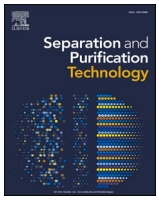
Separation and Purification Technology 424 (2027) 140749



Contents lists available at ScienceDirect

Separation and Purification Technology

journal homepage: www.elsevier.com/locate/seppur



Integrated downstream processing for the selective recovery of formate and DHA from a multi-enzymatic CO_2 utilization system: Evaluating process robustness from pure substrates to real industrial matrices

Sady Roberto Rodriguez, Marina Guillén, Oscar Romero *

Bioprocess Engineering and Applied Biocatalysis Group (ENG4BIO), Department of Chemical, Biological and Environmental Engineering, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain

ARTICLE INFO

Editor name: Raquel Aires Barros

Dataset link: Replication data for "Integrated Downstream Processing for the Selective Recovery of Formate and DHA from a Multi-Enzymatic CO_2 Utilization System" (Original data)

Keywords:
Downstream
Ion-exchange resin
Liquid-liquid extraction
Green solvents
Green metrics and biocatalysis

ABSTRACT

Downstream processing (DSP) is essential for efficient product recovery but often represents a major bottleneck in bioprocess sustainability and economic viability. In biocatalytic systems using immobilized enzymes, DSP typically involves biocatalyst recovery followed by product separation and purification. Therefore, effective DSP requires product-specific techniques that minimize resource use and costs. This work addresses the design of a DSP for the recovery of two products obtained from a multi-enzymatic CCU system, dihydroxyacetone (DHA) and formate, produced from pure and crude substrates (glycerol from industrial biodiesel production and CO_2-gas mimicking real off-gases from iron&steel industry). First, DHA separation was carried out by ion exchange adsorption using the highly selective anionic resin Amberlite® IRA-410, achieving adsorption of at least $89.4 \pm 1.4\%$ and recovery yields of $79.1 \pm 0.5\%$ after elution with sodium chloride. For formate, liquid-liquid extraction (LLE) using the green solvents 2-methyltetrahydrofuran (2-MTHF) and isobutyl acetate was evaluated, showing extraction of $90.1 \pm 0.9\%$ and $65.2 \pm 2.1\%$, respectively. After distillation, both solvents showed similar performance, enabling purified formate streams of 74.9 ± 1.4 wt%. The environmental impact, based on *E*-factor–derived EQ and Global Warming Potential (GWP) metrics, provided guidance for reducing the environmental footprint and improving the sustainability of DSP for early-stage enzymatic systems through potential material recycling and the use of low hazardous chemicals and green solvents. Finally, the designed DSP proved to be effective for both evaluated matrices, demonstrating its reliability and applicability for biocatalytic process intensification at bench scale.

1. Introduction

Downstream processing (DSP) is essential for the efficient recovery of commercially valuable products [1] yet it often represents a major bottleneck in developing profitable production systems. For products requiring high purity, DSP costs can account for up to 80% of total production expenditure, depending on the target molecule and its application [2]. Therefore, efficient DSP should reduce costs, processing time, and energy consumption while enabling the reuse of materials and reagents, thereby improving the sustainability and economic viability of intensified bioprocesses [3].

In enzymatic processes, DSP primarily involves biocatalyst recovery, followed by product separation and purification. When immobilized on solid supports, biocatalysts can be recovered using conventional techniques such as filtration, sedimentation, and centrifugation, enabling their reuse, facilitating continuous processing, and improving final product purity [4]. Efficient product recovery and purification are essential to maximize yield, quality, and economic viability [5]. Common techniques include pervaporation, gas stripping, liquid–liquid extraction, adsorption onto resins or activated carbon, crystallization, precipitation, distillation, and ultrafiltration/nanofiltration [3]. These techniques can be used to purify the target product or remove inhibitory and undesired by-products [2]. Therefore, their selection depends on product properties, such as volatility, solubility, polarity, stability, as well as medium composition and the required purity level [6].

Overall, DSP of products from enzymatic remains relatively underexplored compared with advances in biocatalyst development and reaction engineering, with separation and purification often receiving less

* Corresponding author.
E-mail address: Oscar.Romero.Ormazabal@uab.cat (O. Romero).

https://doi.org/10.1016/j.seppur.2026.140749
Received 16 September 2026; Received in revised form 27 October 2026; Accepted 27 October 2026
Available online 28 October 2026
1383-5866/© 2026 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC license (http://creativecommons.org/licenses/by-nc/4.0/).

attention [7]. This gap is particularly relevant in carbon capture and utilization (CCU), where efficient product recovery is essential for economically viable carbon valorization [8]. Although considerable efforts have focused on improving CO_2 capture technologies and catalytic conversion pathways, DSP remains one of the least explored aspects of CCU systems. Enzymatic CCU media often contain unreacted CO_2, inorganic salts, cofactors, residual substrates, and multiple products or by-products, complicating separation. These challenges are amplified in multi-enzymatic systems, where products may have similar physicochemical properties and require incompatible recovery conditions [9]. Therefore, selective separation techniques tailored to product properties and well-designed downstream stages are crucial for industrial viability [10]. Moreover, product separation can significantly contribute to CCU process energy demand and operating costs, highlighting the need for efficient and sustainable DSP strategies [11].

This study addresses the design and implementation of a DSP scheme for separating and purifying two compounds, formate and dihydroxyacetone (DHA), produced by a multi-enzymatic system based on a carbon capture and utilization (CCU) strategy. This biocatalytic system was previously optimized in a stirred-tank reactor using a co-immobilized biocatalyst comprising formate dehydrogenase (FDH) and glycerol dehydrogenase (GlyDH) [12]. Therefore, the reaction medium consists of a complex aqueous solution containing the biocatalytic products and residual substrates, highlighting the need for an efficient downstream processing strategy (Fig. 1).

To assess the performance of the multi-enzymatic system under industrially relevant conditions, two substrate types were evaluated: pure substrates (a gas mixture of 24% CO_2 in N_2 and pure glycerol) and crude substrates (a blast furnace off-gas mixture containing 24.5% CO_2, 46.6% N_2, 23.9% CO, 1.2% O_2, and 3.8% H_2 [13] and crude glycerol from biodiesel production, composition in Table S1, Supplementary Information [SI]). Using crude substrates derived from industrial streams provides a more realistic assessment of DSP performance, as their complex composition and impurities may affect purification efficiency [14]. This approach may also enable a more representative evaluation of process robustness and industrial relevance.

Formate and DHA are high-value chemicals with substantial global markets, valued at approximately USD 2.4 billion for formic acid/formate [15] and USD 220 million for DHA [16]. Formate is used in leather tanning, textile processing, chemical synthesis, and biotechnology [17], whereas DHA is widely applied in self-tanning products and cosmetics, as well as in pharmaceutical and biotechnological industries [18]. Their enzymatic production offers a potentially more sustainable alternative to conventional chemical processes, with reduced environmental impact and promising industrial applications [19].

Therefore, this work develops a sustainable DSP platform at laboratory scale, compatible with the intensification of the enzymatic system. The DSP strategy was designed based on the physicochemical properties of the compounds present in the final reaction matrices. Selective recovery methods for DHA and formate were evaluated, and the environmental impact of the proposed DSP was assessed to identify key factors affecting its sustainability and further potential industrial applicability. The strategy was applied to reaction matrices obtained using pure and crude substrates to assess the influence of matrix composition on product separation under laboratory-scale conditions.

2. Materials and methods

2.1. Chemicals and reagents

All reagents for the DSP, including the ion exchange resins and the green solvents, were purchased from Sigma-Aldrich (St. Louis, MO, USA). All samples and buffers were prepared in Milli Q water (18.2 MΩ·cm, Merck Millipore, USA). All materials involved in enzyme production, biocatalyst development, and the multi-enzymatic reactions have been previously reported by the authors [12,20].

2.2. Screening of ion exchange resins (IERs) for DHA adsorption

DHA adsorption was evaluated on three IERs previously mentioned: Amberlite® HPR650 H, AmberChrom® 50WX2 and Amberlite® IRA-410. Before use, Amberlite® HPR650 H and AmberChrom® 50WX2 were washed with three bed volumes of 30% (*w*/w) NaCl, followed by distilled water until neutral pH. Amberlite® IRA-410 was washed sequentially with three bed volumes each of 10% (*w*/*v*) NaCl, 3% (*w*/*v*) NaCl, and 3% (*w*/*v*) NaOH, followed by distilled water until neutral pH.

First, adsorption was evaluated in the presence of the other components of the multi-enzymatic reaction. The concentrations used for each compound were selected to approximate the maximum concentrations quantified at the end of the reactions in previous work involving the multi-enzymatic reactions [19,21]. The only exception was NADH (reduced nicotinamide adenine dinucleotide), for which the initial concentration in the reaction was used. The concentrations were as follows: formate 70 mM, NADH 1 mM, glycerol 25 mM, glycerol carbonate 50 mM, and DHA 30 mM. For the experiments, 1 g of resin was incubated in a total volume of 10 mL phosphate buffer 100 mM containing all compounds under the following conditions: 35 °C, pH 7.0, and stirring at 300 rpm, until a constant DHA concentration was reached. As a control, the compounds were incubated without the resins.

Subsequently, the DHA adsorption isotherms for each resin were determined by evaluating the following concentrations: 0, 5, 10, 25, 50,

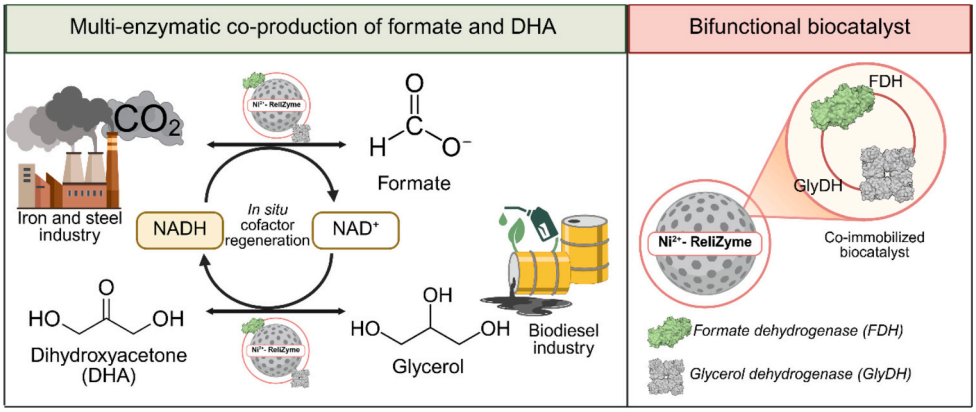


Fig. 1. Schematic representation of the multi-enzymatic platform for the conversion of CO_2 into value-added products. The system enables the co-production of formate and dihydroxyacetone (DHA) from CO_2 and glycerol through in situ NADH regeneration, catalyzed by a bifunctional co-immobilized biocatalyst composed of formate dehydrogenase (FDH) and glycerol dehydrogenase (GlyDH).

