (about 63% and 59% in Cases 2a and 2b, respectively) even though the absolute costs are very similar. Among the costs of utilities, the biggest contributors are the costs of electricity (25%–28% of OPEX), refrigeration (4%–5% of OPEX), low-pressure steam (3% of OPEX), and chilled water (2% of OPEX), while the cost of cooling water is much smaller (0.1% of OPEX). However, it should be noted that utilities' costs may vary significantly depending on the exact plant location.

Lastly, TAC that include CAPEX and OPEX with a PBP of 10 years are 10,842, 5615, and 6071 k$/year, or expressed per kilogram of ethyl acetate, 1.085, 0.607, and 0.613 $/kg in Cases 1, 2a, and 2b. The more expensive recovery process in Case 1 is primarily due to the more expensive recovery of ethyl acetate from the gas phase. More precisely, the higher CAPEX because of the costly multistage compressor for compressing the fermenter's off-gas and more expensive OPEX because of the electricity required to power this compressor. Case 2b is more attractive than Case 2a because the extent of ethyl acetate recovery is larger by a factor 99/92 in the Case 2b. Therefore, the amount of the fermentation broth needed for a fixed annual ethyl acetate production is a factor 99/92 smaller for Case 2b, leading to savings on the fermentation costs that will be larger than the difference between the aforementioned 0.607 and 0.613 $/kg. According to the available market data, the ethyl acetate price in North America in 2023 was 1739 $/kg.[61] Thus, the margin between the market price and the costs of the recovery process for Case 2b is 1.126 $/kg. The cost of the fermentation must be significantly smaller than this margin for economic feasibility of the overall process. Design and economic evaluation of the fermentation are outside the scope of this article, even though they are needed to get a full picture of the overall process.

Ethyl acetate is commonly produced by reactive distillation from ethanol and acetic acid. The operation and control of reactive distillation process have been widely studied.[62,63] Since this is a reversible reaction, hydrolysis may be used to obtain bio-based ethanol and acetic acid from ethyl acetate. Yet, production of bio-based ethanol through fermentation (from lignocellulosic biomass or gas fermentation) has already been proven effective on an industrial scale.[64] Thus, it seems hardly profitable to produce ethanol by hydrolyzing ethyl acetate made by fermentation.

3.5 | Sustainability assessment

The results of the environmental impact analysis are summarized in Table 5.

- *Energy intensity*: Both thermal and electrical energy requirements are the highest in Case 1 (3.410 $kW_{th}h/kg$ and 2.627 kW_eh/kg). The largest part of the thermal energy is used in the reboiler of A-DWC_L due to the water that is removed as the bottom product of A-DWC_L. This stream's flow rate is much larger in Case 1 compared to Cases 2a and 2b due to the water used to strip the remaining ethyl acetate from the gas phase in column C2. On the contrary, the flow rates of all feeds to A-DWC are much smaller in Cases 2a and 2b as additional water was not used. Consequently, the total thermal energy requirements are much smaller (0.323 and 0.298 $kW_{th}h/kg$ in Cases 2a and 2b, respectively). Furthermore, the largest part of the electrical energy requirements in Case 1 is due to the electricity needed to power the multistage compressor for compressing the fermenter off-gas. Contrarily, compressors in the refrigeration systems in Cases 2a and 2b require less power, which makes the total electrical energy requirements much smaller (0.752 and 0.840 kW_sh/kg in Cases 2a and 2b, respectively). Finally, the total primary energy requirements are significantly higher in Case 1 (9.976 $kW_{th}h/kg$), compared to Cases 2a and 2b (2.204 and 2.399 $kW_{th}h/kg$, respectively).
- *Greenhouse gas emissions*: As CO_2 emissions are strongly related to energy usage, these emissions are the highest in Case 1, in which 1.692 or 0.494 kg_{CO_2}/kg is emitted if gray or green electricity is used. On the contrary, CO_2 emissions are much lower in Cases 2a and 2b. However, CO_2 emissions are higher in Case 2a (0.627 or 0.284 kg_{CO_2}/kg if gray or green electricity is used) compared to Case 2b (0.453 or 0.070 kg_{CO_2}/kg if gray or green electricity is used) due to the larger amounts of the remained ethyl acetate in the gas phase that is accounted for through CO_2 equivalents.[42]
- *Water consumption*: Due to the significantly higher thermal energy requirements, water requirements and water loss are the highest in Case 1 (0.570 and 0.042 m^3_w/kg, respectively). Contrarily, these values are much lower in Cases 2a and 2b (approximately 0.056–0.123 and 0.004–0.009 m^3_w/kg, respectively).
- *Material intensity*: As waste is not formed in any of the developed recovery processes, values of material intensity metrics are equal to zero in all cases. Nonetheless, it should be noted that the bottom stream from DWC_L, if not further processed to recover ethanol or used upstream, should be sent to the wastewater treatment.
- *Pollutant and toxic materials*: Since no pollutants or toxic materials are emitted, the values of these metrics are zero in all cases.

