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Recovery of Volatile Fatty Acids from Fermentation Broth Using Ethyl Acetate as Solvent

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Volatile fatty acids (VFAs), short-chain fatty acids (C2–C6), are recognized as platform chemicals with diverse industrial applications. VFAs can be produced through the acidogenic phase of biowaste fermentation, which yields several key VFAs including butyric, propionic, lactic, and acetic acids. However, downstream processing remains a major challenge in VFA production due to the complex composition of fermentation broths (especially in the case of mixed culture fermentation), which complicates separation and increases costs. Among the various recovery methods, liquid-liquid extraction is a promising technique due to its high yield and selective separation of VFAs. Ethyl acetate has been identified as a potential solvent for this extraction process, owing to its low cost and favorable partitioning coefficient for VFAs. This work evaluates the efficiency of ethyl acetate for the liquid-liquid extraction of VFAs from fermentation broth of mixed culture and validates the results using the UNIFAC model for liquid activity in Aspen Plus ® software. Experimental conditions were optimized by varying the pH (2.5–4.5) and stirring time (10–60 minutes). Results indicate that the highest separation efficiency for VFAs was achieved at a pH of 2.5 and a stirring time of at least 30 minutes, with no significant improvement observed beyond 40 minutes. Separation efficiencies of over 90% were obtained for butyric acid, while propionic acid exhibited efficiencies above 70%. Therefore, ethyl acetate may be a potential solvent for VFAs recovery, and the UNIFAC model described with great accuracy the liquid-liquid equilibria of this mixture.

* 1. Introduction

The increasing necessity to lessen global reliance on non-renewable resources, along with the creation of chemical products that do not utilize oil as a primary material, represents one of the most significant challenges facing society today. This issue is garnering growing interest from industries and researchers worldwide. As a result, sustainable alternatives are being investigated, with biomass fermentation emerging as a promising source for a variety of valuable products that would typically be derived from oil (López-Garzón & Straathof, 2014). VFAs are short-chain fatty acids produced as intermediates during anaerobic fermentation, encompassing a range of acids such as acetic, butyric, propionic, lactic, and citric acids. While large-scale production of certain carboxylic acids via fermentation is already established, the industrial methods for synthesizing butyric and propionic acids are still being refined and continue to be a key point of research in the literature (Sun et al., 2024; López-Garzón & Straathof, 2014).

With a global market valued at USD 376.5 million in 2023, butyric acid has a wide array of applications across various industries. In the chemical sector, it is used as a precursor for producing thermoplastic cellulose acetate butyrate (CAB) and butanol. In the food industry, it enhances flavors and serves as an additive in animal feed, alongside propionic acid. Additionally, in the pharmaceutical industry, butyric acid is recognized for its therapeutic properties. Given its diverse applications, the butyric acid market is expected to grow at an annual rate of 9.7% up to 2032. Similarly, the propionic acid market, valued at USD 1.1 billion in 2023, is projected to reach USD 1.42 billion by 2024. These growth trends highlight the importance of these acids as key building blocks for a future with reduced reliance on oil (Technavio, 2024; Market Research Future, 2024; Gerunova et al., 2024; Mazur-Kuśnirek et al., 2024; Jiang et al., 2018).

Despite the increasing demand and ongoing research, the large-scale bioproduction of butyric acid and propionic acid from renewable sources remains economically uncompetitive with traditional, non-renewable methods. To address this challenge, several key issues must be tackled, including the low yield of butyric acid and propionic acid production, the instability of the acid’s composition, and the complex, costly downstream processes required for extraction, purification, and final polishing (Lou et al., 2018; Jiang et al., 2018). Notably, downstream processes can account for up to 50% of the total production cost, driving the investigation of various methods in the literature for concentrating, separating, and purifying VFAs (Chen et al., 2022). Among the various downstream processing technologies for VFA production, liquid-liquid extraction emerges as a promising technique. This method is based on the chemical equilibrium established between the organic acids in liquid phase and the organic solvent used as the extractant. The equilibrium is driven by the higher solubility of VFAs in the organic solvent compared to water, resulting in the transfer of the acids into the extract phase (Chen et al., 2022; Atasoy et al., 2018). One of the solvents proposed in the literature for liquid-liquid extraction of VFAs is ethyl acetate, due to its low cost and favorable partition coefficient for volatile fatty acids. Ethyl acetate extracts the acids from the aqueous phase by forming an organic phase, which can then undergo distillation to separate the target acids (Alao et al. 2023).

