

Downstream Extraction of Volatile Fatty Acids: Simulation and Experimental Evaluation using Hexadecane

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The production of volatile fatty acids (VFAs) through a green and sustainable route is gaining increasing attention due to rising market demand, broad application possibilities, and a balanced approach that combines economic viability with environmental benefits. Among these, VFAs of three and four carbons (propionic and butyric acids) produced via fermentation are promising alternatives to carboxylic acids synthesized from non-renewable sources. However, the downstream processing of these VFAs remains a critical bottleneck, particularly in terms of selectivity and purity. This downstream processing implies high operating costs, representing 20 to 50% of the process expenses. The selection of an appropriate solvent plays a fundamental role in enhancing the extraction process of these VFAs. Hexadecane efficiently separates phases with water and selectively extracts target products from the fermentation medium. A simulation using Aspen Plus® and a 3² factorial design with 3 central points was conducted to assess its extraction efficiency. The experimental results showed a 61.7 % extraction efficiency for butyric acid at 35 °C, solvent-to-feed ratio (ROW) of 6, and pH 2.4. For propionic acid, the extraction efficiency was 35.7% under the same experimental conditions. ANOVA revealed that all main factors were statistically significant ($p < 0.05$) at a 95% confidence level, confirming their strong influence on the extraction of organic acids with hexadecane. The simulation of the extraction process further validated these findings by closely aligning with the experimental results, demonstrating the reliability of the work for potential process improvements in VFAs recovery.

1. Introduction

The need to mitigate climate change, the increase in global energy demand, the pursuit of a more diversified energy mix, and the instability of oil prices are some of the factors driving the high demand for chemicals produced from renewable sources (Calvo & Martin, 2022; Cardona et al., 2010). In this context, the conversion of renewable raw materials to produce organic acids stands out (Leal Silva et al., 2018). Organic acids are highly demanded chemicals that can serve various markets, including fuels, pharmaceuticals, food, and bioplastics. Among the organic acids produced via fermentative routes, propionic and butyric acids hold significant industrial relevance.

Butyric acid, a volatile short-chain fatty acid produced by anaerobic bacteria, is widely used in cosmetics, animal feed, pharmaceutical, and chemical industries. With a global production of 80.000 tonnes annually (\$1800/t), it is traditionally derived from petroleum-based chemical processes, such as propylene hydroformylation or n-butyraldehyde oxidation, which generate toxic residues (Jiang et al., 2018; Playne, 1985). Fermentative biosynthesis emerges as a sustainable alternative but faces challenges like low yields, productivity, and high recovery costs (Jha et al., 2014; Luo et al., 2018). Similar challenges are observed with propionic acid, a versatile carboxylic acid used in the preservation of animal feed and foods like bread and cheese, as well as in anti-arthritis drugs, perfumes, and solvents.

To address the aforementioned issues, research has tended to focus on liquid-liquid extraction using organic solvents to recover carboxylic acids from fermentation broths (Yang et al., 2006). This method is low-cost and

easily scalable but shows greater efficiency with acidified broths, as most solvents extract only non-dissociated acids (Yang et al., 1991). Previous studies have reported on the use of esters as solvents for the extraction of carboxylic acids (Alao et al. 2023). Nevertheless, esters and water are partially miscible and generally they form azeotropic mixtures with water. In this context, hexadecane emerges as a potential solvent due to its high affinity for volatile fatty acids, such as butyric and propionic acids, and its low miscibility with water, which facilitates phase separation and solvent recovery. Therefore, the focus of this work is to investigate the efficiency of liquid-liquid extraction for butyric and propionic acids by using different parameters (pH and solvent-to-feed ratio) to optimize the downstream process.

2. Methodology

2.1 Materials

Butyric, propionic, and sulfuric acids were provided by Êxodo Científica (Brazil) and were of analytical grade. Sodium hydroxide (97%), used as the neutralizing agent, was acquired from NEON (Brazil). N-hexadecane was purchased from Dinâmica (Brazil).

2.2 Extraction Tests

The extraction tests for organic acids (butyric and propionic) were performed using hexadecane as a solvent. The experimental conditions were planned based on a 3² factorial design with 3 central points, varying the pH (2.4, 3.5, and 4.5) and the solvent-to-feed ratio (ROW) (1:1, 3:1, and 6:1), totaling 12 experiments. Under these conditions, butyric acid is mainly in its undissociated form, which helps improve the recovery of this acid by the solvent. In this case, the temperature was fixed at 35°C, and agitation was carried out in an incubator shaker (SP-223 – Splabor) at 150 rpm. The feed solution was prepared to contain 33 g/L of butyric acid and 13 g/L of propionic acid in ultrapure water, with pH adjusted using sodium hydroxide. These concentrations were selected based on results from fermentations that have already been carried out. After preparation, the mixtures were agitated in the incubator shaker according to the conditions defined in the experimental design. At the end of the agitation, the phases were separated for 1 hour in 125 mL separatory funnels, maintained at 35°C.

2.3 Sample analysis

The concentrations of butyric and propionic acids were determined by high-performance liquid chromatography (Agilent 1260 Infinity). Separation was performed on a Bio-Rad Aminex® HPX-87H column maintained at 30 °C, with 3 mmol/L H₂SO₄ as the mobile phase at a flow rate of 0.5 mL/min. Detection was carried out using a refractive index detector (RID). Extraction rates (%Ext) were determined based on the concentration of acids remaining in the aqueous phase, and the extraction percentage was calculated using Eq(1).

$$\% Ext = \frac{C_{initial} - C_{aqueous}}{C_{initial}} = \frac{C_{solvent}}{C_{initial}} \quad (1)$$

where $C_{initial}$ is the initial concentration of the analyte in the solution before extraction (g/L), $C_{aqueous}$ is the concentration of the analyte remaining in the aqueous phase after extraction, and $C_{solvent}$ is the concentration of the analyte extracted into the organic (hexadecane) phase.