3.6 | Sensitivity analysis: influence of the product concentration in the fermentation broth on the downstream processing performance

The recovery of ethyl acetate after the fermentation using the developed Case 2b has been proven to be the most cost-effective and energy-efficient due to the high product recovery and reasonable costs. A sensitivity analysis was performed on this case to examine the influence of the achievable ethyl acetate concentration in the broth on the performance of the downstream processing. The results of this analysis are presented in Figure 8. Generally, the increase of

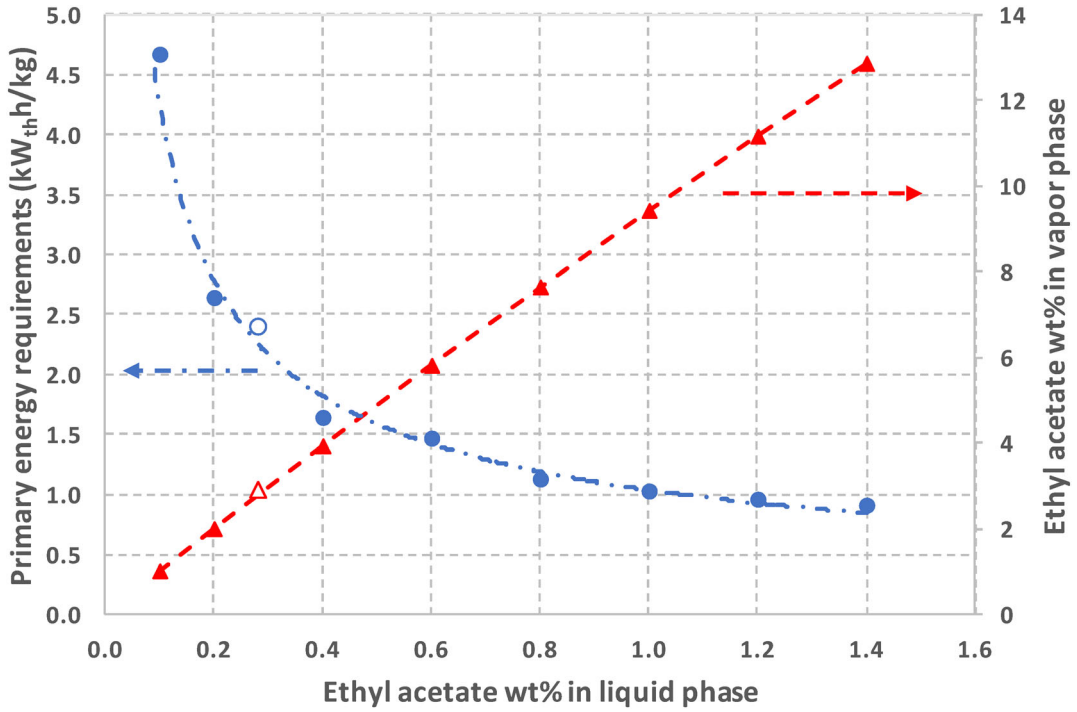

FIGURE 8 Sensitivity analysis: Influence of the achievable product concentration in the fermentation on the downstream processing performance of Case 2b. Markers are simulated cases, with the open marker being the base case.