One effective approach to enhancing downstream efficiency is the simulation and optimization of processes using computational tools, which enable accurate modeling of process behavior in varied conditions. Software such as Aspen Plus® provides a comprehensive set of models that can be employed to test, optimize, and monitor the performance of an entire industrial plant with key advantages, including the ability to model both individual equipment and entire processes. It provides accurate descriptions of process behavior, supported by its robust and extensive database. The software excels in performing techno-economic analyses and comparing different process scenarios, making it a valuable tool for optimization (Leal Silva and Maciel Filho, 2024). Furthermore, it incorporates a comprehensive database to calculate phase equilibrium, which enhances the accuracy and reliability of its simulations (Malik et al., 2023; Corredor et al., 2019; Chaves et al., 2016). A process model can be designed and conditions for extraction can be optimized, thus reducing efforts in process design.

Nevertheless, conditions of the process demand experimental validation before the model can be extended to optimized conditions. Therefore, the aim of this work was to evaluate ethyl acetate as a solvent for liquid-liquid extraction of VFAs from fermentation broth and compare the experimental performance of the process with the performance modeled in Aspen Plus. Different conditions of pH and stirring time were tested in extraction trials, and these results were compared to the results obtained via process simulation. Based on these results, the process can be expanded to determine conditions that optimize the recovery of different VFAs.

* 1. Materials and Methods
     1. Materials

N-butyric acid and propionic acid were provided by Êxodo Científica (Brazil), sulfuric acid was purchased from Dinâmica (Brazil), ethyl acetate was obtained from Sigma-Aldrich (USA), calcium oxide was supplied by Anidrol (Brazil), and ultra-pure water was sourced from Master System MS20000 ALL (GEHAKA, Brazil). pH measurements were performed using a mPA2010 pH meter (MS Tecnopon® Instrumentação, Brazil). The trials were carried out on an MA 505/5FEQ reactor system (MARCONI, Brazil). The concentration of the samples was determined via HPLC using an Agilent 1260 Infinity II system (Agilent Technologies, USA).

* + 1. Methods

2.2.1 Preparation of Butyric Acid and Propionic Acid (VFAs) solution

The synthetic VFA solution with 33 g/L of butyric acid and 13 g/L of propionic acid were prepared with Mili-Q water and the pH was adjusted for 2.5, 3.5 and 4.5 using calcium oxide and sulfuric acid and left at room temperature. This concentration of VFAs was chosen to mimic the concentration of VFAs produced with a mixed culture based on residues from the production of organic sugar.

2.2.2 VFAs extraction

Experimental extraction trials from synthetic fermentation broth were conducted based on the methodology proposed by Alao et al. (2023) and adapted for this work to use ethyl acetate as solvent, with a sample volume of 25 mL and a solvent-to-feed ratio of 1:1. The pH was adjusted to 2.5, 3.5, and 4.5 before the extraction. Extraction was carried out at room temperature in a beaker, stirring at 150 RPM for 10 to 60 minutes (in steps of 10 minutes). Trials were carried out for each pH value and stirring time. Subsequently, the solutions were allowed to settle in different separatory funnels for 30 minutes. Samples of 2 mL were collected from both the aqueous and organic phases in vials and stored in a freezer for analysis.

2.2.3 Quantification

Samples from both the aqueous and organic phases were analyzed via HPLC using an Agilent 1260 Infinity II HPLC system (Agilent Technologies, USA) with an HPX-87H column (1.3 × 7.8 mm) at 15 ºC. The injection volume was 20 µL and the flow rate was 0.5 mL/min. An aqueous solution of 3 mmol/L of sulfuric acid was used as the mobile phase. For the calibration curves, solutions with concentrations of 8.0, 4.8, 3.2, 1.6, and 0.8 g/L of butyric acid and propionic acid were prepared and analyzed by HPLC. Equations 1 and 2 were obtained via data regression of the calibration curves and used to determine the concentrations of butyric acid and propionic acid, respectively. In these equations, is the area of the peak of butyric acid, is the area of the peak of propionic acid, is the concentration of butyric acid, and is the concentration of propionic acid.