100, 150, and 200 mM. These experiments were carried out under the same conditions as previously described (10 mL of total volume for 1 g of resin). As a control, the DHA was incubated without the resins. All assays were conducted in triplicate. The analysis of each evaluated compound was performed according to section 2.5.

2.3. DHA recovery using ion-exchange resins (IERs)

The separation of DHA from the reaction media (reactions conducted with pure and crude substrates) was conducted by ion-exchange adsorption using the IER selected in the previous procedure. For the experiments, 200 mL of reaction media were incubated with 10 g of the selected resin for 24 h at 35 °C with constant agitation at 300 rpm. After filtration, the resin and the remanent reaction media were stored for the next steps. For desorption, the resin containing DHA was incubated with a 500 mM NaCl solution at a 1:9 ratio, following the same conditions previously described. All assays were conducted in triplicate. The quantification of DHA was conducted according to section 2.5.

2.4. Formate isolation by liquid-liquid extraction (LLE)

Formate isolation was performed by LLE with 2-methyltetrahydrofuran (2-MTHF) and isobutyl acetate solvents from the reaction media remaining (reactions conducted with pure and crude substrates) after DHA adsorption. First, the reaction media were acidified to pH 2.0 with HCl 6 M to protonate the formate to formic acid. Four extractions were performed with each solvent (1:1 volume ratio) in a separation funnel, with vigorous shaking for 5 min per extraction. Subsequently, formic acid extracted was separated from the solvents by distillation using a Heidolph WB 2000 rotary evaporator (Schwabach, Germany). The distillation of 2-MTHF was performed at 30 °C for 25 min and isobutyl acetate at 45 °C for 50 min [22,23]. The round-bottom flask was weighed before and after distillation to determine the mass of the recovered formic acid. Finally, the recovered formic acid was resuspended in Milli-Q water. All assays were conducted in triplicate. The quantification of formic acid/formate in both aqueous and organic media was conducted according to section 2.5.

2.5. Compounds analysis

Formate, DHA, and glycerol were quantified using an Agilent 1220 Infinity II liquid chromatograph equipped with an IC-Sep COREGEL 87H3 column and a mobile phase of 0.5 mM H_2SO_4/acetonitrile (65:35). Analyses were performed at 0.6 mL min^{-1}, with a 20 µL injection volume, a column temperature of 30 °C, UV detection at 210 nm, and an RID detector at 35 °C. Glycerol carbonate was quantified by reverse-phase chromatography using a C18 CORTECS column, 0.004 N H_2SO_4 as the mobile phase, a flow rate of 0.8 mL min^{-1}, a 100 µL injection volume, and a column and RID detector temperature of 35 °C. Formic acid in 2-MTHF and isobutyl acetate was quantified using the C18 column under the previously described ion-exchange chromatography conditions. NADH was quantified by UV/visible spectrophotometry at 340 nm using a SPECTROstar microplate reader, with the reaction buffer as the blank. All assays were performed in triplicate.

2.6. Assessment of the environmental impact of the downstream process

The environmental impact of the DSP was assessed based on laboratory experimental data and the amount of product recovered. First, the environmental impact associated with DHA recovery was evaluated through the determination of the environmental quotient (EQ) and the environmental factor (*E*-factor), both of which were calculated according to the equations proposed by Sheldon [24,25]:

$$EQ = E - \text{factor} \times Q \quad (1)$$

where: Q = "Unfriendliness quotient" or environmental impact factor of the waste (related to toxicity, hazard, persistence, etc.). In this case $Q_{NaCl} = 1$

$$E - \text{factor} = \frac{\text{mass of waste generated (kg)}}{\text{mass of product obtained (kg)}} \quad (2)$$

where:

Mass of waste = (Mass of water– mass of sodium chloride [NaCl]– mass of desorbed DHA).

Mass of products = Mass of desorbed DHA.

Regarding formate recovery, the environmental impact associated with the liquid–liquid extraction and the subsequent solvent distillation was evaluated in terms of climate change using the Global Warming Potential (GWP) indicator. The GWP was calculated based solely on energy inputs (heating and distillation) and the treatment of the portion of organic solvent that was not recovered, applying the equations proposed by Domínguez de María [26,27] for biocatalytic reactions in early development stages:

$$\begin{aligned}\text{GWP}\,(extraction[dsp]) : &\left(\frac{(0.0002 \bullet n \bullet \Delta T) + (0.00008 \bullet n \bullet \Delta H)}{[\text{P}]}\right) \\ &+ \left(\frac{0.021 \bullet n \bullet \%\text{Solvent treated}}{[\text{P}]}\right)\end{aligned} \quad (3)$$

where:

n = number of extraction volumes (four extractions).

ΔT = Difference between the temperature of the reaction and the boiling point of the solvent (In this case: reaction temperature: 30 °C; boiling point of 2-MTHF: 80 °C; and isobutyl acetate: 118 °C).

ΔH = Enthalpy of vaporization of the solvent (376 kJ kg^{-1} for 2-MTHF and 354 kJ kg^{-1} for isobutyl acetate).

[P]: Formate concentration subjected to DSP (kg L^{-1}).

Unrecovered solvent fraction (%Solvent treated): 3% for 2-MTHF and 5% for isobutyl acetate.

3. Results and discussion

3.1. Design of integrated downstream processing (DSP)

As a first step, the DSP was designed for the selective recovery of DHA and formate co-produced in two reaction matrices prepared with pure and crude substrates. In addition to the products of the enzymatic reactions, glycerol carbonate (GC) was also formed as a by-product through a parallel chemical pathway involving the reaction between glycerol and CO_2, catalyzed by Ni^{2+} present in the enzyme immobilization support (Ni^{2+}-ReliZyme) and Zn^{2+} located at the active site of the *Bacillus stearothermophilus* glycerol dehydrogenase (GlyDH) used in this study [20]. Although a GC-rich stream was also obtained, it was not included in the DSP, as the primary focus was the recovery and purification of the products generated directly by the enzymatic reaction. As has been extensively reported in the literature, DHA has been successfully recovered from reaction mixtures using ion-exchange resins (IERs), owing to its ability to interact with the charged functional groups of these materials under appropriate operating conditions [28,29]. Therefore, the first stage of the DSP involved screening a range of IERs to identify those exhibiting high selectivity toward DHA while minimizing the adsorption of formate. Subsequently, the adsorbed DHA can be recovered by elution with a sodium chloride solution, generating a concentrated DHA stream while simultaneously regenerating the resin for potential reuse.

Once DHA has been separated, formate can be protonated to its acidic form and subsequently recovered by liquid–liquid extraction (LLE), as has also been extensively reported in the literature [30,31], owing to the preferential distribution of this molecule into suitable organic solvents. Finally, formic acid can be separated from the

extraction solvent by distillation, enabling the recovery of a formate stream with increased purity, as well as the extraction solvent for potential reuse. Fig. 2 illustrates the proposed DSP scheme for recovering DHA and formate. The selected separation strategies were evaluated experimentally using complex reaction mixtures, with their recovery performance and environmental implications discussed in the following sections.

3.2. Downstream processing: DHA recovery by ion-exchange adsorption

As outlined above, the first stage of the DSP involved evaluating the adsorption of DHA on three IERs: Amberlite® HPR650 H, AmberChrom® 50WX2, and Amberlite® IRA-410. Ion-exchange adsorption using ionic resins has been reported as an effective approach for the isolation of this compound from liquid mixtures [28]. First, Adsorption was assessed in a reaction medium containing the main components of the system to evaluate resin selectivity under conditions representative of the final reaction mixture [12,19]. Fig. 3 shows the adsorption yields of each component relative to its initial amount in the liquid medium, while Table S2 (SI) reports the amount adsorbed per g of resin (mg g^{-1}). Amberlite® IRA-410 exhibited the highest DHA adsorption yield ($90.6 \pm 1.4\%$), corresponding to 24.5 ± 0.3 mg g^{-1} resin, compared with $21.7 \pm 2.8\%$ (5.8 ± 0.6 mg g^{-1}) for Amberlite® HPR650 H and $25.6 \pm 2.6\%$ (6.9 ± 0.6 mg g^{-1}) for AmberChrom® 50WX2. These results indicate that Amberlite® IRA-410 is the most suitable of the tested resins for DHA adsorption under the conditions investigated.

The affinity of DHA for the evaluated IERs may be influenced by their physicochemical properties. Table S3 in SI summarizes the main technical characteristics of the IERs used in this study. Amberlite® HPR650 H and AmberChrom® 50WX2 are strong acidic cation exchange resins bearing sulfonic acid groups on a cross-linked styrene matrix, with stability over a broad pH range (0–14) and at elevated temperatures [32]. Although AmberChrom® 50WX2 has been employed for the large-scale separation of DHA and glycerol, its selectivity toward the target compound remains relatively low [28], as further illustrated in Fig. 3. On the other hand, Amberlite® IRA-410 (chloride form) is a basic (type II) anion exchange resin with a benzyldimethyl (2-hydroxyethyl) ammonium group, which primarily interacts with anion [33]. Therefore, DHA adsorption onto these materials can be interpreted in terms of the chemical functionality of the IERs and the physicochemical properties of the adsorbate.

Previous studies have shown that IERs can retain both charged and neutral organic compounds, although the extent of adsorption depends on factors such as molecular structure, hydrophobicity, and ionization state. In general, neutral carbonyl-containing compounds tend to exhibit lower removal efficiencies than ionized species, for which electrostatic interactions and ion-exchange mechanisms can contribute more directly to retention [34]. Since DHA is predominantly present in its non-ionized form under the experimental conditions investigated, its retention is likely influenced by mechanisms beyond conventional ion exchange. Previous studies have demonstrated that hydrophobic interactions and physical adsorption can contribute to the retention of organic compounds by ion-exchange resins. In the case of DHA, additional hydrogen-bonding interactions involving its hydroxyl and carbonyl groups may also contribute to its retention, depending on the functional groups present in the resin [18,35]. Collectively, these considerations provide a physicochemical framework for interpreting the observed adsorption of DHA by IERs.