ethyl acetate concentration in the liquid broth would lead to the linear increase of ethyl acetate concentration in the off-gas from the fermenter (defined by Henry's law). Due to the less diluted feed streams (both liquid and gas), the energy requirements of the downstream processing would decrease. This decrease is more drastic with extremely low product concentrations in the broth while it becomes milder for higher concentrations. For example, about 1.5 or 1.1 $kW_{th}h/kg$ are required to recover high-purity ethyl acetate product when its concentration in the liquid broth is 0.6 or 1.0 wt% (about 5.8 and 9.4 wt% in the exhaust gas), respectively. Therefore, even a slight increase in ethyl acetate concentration from the ones assumed in this study would significantly decrease the downstream processing costs. Nonetheless, obtaining higher product concentrations in the fermentation is challenging due to the inhibitory effects, and a lot of research effort has been put into developing more tolerant microorganisms. In that respect, it is worth noting that the energy requirements (and costs) of the downstream processing would not drastically decrease above a certain concentration of the product in the broth (e.g., 1.0 wt%, see Figure 8). Thus, very high product concentrations in the fermentation broth may not be necessary to make the production process competitive. Hence, simultaneous development of the upstream fermentation and the downstream process is necessary for successful scale-up.

The results of this study offer a realistic picture of the downstream processing performance after the specific fermentation process. Nonetheless, it should be mentioned that novel approaches to obtain ethyl acetate by fermentation include anaerobic fermentation of glucose with hydrogen co-production[65] and of carbon monoxide[66] will lead to off-gas streams very different from those of the aerobic process considered in the current article. However, the same approach might be used for developing the ethyl acetate recovery process.

3.7 | Alternative option: supplying pure oxygen to the fermenter instead of air

As microorganisms need only oxygen from the dry air supplied to the fermenter, an alternative approach would be to supply only pure oxygen such that the gas-to-liquid ratio in the fermentation would be much smaller. In the context of downstream processing (DSP), more ethyl acetate would be recovered from the liquid phase and less from the gas phase. However, an oxygen-rich gas stream would require additional safety measures. To compare the DSP performance when dry air or pure oxygen is supplied to the fermenter, the recovery processes were modified to recover ethyl acetate when pure oxygen is used upstream (Cases 1, 2a, and 2b were modified to Cases 3, 4a, and 4b, respectively) while keeping the same production capacity and O_2 supply per kg of liquid broth. As less exhaust gas is available when only oxygen is supplied upstream, the amount of ethyl acetate stripped by this gas is much smaller. Due to the much larger ethyl acetate concentration in the gas phase compared to the liquid phase, the needed gas flow rate is only slightly reduced (35,600 kg/h), while the required liquid flow rate is significantly increased (110,500 kg/h).

Key performance indicators for these processes are summarized in Table 6. The comparison with Table 5 suggests that supplying pure oxygen to the fermenter instead of dry air does not bring real benefits. In Case 1, the initial separation of ethyl acetate from the gas feed

TABLE 6 Key performance indicators of the designed ethyl acetate recovery processes (oxygen supply to the fermenter).

	Case 3	Case 4a	Case 4b
Ethyl acetate recovery (%)	99.4	93.3	98.7
Economic indicators			
CAPEX (k$)	17,404	11,903	12,643
OPEX (k$/year)	9098	5381	5668
OPEX ($/kg)	0.914	0.574	0.573
TAC (k$/year)	10,839	6571	6932
TAC ($/kg)	1.089	0.701	0.700
Sustainability metrics			
Thermal energy requirements ($kW_{th}h/kg$)	3.491	1.128	1.069
Electrical energy requirements (kW_eh/kg)	2.464	0.943	1.003
Primary energy requirements ($kW_{th}h/kg$)	9.652	3.485	3.576
CO_2 emissions (kg_{CO_2}/kg)[a]	0.506/1.629	0.351/0.781	0.176/0.634
Water consumption (m^3_w/kg)	1.067	0.202	0.144
Water loss (m^3_w/kg)	0.077	0.015	0.011
Material intensity (kg_{waste}/kg)	0	0	0
Pollutant emissions ($kg_{pollutant}/kg$)	0	0	0
Toxic emissions ($kg_{toxic\ material}/kg$)	0	0	0