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| --- | --- |
|  | (1) |
|  | (2) |

To determine the percentage of extraction for each organic acid, the concentration of acid in each sample ( from the organic phase was divided by the concentration of the initial synthetic acid solution as described by Equation 3. This calculation assumes that the volume of extract and feed are the same, which is adequate because of the proportion between solvent and feed that was chosen.

|  |  |
| --- | --- |
|  | (3) |

2.2.4 Simulation of extraction process

Mass balances were obtained using the Aspen Plus ® v14.0 (Aspen Technology INC, Bedford, MA, USA). The process was modeled using the UNIFAC-LL (UNIQUAC Functional-group Activity Coefficient) method to estimate liquid activity coefficients. This variation of UNIFAC has a dataset focused on liquid-liquid equilibria. Vapor phase calculation considered the Hayden-O’Connell equation of state (Leal Silva et al., 2018). The VFA extraction process was simulated based on the experimental parameters shown in Table 1, and the process flowsheet is presented in Figure 1. Both inlet streams were assumed to be 100 L/h under standard conditions. This model is used to calculate equilibrium conditions, and it does not consider the electrolyte behavior of organic acids in aqueous phase.

*Table 1 - Input parameters of the streams*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Temperature  (ºC) | Pressure  (bar) | Total flow  Rate (kg/h) | Water  (kg/h) | Butyric acid  (kg/h) | Propionic acid  (kg/h) | Ethyl acetate  (kg/h) |
| Feed | 25 | 1 | 98.83 | 94.24 | 3.3 | 1.3 | 0 |
| Solvent | 25 | 1 | 89.69 | 0 | 0 | 0 | 89.69 |



*Figure 1 - Process simulation flowsheet in Aspen Plus ®*

* 1. Results and discussion
     1. Experiments results

The solutions prepared with butyric acid and propionic acid at pH 2.5 were transparent. However, as the pH increased with the addition of CaO, an increase in turbidity was observed, caused by the low solubility of calcium salts. This suggests that, as the pH approaches the pKa of the acid, the amount of organic acid in its molecular form decreases, leading to a greater proportion of the organic acid in its ionized form. This is known to affect the recovery of these acids by the organic solvent. According to Xu & Jiang (2011), the pH of the fermentation broth needs to be reduced to at least pH 4.8, which is around the pKa of the acids, to ensure sufficient concentrations of free unionized acids. The percentage recovery of butyric acid (Figure 2) and propionic acid (Figure 3) using ethyl acetate as solvent in pH 2.5, 3.5 and 4.5 are shown below.

Figure 2 - Butyric acid recovery (%) in different pHs and stirring time; initial butyric acid concentration: 33 g/L; solvent to feed proportion 1:1

Figure **3** - Propionic acid recovery (%) in different pH and stirring time. Initial propionic acid concentration: 13 g/L; solvent to feed proportion 1:1 pKa: 4.89

Figures 2 and 3 demonstrate that, as discussed above, higher pH results in lower recovery for both acids, corroborating Xu & Jiang (2011), who proposed that the distribution coefficient depends on the pH; that is, the higher the pH, the lower the distribution coefficient. This happens because at higher pH the organic acid is available on its ionized form, which has greater affinity for the polar solvent (water) than the molecular form, whose concentration is greater at low pH. Additionally, a greater number of carbons in the molecule increases the affinity of the organic acids for the organic solvent and decreases the affinity for the aqueous phase (Chen et al. 2022).

The graphs also show that after 30 minutes, there is no significant increase in recovery for either acid, likely because the extraction reached equilibrium, and the solvent attained its maximum distribution coefficient. Similar results were found by Bekatarou et al. (2016), who studied different solvents with various functional groups, such as ethyl acetate and 1-butanol, for VFA recovery from synthetic solutions. The deviation from the observed trend for pH 2.5 sample at 30 minutes for both butyric acid and propionic acid may be due to slight broadening of the peak observed in the chromatogram. Keshav et al. (2008) used ethyl acetate as a solvent to recover propionic acid from a synthetic medium with the pH adjusted to 2.2 and found a maximum recovery of 74 % after 2 hours of mixing at 30 ºC, which corroborates the results of this study, despite the longer stirring time. The trend in results show that, for the sake of equipment design, lower mixing times are enough to achieve sufficient recovery of organic acids.