The adsorption of other reaction components by Amberlite® IRA-410 was also evaluated. Glycerol carbonate and NADH showed adsorption yields of $17.1 \pm 2.0\%$ (10.1 ± 0.2 mg g^{-1}) and $58.3 \pm 1.8\%$ (3.9 ± 0.03 mg g^{-1}), respectively, whereas formate adsorption was limited ($8.6 \pm 0.2\%$). In contrast, formate adsorption reached $53.1 \pm 1.6\%$ with Amberlite® HPR650 H. Glycerol showed minimal adsorption on all three resins. The relatively high adsorption of NADH by Amberlite® IRA-410 is consistent with its anionic character at neutral pH and the presence of quaternary ammonium groups in the resin [36]. In the case of glycerol carbonate, its semi-polar nature allows it to interact with hydrophobic surfaces, such as benzyl dimethyl ammonium groups [37], as observed in these experiments. However, although co-adsorption of other compounds may affect resin selectivity, its practical impact is expected to depend on their concentration in the final reaction medium. The low formate adsorption observed with Amberlite® IRA-410 may reflect its limited retention under the experimental conditions and competition from other compounds, while the differences between resins may also be influenced by their functional groups and physicochemical properties [38]. As a result, the observed retention of DHA by Amberlite® IRA-410 may be partly attributed to the interactions previously discussed between DHA's hydroxyl and carbonyl groups and the resin matrix, which may contribute to its adsorption relative to other compounds in the reaction mixture.

To determine the maximum DHA adsorption capacity, adsorption isotherms were obtained for each resin (Fig. 4; Langmuir plots are provided in Fig. S1 of the SI). Amberlite® IRA-410 showed the highest maximum adsorption capacity (30.9 ± 0.9 mg g^{-1}), followed by Amberlite® HPR650 H (15.8 ± 0.3 mg g^{-1}) and AmberChrom® 50WX2

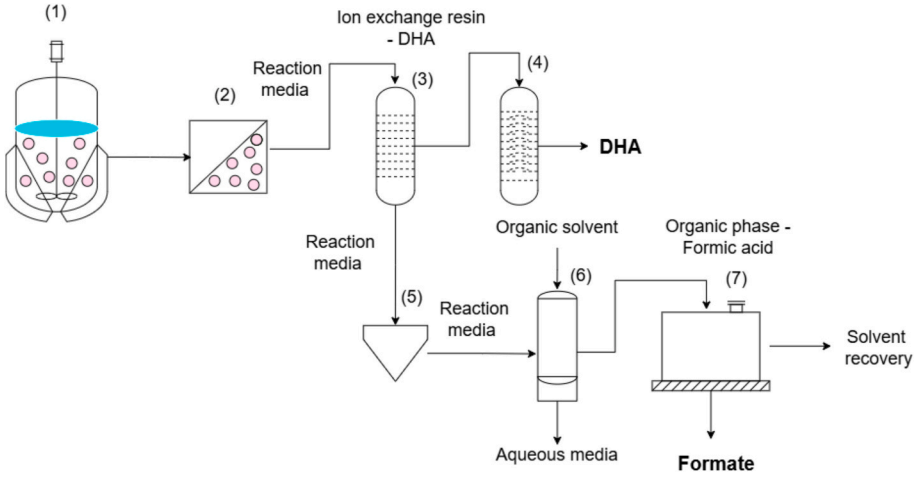


Fig. 2. Overview of the downstream processing (DSP) for DHA and formate separation and purification. (1) Reaction medium. (2) Immobilized biocatalyst recovery by filtration. (3) DHA adsorption onto an ion-exchange resin (IER). (4) DHA desorption from the resin with sodium chloride. (5) Acidification of reaction medium to pH 2. (6) Liquid-liquid extraction with green solvents and (7) Solvent distillation in a rotary evaporator. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

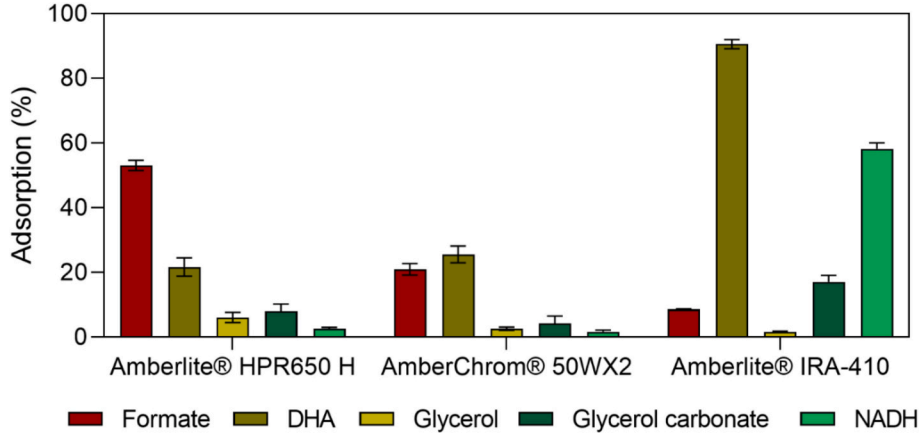


Fig. 3. Formate, DHA, glycerol, glycerol carbonate, and NADH adsorption in three ion exchange resins -Amberlite® HPR650 H, AmberChrom® 50WX2 and Amberlite® IRA-410- at the following initial concentrations: Formate 69.6 mM, DHA 30.7 mM, glycerol 25.7 mM, glycerol carbonate 50.3 mM, and NADH 1 mM.

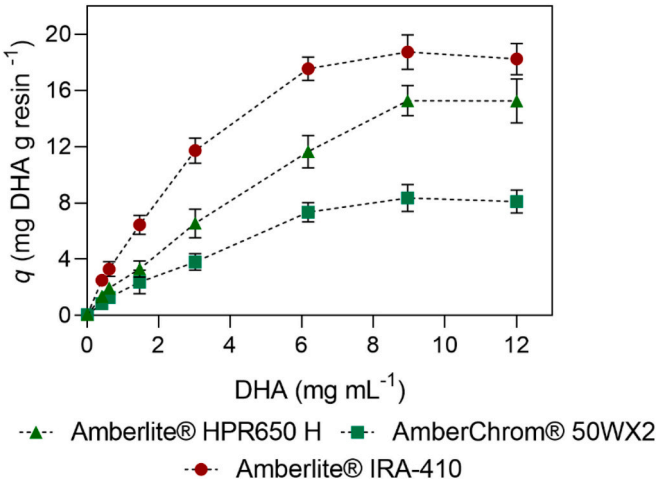


Fig. 4. Adsorption isotherm of DHA on ion exchange resins: Amberlite® HPR650 H, AmberChrom® 50WX2, and Amberlite® IRA-410.

Table 1
Parameters evaluated in the adsorption isotherms of DHA on the ion exchanges reins according to Langmuir model.

Resin	Q_{max}* (mg g^{-1})	K_L** (mL mg^{-1})
Amberlite® HPR650 H	15.8 ± 0.3	0.22 ± 0.03
AmberChrom® 50WX2	9.9 ± 0.1	0.29 ± 0.02
Amberlite® IRA-410	30.9 ± 0.9	0.29 ± 0.04

*Q_{max}: Maximum adsorption capacity. **K_L: Langmuir adsorption constant.

(9.9 ± 0.1 mg g^{-1}) (Table 1). The Langmuir constants were 0.29 ± 0.04, 0.22 ± 0.03, and 0.29 ± 0.02 mL mg^{-1}, respectively. The higher adsorption capacity of Amberlite® IRA-410 may be attributed, at least in part, to its macroporous structure, which provides greater accessibility to adsorption sites as DHA diffuses into the resin particles. In contrast, the gel-type structure of the cation-exchange resins may restrict DHA diffusion due to smaller pore sizes and a more highly hydrated environment, potentially limiting adsorption [39]. These structural differences may therefore contribute to the higher DHA adsorption capacity observed for Amberlite® IRA-410, although further characterization would be required to confirm their relative contributions.

The higher DHA adsorption observed for Amberlite® IRA-410 may also be related to its amino-functionalized groups. As a reducing sugar, DHA can potentially participate in Maillard-type reactions with amino-containing compounds [40], which may account for its higher affinity. However, compared with the materials used in a previously reported multienzymatic reaction system from our group, namely the Ni^{2+}-ReliZyme immobilization carrier and the corresponding bifunctional biocatalyst (the two enzymes immobilized on Ni^{2+}-ReliZyme), Amberlite® IRA-410 exhibited a considerably lower maximum adsorption capacity than both the immobilization carrier (62.6 ± 0.3 mg DHA g^{-1}) and the biocatalyst (140.8 ± 0.5 mg DHA g^{-1}) [12]. This comparison suggests that amino-functionalization alone does not determine the overall DHA adsorption capacity, which may also depend on other material properties. As result, the higher DHA adsorption observed with the anion-exchange resin may be attributed to differences in the chemical functionality and physicochemical properties of the resins, including hydrophilicity, pore structure, diffusion kinetics, and accessible surface area [41]. Furthermore, as discussed above, DHA's affinity for amino-functionalized materials may favor its retention by the anion-exchange resin. Overall, the combination of these properties may provide a more favorable environment for DHA retention, highlighting the need for further investigations involving detailed resin characterization and molecular-level analyses to elucidate the mechanisms underlying DHA adsorption by this material. Consequently, based on these results, Amberlite® IRA-410 was selected as the optimal IER for DHA separation from the liquid medium.

Following the integrated DSP scheme, Fig. 5 presents the DHA recovery process from reaction mixtures prepared using pure and crude substrates. DHA adsorption onto Amberlite® IRA-410 reached 89.4 ± 1.4% and 90.1 ± 1.3% for the pure- and crude-substrate reactions, respectively, consistent with the results obtained during the initial resin screening. The corresponding adsorption capacities were 20.4 and 14.6 mg DHA g^{-1} resin, respectively, below the maximum capacity of 30.9 ± 0.9 mg g^{-1} determined under single-compound conditions. This difference may reflect the influence of the reaction matrix and the different experimental conditions. Overall, approximately 10% of the initially present DHA remained in the liquid phase after adsorption, indicating effective DHA capture from both reaction media.