Abbreviations: CAPEX, capital costs; OPEX, operational expenses; TAC, total annual costs.
[a]Green/gray electricity usage.

is significantly more expensive than the initial separation from the liquid feed stream. In Case 3, a large relative increase in the recovery costs from the liquid phase (due to the larger flow rates) is compensated by a small relative decrease in recovery costs from the gas phase. On the contrary, in Cases 2a and 2b, the costs of the initial separation of ethyl acetate from the gas phase are not as dominant as in Case 1. Thus, a slight relative decrease in these costs in Cases 4a and 4b cannot compensate for a large relative increase in the costs of the initial separation of ethyl acetate from the liquid feed. More details about the process design and performance are available in the Supporting Information S1.

4 | CONCLUSION

The newly developed downstream processes were proven to efficiently recover ethyl acetate after the aerobic fermentation process. The initial separation of volatile products from the liquid broth may be performed using a hybrid combination of gas stripping and vacuum evaporation. This approach may allow recycling of the remaining liquid broth with the microorganisms upstream. The initial separation of ethyl acetate from the fermenter's off-gas may be performed using high-pressure cooling with less expensive chilled water or low-temperature atmospheric cooling with more expensive refrigeration. Following the initial recovery steps, an advanced A-DWC was demonstrated to efficiently separate high-purity ethyl acetate product from water and ethanol. Finally, the total ethyl acetate purification process was proven to be more cost-effective (total recovery costs of 0.61 $/kg compared to 1.09 $/kg) and less energy-intensive (total energy requirements of 2.20–2.40 $kW_{th}h/kg$ compared to 9.98 $kW_{th}h/kg$) when refrigeration is used for the initial separation from the gas feed stream. Thus, the results of this original study suggest that vapor ethyl acetate should be recovered directly from the atmospheric off-gas using low-temperature condensation. Moreover, even though more expensive, lower-temperature condensation (refrigeration temperature of about −70°C compared to −50°C) is more favorable due to the higher ethyl acetate recovery (99% compared to 92%).

Moreover, the downstream processing in case only pure oxygen is supplied to the fermenter instead of dry air is more expensive due to the slightly lower gas feed and much larger liquid feed. This increase is especially significant if refrigeration is used for the recovery of ethyl acetate from the gas phase due to the smaller contribution of these costs in the total recovery costs.

AUTHOR CONTRIBUTIONS

Tamara Jankovic: Conceptualization; Methodology; Software; Data Curation; Validation; Visualization; writing–original draft; writing–review and editing. **Adrie Straathof:** Conceptualization; Methodology; Formal analysis; Validation; Supervision; writing–review and editing. **Anton A. Kiss:** Conceptualization; Methodology; Formal analysis; Investigation; Resources; Visualization; Validation; Supervision; Project administration; writing–original draft, writing–review and editing.

CONFLICT OF INTEREST STATEMENT

None of the authors have a conflict of interest to disclose.

DATA AVAILABILITY STATEMENT

The numerical data supporting Figures 5, 7, and 8 are available in the Supporting Information S1 accompanying this publication. As Figure 6

is a graphical representation of DWC and thermodynamically equivalent sequence of distillation columns, no numerical data is associated with it. Furthermore, additional details about the thermodynamic property models, validation of the obtained results, and the designs of the recovery processes when pure oxygen is used instead of air (Cases 3, 4a, and 4b) are also included in the Supporting Information S1.