After equilibrium, the recovery percentage of butyric acid from the synthetic medium was approximately 93% at pH 2.5, 88% at pH 3.5, and around 67% at pH 4.5. In contrast, for propionic acid, the recovery percentages were 74% at pH 2.5, 67% at pH 3.5, and 39% at pH 4.5. Bilgin et al. (2006) demonstrated that among the esters studied, ethyl acetate exhibited the best distribution coefficient for butyric acid (Kd: 6.07), which was higher than that of nearly all other solvents. In another study published in 2009, the same authors examined the extraction of propionic and butyric acid with different types of solvents and found that for all solvents, including esters, butyric acid consistently showed the best distribution coefficient. Therefore, this may explain the fact that the percentage of butyric acid recovery is better compared to that of propionic acid.

* + 1. Simulation results

Alongside the experiments results, a simulation was carried out in Aspen Plus® to evaluate the accuracy of the predictive model UNIFAC-LL in describing the VFAs extraction from a synthetic butyric/propionic acid solution with ethyl acetate. The input values were described in Table 1 and the results are shown in Table 2.

*Table 2 - Flow rate and percentage of acid recovery (%) results from the simulation*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Total flow  rate (kg/h) | Water  (kg/h) | Butyric acid  (kg/h) | Propionic acid  (kg/h) | Ethyl acetate  (kg/h) |
| Organic phase | 94.94 | 3.47 | 3.05 | 1.02 | 87.39 |
| Aqueous phase | 93.58 | 90.76 | 0.25 | 0.28 | 2.29 |
| Acid recovery |  |  | Butyric acid | Propionic acid |  |
| Simulation | | - | 92.5 % | 78.0 % | - |
| Experimental | | - | 93.2 % | 73.3 % |  |

The acid recovery predicted by the UNIFAC-LL model implemented in Aspen Plus® at equilibrium was 92.5% for butyric acid and 78.0% for propionic acid. Since UNIFAC-LL is a predictive model based on the functional groups of the molecule, the interaction parameters are estimated according to the model and can be determined with good accuracy (Fredenslund et al., 1975). The simulated results demonstrated accuracy in describing the liquid-liquid extraction between the solvent and the organic acids, yielding results that closely matched the experimental data, although propionic acid showed a deviation from the empirical data. The difference between the simulated and experimental results was approximately 1% for butyric acid and 4.7% for propionic acid. Woo & Kim (2019) simulated a plant in Aspen Plus ® for the recovery of C2-C6 bio-based volatile fatty acids using different solvents and applied NRTL and UNIFAC to estimate the interaction parameters based on the functional groups in the molecules studied. They found great accuracy in modeling the liquid-liquid equilibrium for acetic, propionic, butyric, and other acids with an ester as solvent, similar to the results of this work. Although, in this work, propionic acid showed a greater deviation from the experimental results, it was still acceptable for use in predictive models such as UNIFAC-LL.

The strong correlation between the UNIFAC-LL model and the experimental data can be attributed to the fact that, in the UNIFAC modification (UNIFAC-LL), the group interaction parameters are derived from experimental correlation data specifically for liquid-liquid equilibrium (Magnussen et al., 1981). In addition, all the molecules involved in the experiment are not complex and the interaction parameters correlation between them are well established in the model.

* 1. Conclusion

This study demonstrated that liquid-liquid extraction of VFAs from synthetic fermentation media using ethyl acetate as a solvent is a viable and promising method as it achieves high recovery yields, particularly for butyric acid, at low pH values. The optimal results were obtained when the pH was adjusted to 2.5, with average recoveries of 93% for butyric acid and 73% for propionic acid after 40 minutes of stirring, at which point equilibrium was reached. In contrast, the lowest values were observed at pH 4.5, with average recoveries of approximately 67% for butyric acid and 39% for propionic acid after 40 minutes. These findings were expected, as increasing the pH of the acid solution leads to a higher proportion of the acids in their ionized form, which impedes their extraction by the solvent since the ionized form has more affinity for the polar solvent (water). To validate the UNIFAC-LL model, a simulation was conducted in Aspen Plus® using similar input conditions. The simulation results demonstrated excellent accuracy, with deviations of less than 1% for butyric acid and 5 % for propionic acid. These results indicate that the UNIFAC-LL model effectively describes the liquid-liquid equilibrium between the VFAs solution and ethyl acetate, highlighting the potential of ethyl acetate as a solvent for future studies

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