During subsequent desorption with sodium chloride, DHA recovery reached 79.1 ± 0.5% and 74.1 ± 1.9% for the pure- and crude-substrate reactions, corresponding to 161.0 ± 2.1 mg and 108.4 ± 2.6 mg of recovered DHA, respectively. Incomplete desorption indicates that a fraction of the adsorbed DHA (from crude substrates matrix) remained associated with the resin despite the addition of the eluent (33.3 ± 2.1% of DHA remained non-desorbed). This lower recovery may be related to the complexity of the reaction matrix, as co-adsorbed compounds could influence the local chemical environment or the accessibility of adsorption sites [42]. These changes may hinder ion-exchange-driven DHA desorption, resulting in lower recovery than with pure substrates

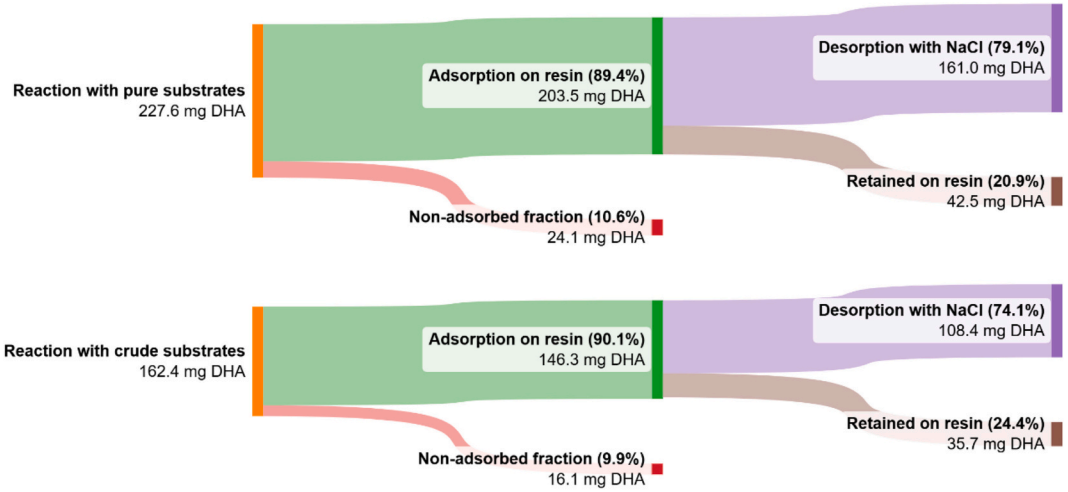


Fig. 5. Sankey diagram of the mass balance for the downstream process used for DHA recovery from a multi-enzymatic CO_2 reduction system using pure substrates (24% CO_2 gas mixture and pure glycerol) and crude substrates (crude gas mixture mimicking off gases from iron and steel industry and crude glycerol). DHA recovery was performed via adsorption onto Amberlite® IRA-410 resin, followed by desorption using sodium chloride.

matrix.

Despite these limitations, the desorption yields obtained with Amberlite® IRA-410 were higher than those previously reported by our group for DHA recovery from a bifunctional biocatalyst ($27.2 \pm 0.3\%$ and $30.1 \pm 0.3\%$ for reactions with pure and crude substrates, respectively) [12]. This difference suggests that, despite Amberlite® IRA-410's high Langmuir affinity constant, DHA desorption strongly depends on the elution mechanism. High NaCl concentrations promote salting-out, weakening electrostatic and secondary interactions and facilitating DHA release. Additionally, the absence of protein-derived amino groups that may interact with DHA likely enhances desorption in the IER system. In contrast, biocatalyst regeneration requires milder conditions to preserve enzyme activity and structural integrity, potentially resulting in lower recovery.

The recovery of DHA has been investigated using several DSP technologies, including continuous chromatography separation, solvent-based crystallization, and reverse osmosis. These approaches demonstrate the feasibility of DHA recovery through different separation mechanisms, although their performance and practical applicability depend on product purity requirements, feed composition, and

Table 2
Comparison of reported separation technologies for DHA and formate recovery in terms of separation performance, sustainability, scalability, and main limitations.

Separation technology	Separation performance	Advantages	Limitations	Sustainability and scalability	References
			DHA		
Chromatography	DHA purity of up to 97%	High DHA selectivity in multicomponent mixtures, with potential for continuous separation and high product purity	Regeneration requirements, operating costs, and performance in complex matrices need consideration	Continuous operation supports scale-up, although solvent consumption and mobile-phase requirements may increase resource use and waste generation.	[29,43]
Crystallization using ethanol or methanol	Purity of up to 99% and recovery around 80%.	High-purity DHA isolation as a solid product, potentially simplifying downstream handling and further purification	Performance depends on DHA concentration and crystallization conditions; solvent recovery may be necessary	Potential for large-scale application, although solvent recovery and associated environmental, health, and safety concerns must be considered.	[44,45]
Reverse osmosis membranes	Yields of up to 95% at DHA concentrations above $30\ g\ L^{-1}$.	DHA concentration from aqueous streams without phase change, potentially reducing reliance on thermal concentration.	Requires pressurization and relatively high DHA feed concentrations for the reported performance; selectivity in complex matrices may be limited.	Potential for scale-up, although pressurization, membrane lifetime, cleaning, and concentrating management affect resource efficiency.	[46,47]
			Formate		
Ion-exchange resins (IERs)	Formate retention capacities above $40\ mg\ g^{-1}$ resin, including in matrices containing different organic acids.	Selective capture of ionic species from dilute aqueous streams, with potential for resin reuse and integration into fermentation or enzymatic processes.	Limited adsorption capacity and competition from other anions in complex feed streams may reduce formate recovery efficiency and selectivity.	Potential for scale-up and resin reuse, although regeneration consumes chemicals and water and generates wastewater.	[38,48]
Bipolar membrane electrodialysis (BMED	Formic acid purity of up to 97.5%.	Simultaneous ion separation and acid generation, potentially reducing the need for external acidifying reagents.	Product leakage at high concentrations can reduce formic acid recovery and separation efficiency	Membrane-stack expansion supports scale-up, although electricity demand may increase the environmental footprint.	[49,50]
Crystallization and precipitation	Purity >99.7% by layer melt crystallization; however, these methods are unsuitable for streams below 10 wt%.	Recovery of formate as a solid salt, facilitating solid–liquid separation and subsequent product processing.	Low formate concentrations and high salt solubility can result in poor recovery and may require an additional concentration step	Potential for large-scale application, although reagent consumption and waste generation depend on process configuration and product titers.	[51,52]

operating conditions [29,43–47]. A comparative overview of their separation performance, sustainability, scalability, and main limitations is provided in Table 2. Compared with these approaches, our IER-based system with Amberlite® IRA-410 represents a promising alternative for DHA recovery at bench-scale, potentially enabling the selective separation from complex liquid media under mild conditions. However, its overall process performance and feasibility require further evaluation.(See Table 3.)

In this context, resin regeneration is essential for the practical implementation of ion-exchange-based separation processes, as it enables the recovery of the adsorbed compound and prepares the resin for subsequent use, as previously reported for this anionic resin [53]. As result, DHA desorption is a key step, while the IER's performance over successive adsorption–desorption cycles are crucial for assessing its long-term reusability [54]. Maintaining adsorption capacity and efficient DHA recovery would help reduce resin consumption and operating costs while improving process sustainability [28,29]. Although current batch experiments validated equilibrium selectivity, transitioning toward continuous operations, such as fixed-bed columns or simulated moving bed (SMB) chromatography, represents the logical next step for industrial scale-up. Such continuous configurations would enhance process throughput and eluent utilization, while multi-cycle testing under continuous flow remains essential to verify long-term resin stability.

3.3. Downstream processing: Formate recovery via liquid-liquid extraction (LLE) with green solvents

The separation of formic acid or formate from aqueous solutions is challenging, particularly at low concentrations and at pH values above the pKa (acid dissociation constant) of formic acid, where the dissociated form predominates [51]. To address this limitation, the extraction conditions were selected based on the acid–base equilibrium of formic acid and previously reported operating conditions [30,55]. Acidification to pH 2.0 favors the protonated form of formic acid (approximately 98.3% at pKa = 3.75), promoting its partitioning into the organic phase. The aqueous-to-organic phase ratio was selected based on literature [56], and successive extractions were performed until no further formic acid extraction was detected. Rotary evaporation temperatures and durations were selected according to solvent boiling points and previously reported conditions [22,23].

Formate isolation from the remaining medium was evaluated by liquid–liquid extraction (LLE) using two green solvents, 2-methyltetrahydrofuran (2-MTHF) and isobutyl acetate, both widely used for the separation of small organic molecules [57,58]. Fig. 6 shows the DSP flow from the DHA adsorption step onto the IER to solvent removal by rotary evaporation, allowing the recovery of purified formic acid. As observed, more than 95% of the initial formate content remained in the aqueous phase for both pure- and crude-substrate reaction matrices. This retention is consistent with the predominance of dissociated formate under the near-neutral pH conditions used during DHA adsorption, allowing subsequent recovery from the liquid phase.

Following acidification, LLE with 2-MTHF achieved extraction yields of $90.1 \pm 0.9\%$ and $84.3 \pm 0.6\%$ from the pure- and crude-substrate matrices, respectively, corresponding to 287.2 ± 2.3 and 241.3 ± 0.3 mg of extracted formic acid. In comparison, isobutyl acetate achieved lower extraction yields of $65.2 \pm 2.1\%$ and $62.7 \pm 2.2\%$, respectively. For this stage of the DSP, the complexity of the crude substrate matrix did not significantly affect formate isolation using this methodology, as comparable recovery yields were obtained with both extraction solvents to those achieved with the pure substrate matrix.

Table 3
Environmental impact assessment of downstream processing for DHA recovery from pure and crude substrate matrices using ion-exchange adsorption with Amberlite® IRA-410 resin, based on environmental quotient (EQ) analysis.

Matrix	Environmental quotient (EQ) for DHA recovery
Pure substrates	541.7 ± 4.3
Crude substrates	804.9 ± 8.9

2-MTHF, a bio-renewable solvent derived from lignocellulosic biomass [59], is widely employed in organic synthesis and extraction due to its moderate polarity [60], which allows it to interact with molecules of diverse nature. The extraction of formic acid using 2-MTHF has been previously reported, indicating that it is a promising and cost-effective solvent for extracting this compound from dilute streams and complex mixtures [30]. On the other hand, isobutyl acetate is commonly regarded as a comparatively benign or moderately green solvent [31]; however, its overall sustainability is context-dependent and generally lower than that of more recently developed bio-based solvents such as 2-MTHF. This solvent has been reported for the extraction of small organic acids, but not specifically for formic acid [61,62]. However, its lower extraction yields suggest a reduced partition coefficient for formic acid in this solvent compared to 2-MTHF (0.01 in isobutyl acetate and 0.1 in 2-MTHF) [63] or the need for larger extraction volumes. Although LLE has been widely reported for formate separation, many approaches require high temperatures and pressures or expensive and toxic solvents, often resulting in low extraction yields [64,65]. These results support the technical feasibility of LLE for formate recovery from complex enzymatic reaction mixtures under the investigated conditions.

Following the DSP, solvent removal was performed by rotary evaporation to recover formate (formic acid) from the organic extracts. For the 2-MTHF extraction, $66.4 \pm 2.4\%$ and $67.7 \pm 0.9\%$ of formate were recovered after solvent removal, corresponding to 190.8 ± 3.3 and 163.4 ± 2.3 mg of formate from the reaction matrices containing pure and crude substrates, respectively. Similarly, for isobutyl acetate extraction, formate recoveries of $64.5 \pm 1.4\%$ and $61.4 \pm 2.2\%$ were obtained, corresponding to 134.0 ± 3.4 and 110.3 ± 2.2 mg of formate, respectively. These results indicate that, although comparable formate recoveries were achieved during solvent removal by rotoevaporation, the overall performance of the formate recovery was primarily determined by the LLE stage, where solvent-dependent partitioning of formic acid governs the extraction efficiency.