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REFERENCES

1. Kruis AJ, Bohnenkamp AC, Patinios C, et al. Microbial production of short and medium chain esters: enzymes, pathways, and applications. *Biotechnol Adv*. 2019;37:107407.
2. Saboe PO, Monroe HR, Michener WE, et al. In situ product recovery of bio-based ethyl esters via hybrid extraction-distillation. *Green Chem*. 2019;21:5306-5315.
3. Coherent Market Insights. Ethyl acetate marker analysis. Accessed July 1, 2024. https://www.coherentmarketinsights.com/market-insight/ethyl-acetate-market-2353
4. Zhang S, Guo F, Yan W, et al. Perspectives for the microbial production of ethyl acetate. *Appl Microbiol Biotechnol*. 2020;104:7239-7245.
5. Zirahi A, Sadeghi Yamchi H, Haddadnia A, Zirrahi M, Hassanzadeh H, Abedi J. Ethyl acetate as a bio-based solvent to reduce energy intensity and CO_2 emissions of in situ bitumen recovery. *AIChE J*. 2020;66:e16828.
6. Šulgan B, Labovský J, Labovská Z. Multi-aspect comparison of ethyl acetate production pathways: reactive distillation process integration and intensification via mechanical and chemical approach. *Processes*. 2020;8:1-32.
7. Mahajan YS. Ethyl acetate production by Fischer esterification: use of excess of acetic acid and complete separation sequence. *Int J Chem React Eng*. 2024;22:1-14.
8. Löser C, Urit T, Bley T. Perspectives for the biotechnological production of ethyl acetate by yeasts. *Appl Microbiol Biotechnol*. 2014;98:5397-5415.
9. CropEnergies. Ground-breaking ceremony for first production plant for green ethyl acetate in Europe. Accessed June 4, 2024. https://www.cropenergies.com/en/press/details/ground-breaking-ceremony-for-first-production-plant-for-green-et
10. Díaz-Montaño DM, Délia ML, Estarrón-Espinosa M, Strehaiano P. Fermentative capability and aroma compound production by yeast strains isolated from *Agave tequilana* weber juice. *Enzyme Microb Technol*. 2008;42:608-616.
11. Dragone G, Mussatto SI, Oliveira JM, Teixeira JA. Characterisation of volatile compounds in an alcoholic beverage produced by whey fermentation. *Food Chem*. 2009;112:929-935.
12. Molina AM, Swiegers JH, Varela C, Pretorius IS, Agosin E. Influence of wine fermentation temperature on the synthesis of yeast-derived volatile aroma compounds. *Appl Microbiol Biotechnol*. 2007;77:675-687.
13. Urit T, Stukert A, Bley T. Formation of ethyl acetate by *Kluyveromyces marxianus* on whey during aerobic batch cultivation at specific trace element limitation. 2012;96:1313-1323.
14. Löser C, Urit T, Keil P, Bley T. Studies on the mechanism of synthesis of ethyl acetate in *Kluyveromyces marxianus* DSM 5422. *Appl Microbiol Biotechnol*. 2015;99:1131-1144.
15. Löser C, Urit T, Nehl F, Bley T. Screening of *Kluyveromyces* strains for the production of ethyl acetate: design and evaluation of a cultivation system. *Eng Life Sci*. 2011;11:369-381.
16. Urit T, Löser C, Wunderlich M, Bley T. Formation of ethyl acetate by *Kluyveromyces marxianus* on whey: studies of the ester stripping. *Bioprocess Biosyst Eng*. 2011;34:547-559.
17. Löser C, Urit T, Stukert A, Bley T. Formation of ethyl acetate from whey by *Kluyveromyces marxianus* on a pilot scale. *J Biotechnol*. 2013;163:17-23.
18. Urit T, Manthey R, Bley T, Löser C. Formation of ethyl acetate by *Kluyveromyces marxianus* on whey: influence of aeration and inhibition of yeast growth by ethyl acetate. *Eng Life Sci*. 2013;3:247-260.
19. Löser C, Urit T, Förster S, Stukert A, Bley T. Formation of ethyl acetate by *Kluyveromyces marxianus* on whey during aerobic batch and chemostat cultivation at iron limitation. *Appl Microbiol Biotechnol*. 2012;96:685-696.
20. Duboc P, von Stockar U. Systematic errors in data evaluation due to ethanol stripping and water vaporization. *Biotechnol Bioeng*. 1998;58:428-439.
21. Lenntech. Argon (Ar) and water. Accessed May 10, 2024. https://www.lenntech.com/periodic/water/argon/argon-and-water.htm
22. Lenntech. Nitrogen (N) and water. Accessed May 10, 2024. https://www.lenntech.com/periodic/water/nitrogen/nitrogen-and-water.htm
23. Daniell J, Köpke M, Simpson SD. Commercial biomass syngas fermentation. *Energies*. 2012;5:5372-5417.
24. López-Garzón CS, Straathof AJJ. Recovery of carboxylic acids produced by fermentation. *Biotechnol Adv*. 2014;32:873-904.
25. ReAgent. Ethyl acetate. Accessed May 21, 2024. https://www.chemicals.co.uk/ethyl-acetate
26. DutchChems. Ethyl acetate. Accessed May 21, 2024. https://www.dutchchems.com/product/ethyl-acetate/
27. Janković T, Straathof AJJ, Kiss AA. Enhanced isobutanol recovery from fermentation broth for sustainable biofuels production. *Energy Conv Manage*. 2024;21:100520.
28. Aspen Technology. *Aspen physical property system*. Aspen Technology; 2020.
29. NIST. Reference fluid thermodynamic and transport properties database (REFPROP). https://www.nist.gov/programs-projects/reference-fluid-thermodynamic-and-transport-properties-database-refprop
30. Humbird D, Davis R, Tao L, et al. Process design and economics for biochemical conversion of lignocellulosic biomass to ethanol. Technical Report NREL/TP-5100-51400. National Renewable Energy Laboratory. 2011.
31. Kiss AA. Design, control and economics of distillation. *Advanced Distillation Technologies: Design, Control and Applications*. Wiley; 2013:37-66.
32. Platform, British Columbia/Yukon Open Authoring. Cost of manufacturing. Accessed February 19, 2024. https://pressbooks.bccampus.ca/chbe220/chapter/costs-of-ma
33. Platform, British Columbia/Yukon Open Authoring. Cost of operating labour. Accessed February 19, 2024. https://pressbooks.bccampus.ca/chbe220/chapter/cost-of-operating
34. Federal Reserve Bank of St. Luis Economic Data. Producer price index by industry: utilities. Accesed July 11, 2024. https://fred.stlouisfed.org/series/PCU221221
35. Office for National Stat. Consumer price inflation tables. Accessed July 11, 2024. https://www.ons.gov.uk/economy/inflationandpriceindices/datasets/consumerpriceinflation
36. ChemAnalyst. Ethylene price trend and forecast. Accessed May 21, 2024. https://www.chemanalyst.com/Pricing-data/ethylene-40
37. Intratec. Propane price in Netherlands. Accessed May 21, 2024. https://www.intratec.us/products/energy-price-references/commodity/propane-price-netherlands
38. Schwarz J, Beloff B, Beaver E. Use sustainability metrics to guide decision-making. *Chem Eng Prog*. 2002;98:58-63.