Notably, formate recovery after solvent removal was substantially lower than the amount initially extracted into the organic phase, suggesting significant losses during evaporation. This may be attributed to the high-boiling azeotropic behavior reported for the 2-MTHF–formic acid system, which can hinder complete formic acid recovery [20,30]. Nevertheless, this limitation could be addressed through azeotropic distillation, as previously demonstrated [66]. In contrast, no azeotrope has been reported for the isobutyl acetate–formic acid system. However, despite its lower volatility, similar formate recovery was obtained after solvent removal, suggesting that formic acid co-evaporation under vacuum causes significant losses regardless of the solvent used. Thus, the reported recoveries reflect a laboratory-scale solvent removal step rather than an optimized process. Dedicated solvent recovery operations, such as fractional or azeotropic distillation, could improve formic acid recovery while enabling solvent recycling.

Finally, formate streams with purities of 74.9 ± 1.4 and 69.6 ± 1.9 wt% using 2-MTHF, and 63.5 ± 2.1 and 59.6 ± 0.8 wt% using isobutyl acetate, were obtained from the pure and crude substrate matrices, respectively. Lower purities with the crude matrix likely resulted from co-extraction or co-recovery of additional components, while the lower values obtained with isobutyl acetate indicate lower selectivity than 2-MTHF, consistent with previous observations. While the batch LLE and rotary evaporation steps employed here served as a proof-of-concept to demonstrate solvent selectivity and feasibility under complex matrix conditions, process intensification is required to meet industrial standards and achieve commercial-grade formic acid (85–99 wt%). Upgrading from single-stage batch extraction to continuous multi-stage counter-current extraction columns would dramatically improve the overall distribution yield while minimizing solvent-to-

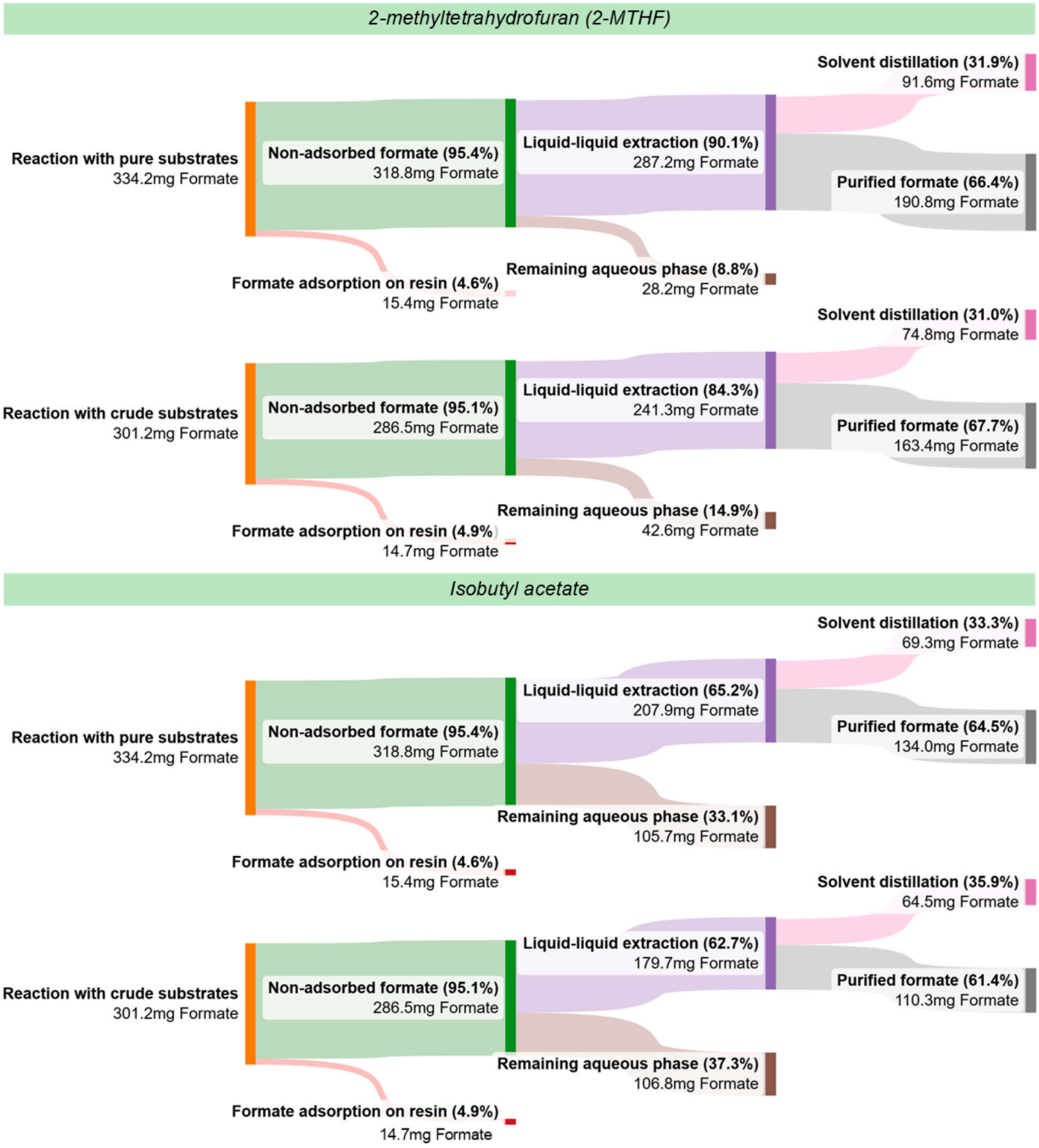


Fig. 6. Sankey diagram of the mass balance for the downstream process used for formate recovery from a multi-enzymatic CO_2 reduction system using pure substrates (24% CO_2 gas mixture and pure glycerol) and crude substrates (crude gas mixture mimicking off gases from iron and steel industry and crude glycerol). Formate isolation was performed via liquid–liquid extraction using green solvents, 2-methyltetrahydrofuran (2-MTHF) and isobutyl acetate, followed by solvent removal using a rotary evaporator and recovery of purified formate. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

feed ratios [30,55]. Subsequently, replacing batch rotary evaporation with continuous fractional distillation or azeotropic distillation specifically tailored for the 2-MTHF–water–formic acid system would reduce thermal energy demand, prevent co-evaporation losses, and enable closed-loop solvent recycling, greatly enhancing both technical and economic feasibility at larger scales.

Other DSP strategies for formate recovery include IER-based separation, bipolar membrane electrodialysis (BMED), and crystallization or precipitation, which offer potential advantages in terms of recovery efficiency and product concentration but are subject to limitations related to selectivity, energy consumption, and feed composition [38,48–51,67] (Table 2). Compared to these technologies, our results demonstrate the feasibility of formic acid recovery at the bench scale, although further optimization of the solvent-to-feed ratio, number of extraction stages, distillation conditions and product titers is needed to improve recovery and meet application-specific purity requirements, as the conditions were selected based on physicochemical principles and literature precedents rather than systematic optimization.

Although not a target product for DSP, a mass balance was also performed for glycerol carbonate (GC) (Fig. S2, SI), a valuable by-product of the multi-enzymatic system used as a green solvent, polymer synthesis intermediate, and precursor of value-added chemicals

[68]. More than 84% of the produced GC from both reaction matrices remained in the aqueous fraction after LLE with 2-MTHF and isobutyl acetate, at concentrations of 34.6 ± 1.3 and 38.7 ± 2.2 mM, respectively. The remaining losses likely reflect interactions with the DHA adsorption resin, which retained approximately 15% of the initial GC. During LLE, GC showed a higher affinity for 2-MTHF than for isobutyl acetate, consistent with its polarity. GC separation has been explored using vacuum distillation, membrane separation, acidification, and LLE, with efficiency depending on its polarity, thermal stability, and reaction medium composition [69,70]. Thus, the GC-containing aqueous stream offers an opportunity to develop an additional recovery and purification route, expanding the range of recovered products and improving the overall sustainability of the DSP system.

3.4. Environmental impact assessment of integrated DSP

DSP typically accounts for the highest consumption of resources and waste generation during product recovery, as separation and purification operations often require large amounts of water, solvents, and energy, significantly increasing the overall environmental footprint. Therefore, evaluating the environmental burden of downstream operations enables the development of more sustainable and scalable bioprocesses aligned with green chemistry principles [52]. The environmental indicators calculated in this study provide a preliminary assessment of the DSP operations under the experimental conditions investigated for both matrices (pure and crude substrates). For DHA recovery, the environmental quotient (EQ), derived from the *E*-factor, was calculated based on the mass balance of the elution step, the amount of DHA recovered, and the eluent used (sodium chloride, NaCl). For formate recovery, the global warming potential (GWP) was estimated by considering the energy requirements associated with heating and solvent distillation, as well as the treatment of the unrecovered solvent fraction, according to the assumptions defined in the calculation models [26].

Importantly, the results were normalized to the amount of product recovered to facilitate comparisons between substrate matrices and recovery conditions. However, this preliminary assessment does not constitute a comprehensive life cycle assessment (LCA), as it is limited to the processes and inputs included in the calculation models. The manufacture of solvents, resins, and other materials, equipment production, transportation, and other activities were excluded unless explicitly considered in the models. Furthermore, laboratory-scale estimates may differ at larger scales due to changes in solvent recovery, energy integration, equipment efficiency, and waste management. Thus, these indicators primarily support the comparison of DSP alternatives and the identification of opportunities for process improvement at larger scales, rather than a comprehensive assessment of the integrated process's environmental sustainability.

As shown in Table 2, DHA recovery resulted in higher EQ values for the crude substrate matrix (804.9 ± 8.9) than for the pure substrate matrix (541.7 ± 4.3). This difference is consistent with the lower product yield obtained from the crude matrix, which increases the environmental burden per unit of recovered product. However, the EQ values should be interpreted alongside the nature of the waste generated, rather than solely in terms of its total amount. Sodium chloride (NaCl), used to elute DHA from the IER, is widely used for resin regeneration because of its relatively simple production process and lower consumption of coal, natural gas, and electricity compared with other regenerating agents.

[71]. In the assessment framework applied here, NaCl is assigned a Q value of 1, indicating a low environmental hazard according to the adopted classification [72]. Nevertheless, this classification should not be interpreted as evidence of negligible environmental impact, since the effects of saline effluents depend on their concentration, volume, and receiving environment. Similarly, although the resulting waste stream is aqueous, its suitability for conventional wastewater treatment depends on its composition and salinity. Overall, the higher EQ values observed for the crude substrate matrix appear to be associated primarily with the lower product yield and the resulting process requirements, rather than necessarily indicating a higher intrinsic hazard of the waste generated.

From an environmental perspective, regenerating and reusing the IER can reduce fresh resin consumption and solid waste generation by extending the service life of a single resin batch [73], as previously discussed. However, the magnitude of these benefits depends on the regeneration chemistry, energy requirements, and efficiency of solvent and reagent use. Previous LCA studies have reported that increasing the number of resin reuse cycles can reduce overall environmental impacts, including those associated with resource consumption and waste treatment [74,75], consistent with the sustainability considerations for IER-based systems summarized in Table 2. In the proposed DSP, the use of NaCl as a regenerating agent, combined with effective resin reuse, therefore offers an opportunity to improve resource efficiency and reduce the environmental footprint of DHA recovery. The extent of these benefits should be further assessed over repeated cycles and under scaled-up operating conditions.

Regarding formate isolation, the environmental footprint was determined using the Global Warming Potential (GWP) indicator for biocatalytic reactions conducted in aqueous media with LLE using organic solvents. Fig. 7 shows a comparison of the total GWP values for formate recovery using 2-MTHF and isobutyl acetate in both matrices, along with the GWP of the upstream multi-enzymatic CCU reaction previously reported in our previous study [19].

As observed, formate extraction using 2-MTHF resulted in a lower GWP than isobutyl acetate for both substrate matrices. The total GWP values were 258.7 and 341.7 kg CO_2 eq. kg product^{-1} for the pure and crude substrate matrices, respectively, when using 2-MTHF, compared with 580.7 and 671.9 kg CO_2 eq. kg product^{-1} when using isobutyl acetate. This difference may be partly explained by the lower boiling point and enthalpy of vaporization of 2-MTHF, which may facilitate solvent recovery with lower energy requirements. In addition, the amount of unrecovered solvent requiring further treatment was approximately 2% lower than with isobutyl acetate, further contributing to the lower estimated GWP. For both solvents, the pure substrate matrix also resulted in a lower GWP than the crude matrix. This difference is consistent with the higher amount of formate recovered from the pure substrate matrix, which allows the energy and resource requirements of downstream processing to be distributed over a greater quantity of products.

To place these findings into the context of the overall process, the environmental performance of the DSP stages was compared with that of the previously assessed upstream (USP) multi-enzymatic reaction. This stage exhibited a substantially lower impact, with a GWP of 13.2 ± 0.2 kg CO_2 eq. per kg of product [19], indicating that the downstream operations account for most of the environmental burden. The relatively low GWP attributed to the USP reflects both its moderate energy requirements and the allocation of the environmental burden among the three products generated by the multi-enzymatic reaction, thereby reducing the impact assigned to each individual product. The predominance of DSP is consistent with the challenges reported for other formate and formic acid production routes, including electrochemical, thermochemical, and photocatalytic approaches [76]. However, this high environmental burden is also driven by the low formate concentration, which increases the separation energy required per unit of recovered product.

These findings are consistent with previous studies on formic acid recovery, which have identified liquid–liquid extraction (LLE) and solvent recovery as important environmental hotspots in electrochemical CO_2 reduction systems. Although these studies primarily rely on life cycle assessment (LCA) rather than reporting the carbon footprint exclusively in terms of global warming potential (GWP), they highlight the substantial energy requirements associated with separation and solvent recovery. Reported heating duties range from 4.5 to 31.5 GJ per

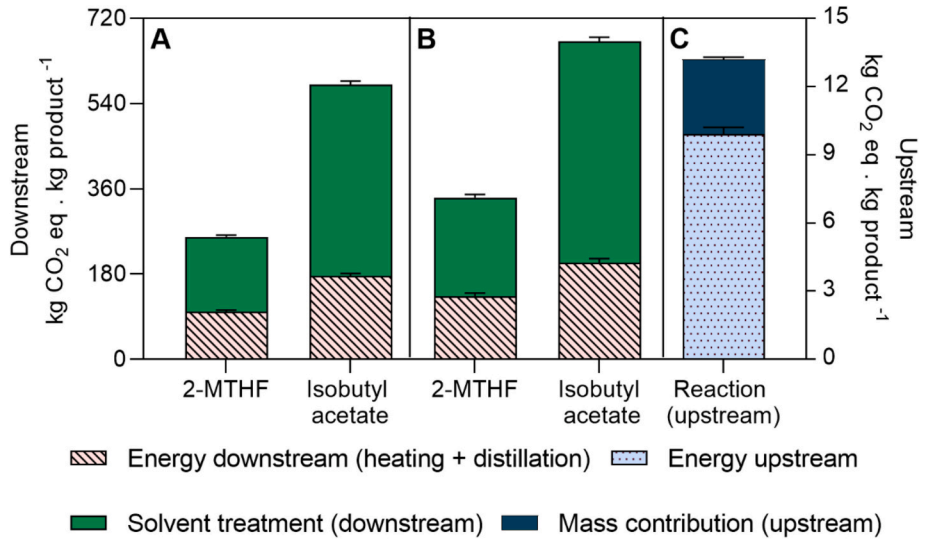


Fig. 7. Total global warming potential (GWP) assessment of the downstream process for formate recovery by liquid–liquid extraction using 2-methyltetrahydrofuran (2-MTHF) and isobutyl acetate from two different matrices with (A) pure substrates and (B) crude substrates. (C) Total GWP assessment of the upstream processing (USP) for the multi-enzymatic reduction of CO_2 to high-value-added compounds, considering the contributions of energy and mass inputs to the reaction.

ton of product, and steam demand associated with these operations can contribute up to 20–50% of the total environmental impacts [76]. As summarized in Table 2, the sustainability of separation technologies depend on their specific operational requirements, resource use, and suitability for the target feed stream.

At present, liquid–liquid extraction is considered one of the most suitable technologies for recovering formic acid, particularly in its undissociated form, from dilute aqueous streams. However, further progress is needed to develop extraction solvents with higher distribution coefficients, improved selectivity, and lower mutual solubility with water [51]. In this context, greener solvents offer a promising strategy for reducing the environmental impacts of conventional extraction processes, with reductions of up to 50% in greenhouse gas emissions and energy consumption reported for some systems [77]. The present results support this approach, as the use of 2-MTHF reduced the GWP of the downstream process by approximately 50% compared with isobutyl acetate. Further improvements could be achieved by increasing solvent recovery efficiency, reducing solvent consumption, implementing multistage extraction, improving heat integration, and adopting alternative low-energy separation technologies. In addition, increasing the formate titer in the USP could also reduce the separation burden per unit of recovered product, while reusing recovered water and distilled solvent in subsequent cycles could further improve resource efficiency, provided that the recovered streams meet the required quality specifications. Future work could also expand solvent screening beyond the two solvents investigated in this study by combining the CHEM21 solvent selection guide with COSMO-RS simulations. This integrated approach could help identify alternative solvents that balance extraction performance with improved safety and environmental profiles for the proposed system, guiding the selection of promising candidates for further evaluation [77].

Combining enzymatic conversion with industrial waste streams and green solvents establishes a viable path toward sustainable product recovery. Nevertheless, this study highlights DSP optimization, specifically solvent recovery and extraction efficiency, as the main bottleneck for reducing the carbon footprint. The elevated GWP values reflect early-stage, non-optimized bench-scale batch equipment. Transitioning to continuous multi-stage processing, heat integration, and complete solvent recovery in scaled-up systems will substantially decrease the energy duty, aligning the downstream environmental footprint with that of the upstream bioprocess.

4. Conclusions

The effective separation and purification of the two high-value industrial compounds, formate and DHA, produced by the multi-enzymatic system under study, was achieved through a DSP designed with selective, cost-effective, and sustainable techniques. First, DHA separation was carried out by ion exchange adsorption using the anionic resin Amberlite® IRA-410, which showed the highest affinity and selectivity over the remaining components of the system. DHA recovery yields were $79.1 \pm 0.5\%$ and $74.1 \pm 1.9\%$, corresponding to 161.0 ± 2.1 mg and 108.4 ± 2.6 mg of recovered DHA from matrices with pure and crude substrates, respectively. In the case of formate, LLE using green solvents proved to be both effective and selective with 2-MTHF and isobutyl acetate, showing high extraction yields of $90.1 \pm 0.9\%$ and $84.3 \pm 0.6\%$ with 2-MTHF. After distillation, 190.8 ± 3.3 and 163.4 ± 2.3 mg of formate were recovered. During this process, LLE proved to be a key step for formate recovery efficiency, with solvent performance strongly dependent on partition coefficients and formate affinity. Finally, the environmental impact assessment of both processes, based on the *E*-factor–derived EQ and GWP metrics, provides clear guidance for reducing the overall environmental footprint and enhancing process sustainability using low hazard compounds such as sodium chloride, as well as the regeneration and reuse of the IER and extraction solvent. Likewise, the use of green solvents in LLE for early-stage enzymatic systems operating with crude feedstocks offers further opportunities to improve overall environmental performance. As a result, the proposed DSP enabled the effective recovery of DHA and formate from complex crude matrices at bench scale. While these batch-scale results confirm fundamental chemical feasibility, future research on continuous operation (including fixed-bed adsorption, multi-stage counter-current LLE, and continuous azeotropic distillation) will provide the necessary engineering roadmap to achieve full industrial scale-up and economic viability.

Declaration of competing interest

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

Acknowledgments

S. R. R. acknowledges the Generalitat of Catalunya (AGAUR) for his pre-doctoral scholarship (Joan Oro – 2022FI_B 00955). All the authors acknowledge Generalitat de Catalunya, and the 2021 SGR 00143 and project MEPLAB-CO_2 (TED2021-129732 A-I00) funded by MCIN/AEI/10.13039/501100011033 and by the European Union "NextGenerationEU"/PRTR.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.seppur.2026.140749.