39. BP. Approximate conversion factors. Statistical Review of World Energy. 2021. https://www.bp.com/en/global/corporate/energy-economics.html
40. Lieberman NP, Lieberman ET. Steam generation. *A Working Guide to Process Equipment*. McGrawHill; 2022:261-276.
41. Kiss AA, Suszwalak DJ-PC. Innovative dimethyl ether synthesis in a reactive dividing-wall column. *Comput Chem Eng*. 2012;38:74-81.
42. CarbonCloud. Ethyl acetate. E1504. 2024. https://apps.carboncloud.com/climatehub/product-reports/id/32
43. Armstrong DW, Martin SM, Yamazaki H. Production of ethyl acetate from dilute ethanol solutions by *Candida utilis*. *Biotechnol Bioeng*. 1984;26:1038-1041.
44. Sulzer. Structured packings. Accessed January 12, 2024. https://www.sulzer.com/en/products/separation-technology/structured-packings
45. Kiss AA, Infante Ferreira CA. Mechanically driven heat pumps. *Heat Pumps in Chemical Process Industry*. CRC Press; 2016:189-251.
46. Khan FI, Ghoshal AK. Removal of volatile organic compounds from polluted air. *J Loss Prev Process Ind*. 2010;13:527-545.
47. Wylock C, Eloundou Mballa PP, Heilporn C, Debaste F, Fauconnier ML. Review on the potential technologies for aromas recovery from food industry flue gas. *Trends Food Sci Technol*. 2015;46:68-74.
48. Lukin I, Pietzka L, Groß K, Górak A, Schembecker G. Economic evaluation of rotating packed bed use for aroma absorption from bioreactor off-gas. *Chem Eng Process*. 2020;154:108011.
49. Volker E. How to design and optimise sieve trays. WelChem. Accessed March 28, 2023.
50. Du K, Zhang S, Xu W, Niu X. A study on the cycle characteristics of an auto-cascade refrigeration system. *Exp Therm Fluid Sci*. 2009;33:240-245.
51. Gales L, Mendes A, Costa C. Recovery of acetone, ethyl acetate and ethanol by thermal pressure swing adsorption. *Chem Eng Sci*. 2003;58:5279-5289.
52. Sato K, Sugimoto K, Nakane T. Separation of ethanol/ethyl acetate mixture by pervaporation at 100–130°C through NaY zeolite membrane for industrial purpose. *Microporous Mesoporous Mater*. 2008;115:170-175.
53. Zhu Z, Ri Y, Jia H, Li X, Wang Y, Wang Y. Process evaluation on the separation of ethyl acetate and ethanol using extractive distillation with ionic liquid. *Sep Purif Technol*. 2017;181:44-52.
54. Zhang DL, Deng YF, Li CB, Chen J. Separation of ethyl acetate–ethanol azeotropic mixture using hydrophilic ionic liquids. *Ind Eng Chem Res*. 2008;47:1995-2001.
55. Toth AJ, Szanyi A, Koczka K, Mizsey P. Enhanced separation of highly non-ideal mixtures with extractive heterogeneous-azeotropic distillation. *Sep Sci Technol*. 2016;51:1238-1247.
56. Toth AJ. Comprehensive evaluation and comparison of advanced separation methods on the separation of ethyl acetate-ethanol-water highly non-ideal mixture. *Sep Purif Technol*. 2019;224:490-508.
57. Caballero JA, Grossmann IE. Synthesis of complex thermally coupled distillation systems including divided wall columns. *AIChE J*. 2013;59(4):1139-1159.
58. Ehlers C, Schroder M, Fieg G. Influence of heat transfer across the wall of dividing wall columns on energy demand. *AIChE J*. 2015;61(5):1648-1662.
59. Janković T, Straathof AJJ, Kiss AA. Advanced downstream processing of bioethanol from syngas fermentation. *Sep Purif Technol*. 2023;322:124320.
60. Qian X, Lin KH, Jia S, Biegler LT, Huang K. Nonlinear model predictive control for dividing wall columns. *AIChE J*. 2023;69(6):e18062.
61. ChemAnalyst. Ethyl acetate price trend and forecast. Accessed May 21, 2024. https://www.chemanalyst.com/Pricing-data/ethylene-40
62. Vora N, Daoutidis P. Analysis and nonlinear control of an ethyl acetate reactive distillation column. *Proc Am Control Conf*. 1998;4:2113-2117.
63. Lee HY, Huang HP, Chien IL. Control of reactive distillation process for production of ethyl acetate. *J Process Control*. 2007;17:363-377.
64. LanzaTech. World's leading steel company, ArcelorMittal and LanzaTech announce first ethanol samples from commercial flagship carbon capture and utilisation facility in Ghent, Belgium. https://lanzatech.com/worlds-leading-steel-company-arcelormittal-and-lanza 2023.
65. Bohnenkamp AC, Kruis AJ, Mars AE, et al. Multilevel optimisation of anaerobic ethyl acetate production in engineered *Escherichia coli*. *Biotechnol Biofuels*. 2020;13:1-14.
66. Dykstra JC, van Oort J, Yazdi AT, et al. Metabolic engineering of *Clostridium autoethanogenum* for ethyl acetate production from CO. *Microb Cell Fact*. 2022;21:1-11.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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