Data availability

Research Link Provided

Replication data for "Integrated Downstream Processing for the Selective Recovery of Formate and DHA from a Multi-Enzymatic CO_2 Utilization System" (Original data) (CORA)

References

[1] S.P. Schwaminger, I. Zimmermann, S. Berensmeier, Current research approaches in downstream processing of pharmaceutically relevant proteins, Curr. Opin. Biotechnol. 77 (2022) 102768, https://doi.org/10.1016/j.copbio.2022.102768.
[2] U.A. Salas-Villalobos, R.V. Gómez-Acata, J. Castillo-Reyna, O. Aguilar, *In situ* product recovery as a strategy for bioprocess integration and depletion of inhibitory products, J. Chem. Technol. Biotechnol. 96 (10) (2021) 2735–2743, https://doi.org/10.1002/jctb.6797.
[3] K.V.K. Boodhoo, M.C. Flickinger, J.M. Woodley, E.A.C. Emanuelsson, Bioprocess intensification: a route to efficient and sustainable biocatalytic transformations for the future, Chem. Eng. Process. Process Intensif. 172 (2022) 108793, https://doi.org/10.1016/j.cep.2022.108793.
[4] L.-E. Meyer, M. Hobisch, S. Kara, Process intensification in continuous flow biocatalysis by up and downstream processing strategies, Curr. Opin. Biotechnol. 78 (2022) 102835, https://doi.org/10.1016/j.copbio.2022.102835.
[5] R. Siedentop, C. Claaßen, D. Rother, S. Lütz, K. Rosenthal, Getting the most out of enzyme cascades: strategies to optimize in vitro multi-enzymatic reactions, Catalysts 11 (10) (2021) 1183, https://doi.org/10.3390/catal11101183.
[6] N.P. Mendis, J. Wang, R. Lakerveld, Simultaneous solvent selection and process Design for Continuous Reaction–Extraction–Crystallization Systems, Ind. Eng. Chem. Res. 61 (31) (2022) 11504–11517, https://doi.org/10.1021/acs.iecr.1c05012.
[7] R.A. Sheldon, J.M. Woodley, Role of biocatalysis in sustainable chemistry, Chem. Rev. 118 (2) (2018) 801–838, https://doi.org/10.1021/acs.chemrev.7b00203.
[8] H. Naims, Economics of carbon dioxide capture and utilization—a supply and demand perspective, Environ. Sci. Pollut. Res. 23 (22) (2016) 22226–22241, https://doi.org/10.1007/s11356-016-6810-2.
[9] K.M. Abed, et al., Superiority of liquid membrane-based purification techniques in biodiesel downstream processing, Renew. Sustain. Energy Rev. 207 (2025) 114911, https://doi.org/10.1016/j.rser.2024.114911.
[10] A.I. Benítez-Mateos, F. Paradisi, Perspectives on flow biocatalysis: the engine propelling enzymatic reactions, J. Flow Chem. 14 (1) (2024) 211–218, https://doi.org/10.1007/s41981-023-00283-z.
[11] B.O. Yusuf, et al., Carbon dioxide (CO_2) capture and utilization technologies: new developments toward net-zero emissions and climate-change mitigation, Carbon Capture Sci. Technol. 18 (2026) 100589, https://doi.org/10.1016/j.ccst.2026.100589.
[12] S.R. Rodriguez, O. Romero, M. Guillén, Enzyme-powered CO_2 utilization: a bifunctional immobilized biocatalyst for intensified CCU of industrial feedstocks to high-value chemicals, ACS Sustain. Chem. Eng. 14 (1) (2026) 86–98, https://doi.org/10.1021/acssuschemeng.5c07343.
[13] A. Carceller, M. Guillén, G. Álvaro, Lactic acid from CO_2: a carbon capture and utilization strategy based on a biocatalytic approach, Environ. Sci. Technol. 57 (51) (2023) 21727–21735, https://doi.org/10.1021/acs.est.3c05455.
[14] M. Tomatis, H. Kumar Jeswani, A. Azapagic, Environmental impacts of valorisation of crude glycerol from biodiesel production – a life cycle perspective, Waste Manag. 179 (2024) 55–65, https://doi.org/10.1016/j.wasman.2024.03.005.
[15] V. Tzitzili, N. Misailidis, V. Parisis, D. Petrides, M.C. Georgiadis, Synthesis, design and techno-economic evaluation of a formic acid production plant from carbon dioxide, Processes 13 (11) (2025) 3626, https://doi.org/10.3390/pr13113626.
[16] L.S. Oh, et al., Selective production of market-valued dihydroxyacetone on two alloy phase surfaces by electrocatalytic oxidation of biomass-derived glycerol, Appl. Catal. Environ. 378 (2025) 125591, https://doi.org/10.1016/j.apcatb.2025.125591.
[17] B. Thijs, J. Rongé, J.A. Martens, Matching emerging formic acid synthesis processes with application requirements, Green Chem. 24 (6) (2022) 2287–2295, https://doi.org/10.1039/D1GC04791D.
[18] L. Bricotte, K. Chougrani, V. Alard, V. Ladmiral, S. Caillol, Dihydroxyacetone: a user guide for a challenging bio-based synthon, Molecules 28 (6) (2023) 2724, https://doi.org/10.3390/molecules28062724.
[19] S.R. Rodriguez, M. Guillén, O. Romero, Enhancing multi-enzymatic CO_2 conversion via reactor engineering: effects of mass transfer on sustainable and green metrics, Green Chem. 21 (28) (2026) 8766–8780, https://doi.org/10.1039/D6GC00387G.
[20] S.R. Rodriguez, G. Álvaro, M. Guillén, O. Romero, Multienzymatic platform for coupling a CCU strategy to waste valorization: CO_2 from the Iron and steel industry and crude glycerol from biodiesel production, ACS Sustain. Chem. Eng. 13 (4) (2025) 1440–1449, https://doi.org/10.1021/acssuschemeng.4c04908.
[21] S.R. Rodriguez, A.D.O. Maciel, O. Romero, M. Guillén, I. Antonopoulou, Carbonic anhydrase boosts multi-step CO_2 conversion: A synergistic approach of CO-immobilized enzymes for CO_2 valorization, Chem. Eng. J. (2026) 177653, https://doi.org/10.1016/j.cej.2026.177653.
[22] A.V. Polkovnichenko, T.V. Chelyuskina, Distillation separation of the isobutyl acetate–acetic acid–isoamyl acetate industrial mixture, Theor. Found. Chem. Eng. 57 (4) (2023) 503–514, https://doi.org/10.1134/S0040579523040413.
[23] A.R. Alcantara, P.D. de Maria, Recent advances on the use of 2-methyltetrahydrofuran (2-MeTHF) in biotransformations, Curr. Green Chem. 5 (2) (2018) 86–103, https://doi.org/10.2174/2213346105666180727100924.
[24] R.A. Sheldon, Metrics of green chemistry and sustainability: past, present, and future, ACS Sustain. Chem. Eng. 6 (1) (2018) 32–48, https://doi.org/10.1021/acssuschemeng.7b03505.
[25] R.A. Sheldon, The E factor at 30: a passion for pollution prevention, Green Chem. 25 (5) (2023) 1704–1728, https://doi.org/10.1039/D2GC04747K.
[26] P. Domínguez de María, General equations to estimate the CO_2 production of (bio) catalytic reactions in early development stages, RSC Sustain. 2 (12) (2024) 3817–3825, https://doi.org/10.1039/D4SU00535J.
[27] P. Domínguez de María, (re)visiting the sustainability thresholds: are product titers of 1 g L^{-1} enough for (bio)chemical processes? ChemSusChem 18 (22) (2025) https://doi.org/10.1002/cssc.202501831.
[28] P.M. Walgode, R.P.V. Faria, A.E. Rodrigues, Pseudo-ternary chromatographic separation of dihydroxyacetone from the glycerol oxidation byproducts on a simulated moving bed cascade, Chem. Eng. J. 476 (2023) 146447, https://doi.org/10.1016/j.cej.2023.146447.
[29] P.M. Walgode, L.C.D. Coelho, R.P.V. Faria, A.E. Rodrigues, Dihydroxyacetone production: from glycerol catalytic oxidation with commercial catalysts to chromatographic separation, Ind. Eng. Chem. Res. 60 (29) (2021) 10551–10565, https://doi.org/10.1021/acs.iecr.1c00275.
[30] A.T. Laitinen, et al., Liquid-liquid extraction of formic acid with 2-Methyltetrahydrofuran: experiments, process modeling, and economics, Ind. Eng. Chem. Res. 60 (15) (2021) 5588–5599, https://doi.org/10.1021/acs.iecr.1c00159.
[31] M. Behroozi, M. Vahedpour, M. Shardi Manaheji, Separation of formic acid from aqueous solutions by liquid extraction technique at different temperatures, Phys. Chem. Res. 7 (1) (2019) 201–215, https://doi.org/10.22036/pcr.2019.154646.1557.
[32] M. Chan, H. Doan, T. Dang-Vu, An investigation of lanthanum recovery from an aqueous solution by adsorption (ion exchange), Inorganics (Basel). 12 (9) (2024) 255, https://doi.org/10.3390/inorganics12090255.
[33] J. Tong, et al., Efficient capture of ReO_4^-/TcO_4^- on anion exchange resin from wastewater, Sep. Purif. Technol. 352 (2025) 128168, https://doi.org/10.1016/j.seppur.2024.128168.
[34] H.-Y. Cai, W.-L. Wang, W.-Y. Ouyang, Q.-Y. Wu, L. Zhang, H.-Y. Hu, Adsorption of neutral and negatively charged low-molecular-weight carbonyls in reverse osmosis permeates by ion-exchange resins, Water Cycle 3 (2022) 1–7, https://doi.org/10.1016/j.watcyc.2021.12.001.
[35] S. Rahmani, M. Mohseni, The role of hydrophobic properties in ion exchange removal of organic compounds from water, Can. J. Chem. Eng. 95 (8) (2017) 1449–1455, https://doi.org/10.1002/cjce.22823.
[36] W. Zhu, Methods for industrial scale preparation and purification of nicotinamide coenzymes and their precursors, in: Biology of Nicotinamide Coenzymes, Singapore: Springer Nature, Singapore, 2025, pp. 735–755, https://doi.org/10.1007/978-981-97-9877-3_45.
[37] K. Chruszcz-Lipska, E. Szostak, A study of the structure of an anion exchange resin with a quaternary ammonium functional group by using infrared spectroscopy and DFT calculations, Materials 17 (24) (2024) 6132, https://doi.org/10.3390/ma17246132.
[38] M.A. Hoque, K. Abad, P. Kharel, A. Omosebi, J. Thompson, Downstream separation of formic acid with anion-exchange resin from Electrocatalytic carbon dioxide (CO_2) conversion: adsorption, kinetics, and equilibrium modeling, Ind. Eng. Chem. Res. 63 (6) (2024) 2779–2790, https://doi.org/10.1021/acs.iecr.3c02946.
[39] J. Yan, D. Lin, S. Yao, Q. Zhang, Exploring the effects of resin particle sizes on enhancing antibody binding capacity of a hybrid biomimetic ligand, J. Chromatogr. A 1722 (2024) 464891, https://doi.org/10.1016/j.chroma.2024.464891.
[40] Y. Sun, F.A.M. Al-Zahrani, L. Lin, Colour formation of dihydroxyacetone with cysteine and its derivatives via Maillard reaction, Dyes Pigments 208 (2022), https://doi.org/10.1016/j.dyepig.2022.110854.
[41] P. Finkbeiner, et al., Interactions between organic model compounds and ion exchange resins, Environ. Sci. Technol. 53 (16) (2019) 9734–9743, https://doi.org/10.1021/acs.est.9b02139.

[42] T. Kinoshita, H.-J. Fan, T. Kawakita, E. Furuya, Optimization of a modification technique for reducing irreversible adsorption within synthetic resins, Adsorpt. Sci. Technol. 34 (6) (2016) 345–354, https://doi.org/10.1177/0263617416658889.
[43] Y. Peng, S. Wang, L. Lan, W. Chen, B. Fang, Resin adsorption application for product separation and catalyst recycling in coupled enzymatic catalysis to produce 1,3-propanediol and dihydroxyacetone for repeated batch, Eng. Life Sci. 13 (5) (2013) 479–486, https://doi.org/10.1002/elsc.201300012.
[44] J.F. Martínez-Gallegos, A. Burgos-Cara, F. Caparrós-Salvador, G. Luzón-González, M. Fernández-Serrano, Dihydroxyacetone crystallization: process, environmental, health and safety criteria application for solvent selection, Chem. Eng. Sci. 134 (2015) 36–43, https://doi.org/10.1016/j.ces.2015.04.047.
[45] Z. Zheng, M. Luo, J. Yu, J. Wang, J. Ji, Novel process for 1,3-dihydroxyacetone production from glycerol. 1. Technological feasibility study and process design, Ind. Eng. Chem. Res. 51 (9) (2012) 3715–3721, https://doi.org/10.1021/ie201710h.
[46] S. Liebminger, R. Hofbauer, M. Siebenhofer, G.S. Nyanhongo, G.M. Guebitz, Microbial conversion of crude glycerol to dihydroxyacetone, Waste Biomass Valoriz. 5 (5) (2014) 781–787, https://doi.org/10.1007/s12649-013-9288-x.
[47] T. Zweckmair, M. Imendörffer, S. Novalin, Separation of small organic compounds derived from aldol-condensation by membrane processes, Sep. Sci. Technol. 46 (14) (2011) 2191–2198, https://doi.org/10.1080/01496395.2011.595758.
[48] M.-L. Daumer, S. Picard, P. Saint-Cast, P. Dabert, Technical and economical assessment of formic acid to recycle phosphorus from pig slurry by a combined acidification–precipitation process, J. Hazard. Mater. 180 (1–3) (2010) 361–365, https://doi.org/10.1016/j.jhazmat.2010.04.039.
[49] Z. Wang, et al., Separation and conversion of CO_2 reduction products into high-concentration formic acid using bipolar membrane electrodialysis, J. Membr. Sci. 708 (2024) 123016, https://doi.org/10.1016/j.memsci.2024.123016.
[50] H.-G. Nam, G.-W. Lim, S. Mun, Separation of acetic acid, formic acid, succinic acid, and lactic acid using adsorbent resin, J. Chem. Eng. Data 57 (8) (2012) 2102–2108, https://doi.org/10.1021/je201065u.
[51] L.M.J. Sprakel, B. Schuur, Solvent developments for liquid-liquid extraction of carboxylic acids in perspective, Sep. Purif. Technol. 211 (2019) 935–957, https://doi.org/10.1016/j.seppur.2018.10.023.
[52] G.A. Maul, Y. Kim, A. Amini, Q. Zhang, T.H. Boyer, Efficiency and life cycle environmental impacts of ion-exchange regeneration using sodium, potassium, chloride, and bicarbonate salts, Chem. Eng. J. 254 (2014) 198–209, https://doi.org/10.1016/j.cej.2014.05.086.
[53] A.V. Baskar, et al., Recovery, regeneration and sustainable management of spent adsorbents from wastewater treatment streams: a review, Sci. Total Environ. 822 (2022) 153555, https://doi.org/10.1016/j.scitotenv.2022.153555.
[54] M. Ramdin, et al., High-pressure electrochemical reduction of CO_2 to formic acid/Formate: effect of pH on the downstream separation process and economics, Ind. Eng. Chem. Res. 58 (51) (2019) 22718–22740, https://doi.org/10.1021/acs.iecr.9b03970.
[55] H. Veith, M. Voges, C. Held, J. Albert, Measuring and predicting the extraction behavior of biogenic formic acid in biphasic aqueous/organic reaction mixtures, ACS Omega 2 (12) (2017) 8982–8989, https://doi.org/10.1021/acsomega.7b01588.
[56] F.P. Byrne, et al., Tools and techniques for solvent selection: green solvent selection guides, Sustain. Chem. Process. 4 (1) (2016) 7, https://doi.org/10.1186/s40508-016-0051-z.
[57] A.F. Martínez, C.A. Sánchez, A. Orjuela, G. Rodríguez, Isobutyl acetate by reactive distillation. Non-reactive phase equilibrium and topological analysis, Fluid Phase Equilib. 516 (2020) 112612, https://doi.org/10.1016/j.fluid.2020.112612.
[58] P. Janicka, et al., Trends in the new generation of green solvents in extraction processes, Curr. Opin. Green Sustain. Chem. 37 (2022) 100670, https://doi.org/10.1016/j.cogsc.2022.100670.
[59] F. Brondani Teixeira Ribas, H. Gasparetto, A. Luiza Barrachini Nunes, N. Paula Gonçalves Salau, Unleashing the power of hydrous 2-methyl tetrahydrofuran for enhanced oil extraction from rice bran and soybean flakes, J. Mol. Liq. 399 (2024), https://doi.org/10.1016/j.molliq.2024.124490.
[60] D. Ho, et al., Green solvents for organic thin-film transistor processing, J. Mater. Chem. C Mater. 8 (17) (2020) 5786–5794, https://doi.org/10.1039/D0TC00512F.
[61] L.R. Roque, G.P. Morgado, V.M. Nascimento, J.L. Ienczak, S.C. Rabelo, Liquid-liquid extraction: a promising alternative for inhibitors removing of pentoses fermentation, Fuel 242 (2019) 775–787, https://doi.org/10.1016/j.fuel.2018.12.130.
[62] F. Mohammaei, E. Mohammadinasab, Coefficient partition prediction of saturated monocarboxylic acids using the molecular descriptors, J. Chil. Chem. Soc. 63 (3) (2018) 4068–4071, https://doi.org/10.4067/s0717-97072018000304068.
[63] A. Smirnov, A. Golikova, A. Toikka, A. Samarov, M. Toikka, Study of the liquid–liquid equilibrium and extraction properties of deep eutectic solvents based on choline chloride for the separation of the ethanol–ethyl formate system for potential use in biofuel production, Ind. Eng. Chem. Res. 62 (1) (2023) 586–597, https://doi.org/10.1021/acs.iecr.2c02911.
[64] T. Roncal, L. Lorenzo, S. Prieto-Fernández, J.R. Ochoa-Gómez, Purification and concentration of formic acid from formic acid/gluconic acid mixtures by two successive steps of nanofiltration and reactive liquid-liquid extraction, Sep. Purif. Technol. 286 (2022) 120492, https://doi.org/10.1016/j.seppur.2022.120492.
[65] B. Mahida, M. Medjahdi, H. Benyounes, I. Khelifa, L. Baidar, Azeotropic formic acid-water separation by sulfolane extractive distillation feasibility study, *Indian* Chem. Eng. (2025) 1–12, https://doi.org/10.1080/00194506.2025.2455984.
[66] Y. Luo, Y. Liu, J. Shen, B. Van der Bruggen, Application of bipolar membrane electrodialysis in environmental protection and resource recovery: a review, Membranes (Basel) 12 (9) (2022) 829, https://doi.org/10.3390/membranes12090829.
[67] S. Lukato, G.N. Kasozi, B. Naziriwo, E. Tebandeke, Glycerol carbonylation with CO_2 to form glycerol carbonate: a review of recent developments and challenges, Curr. Res. Green Sustain. Chem. 4 (2021), https://doi.org/10.1016/j.crgsc.2021.100199.
[68] H. Tang, X.Y. Ng, C. Luo, H.C. Ong, H.V. Lee, Advancing biorefineries: catalytic frontiers in the synthesis and application of glycerol carbonate, Green Chem. 28 (14) (2026) 6006–6027, https://doi.org/10.1039/D6GC00180G.
[69] E. Hernández, A. Belinchón, P. Navarro, J. Palomar, Mild one-pot production of glycerol carbonate from CO_2 with separation from ionic liquid catalyst, Sep. Purif. Technol. 356 (2025) 129970, https://doi.org/10.1016/j.seppur.2024.129970.
[70] S. Wang, S. Jia, Z. Gao, J. Wang, J. Gong, Highly efficient crystallization for sustainable azeotrope separation of formic acid-water, Sep. Purif. Technol. 342 (2024) 126821, https://doi.org/10.1016/j.seppur.2024.126821.
[71] K. Van Aken, L. Strekowski, L. Patiny, EcoScale, a semi-quantitative tool to select an organic preparation based on economical and ecological parameters, Beilstein J. Org. Chem. 2 (2006), https://doi.org/10.1186/1860-5397-2-3.
[72] A.D. La Rosa, D.R. Banatao, S.J. Pastine, A. Latteri, G. Cicala, Recycling treatment of carbon fibre/epoxy composites: materials recovery and characterization and environmental impacts through life cycle assessment, Compos. B. Eng. 104 (2016) 17–25, https://doi.org/10.1016/j.compositesb.2016.08.015.
[73] T.H. Boyer, A. Ellis, Y. Fang, C.E. Schaefer, C.P. Higgins, T.J. Strathmann, Life cycle environmental impacts of regeneration options for anion exchange resin remediation of PFAS impacted water, Water Res. 207 (2021) 117798, https://doi.org/10.1016/j.watres.2021.117798.
[74] X. Foster, W.A. Tarpeh, H. Dong, C. Vaneeckhaute, Enhancing the regeneration efficiency of a hybrid anion exchange resin for removal of phosphorus from wastewater with a lower environmental impact, J. Water Process Eng. 69 (2025) 106851, https://doi.org/10.1016/j.jwpe.2024.106851.
[75] L. Nickel, et al., Ex ante life cycle assessment of industrial-scale electrochemical reduction of CO_2 to formic acid, ACS Sustain. Chem. Eng. 14 (10) (2026) 4762–4775, https://doi.org/10.1021/acssuschemeng.5c07420.
[76] I. Thiele, S.L. Riedel, How does downstream processing influence the sustainability and techno-economics of polyhydroxyalkanoates production? J. Clean. Prod. 521 (2025) 146257 https://doi.org/10.1016/j.jclepro.2025.146257.
[77] D. Soukup-Carne, et al., Extraction of 5-Hydroxymethylfurfural and furfural in aqueous biphasic systems: a COSMO-RS guided approach to greener solvent selection, ACS Sustain. Chem. Eng. 12 (9) (2024) 3766–3779, https://doi.org/10.1021/acssuschemeng.3c07894.