2.4 Statistical Analysis

Experimental data from the 3² factorial design were analyzed using Statistica software. Analysis of variance (ANOVA) was performed with a 95% confidence level to evaluate the significance of main effects and interactions. The adjusted statistical model described the relationship between extraction efficiency and the independent variables.

2.5 Process simulation

Mass balances were obtained using Aspen Plus® v 14.0 (Aspen Technology Inc., Bedford, MA, USA). The UNIFAC-Dortmund model was used to estimate activity coefficients in the liquid phase, offering improved accuracy for non-ideal mixtures of organic acids and hydrophobic solvents. The VFA extraction process was simulated based on the experimental parameters (Table 1), and the final flowsheet is shown in Figure 1.

Table 1 – Input parameters of the streams

	T (°C)	P (bar)	Total flow rate (kg/h)	Water (kg/h)	Butyric acid (kg/h)	Propionic acid (kg/h)	Hexadecane (kg/h)
Feed	35	1	997.1	947.3	32.90	12.96	0
Solvent	35	1	4653.5*	0	0	0	4653.5

*The solvent-to-feed ratio was varied according to the same conditions defined in the experimental design.

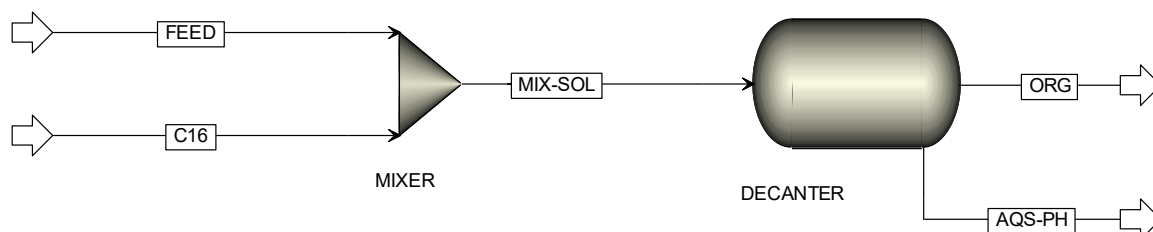


Figure 1 - Process simulation flowsheet in Aspen Plus®

Since the model selected in Aspen Plus® does not model the effects of pH such as the dissociation of organic acids into ionic species, part of the experimental trials had their pH set to a value below the pKa of the acids, approximately 2.4. Therefore, the simulation results were compared only for this pH value. Butyric and propionic acids, as weak acids, follow the equilibrium:



The dissociation constant (K_a) is defined as:

$$K_a = \frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]} \quad (3)$$

Assuming $[\text{HA}] \approx C_{\text{initial}}$ and $[\text{H}^+] \approx [\text{A}^-]$, the hydrogen ion concentration is approximated by:

$$[\text{H}^+] = \sqrt{K_a \cdot C_{\text{initial}}} \quad (4)$$

Using K_a values (butyric: 1.5×10^{-5} ; propionic: 1.3×10^{-5}) and initial concentrations (33 g/L and 13 g/L, respectively), the contributions were $[\text{H}^+]$ butyric acid: 0.0023 mol/L and $[\text{H}^+]$ propionic acid: 0.0015 mol/L. The total $[\text{H}^+]$ was 0.0038 mol/L, yielding a pH of 2.41, calculated as for Eq (5):

$$\text{pH} = -\log_{10} ([\text{H}^+]) \quad (5)$$

This result highlights the combined influence of both acids on solution acidity, consistent with their pKa values and experimental conditions. Notably, this pH was precisely the one obtained during the preparation of the feed solution, eliminating the need for neutralization with sodium hydroxide, as also observed by Zigorová et al. (1996).

3. Results and Discussions

3.1 Statistical Analysis

Table 2 presents the 3^2 factorial design matrix for pH (x_2) and solvent-to-feed ratio (ROW, x_1) along with the extraction percentages of butyric and propionic acids for 12 experiments, calculated using Equation 1. Statistical analysis at a 95% confidence level (Figure 2) revealed that the most significant factors for the extraction process were pH (x_2) and the solvent-to-feed ratio (ROW, x_1), underscoring their importance in process efficiency. For butyric acid, the linear term of pH exhibited the largest effect (-43.05), followed by the linear term of ROW (38.70). For propionic acid, the most significant factor was the interaction between ROW and pH (x_1x_2), with an effect of -14.75, while the linear term of ROW had a positive impact (10.68). After excluding the insignificant terms at a 95% confidence level, the following regression equation in coded units was derived for %Ext:

$$\%Ext_{But} = 39.4 + 9.6x_1 - 12.0x_2 + 4.6x_2^2 - 8.9x_1x_2 + 4.7x_1x_2^2 - 4.1x_1^2x_2 + 2.7x_1^2x_2^2 \quad (6)$$

$$\%Ext_{Pro} = 23.6 + 4.5x_1 - 3.6x_2 - 9.0x_1x_2 + 5.1x_1x_2^2 \quad (7)$$

Table 2. Values for the variables considered in the factorial design and the response (% extraction).

Run	x ₁ (ROW)	x ₂ (pH)	%Ext _{But}	%Ext _{Pro}	Run	x ₁ (ROW)	x ₂ (pH)	%Ext _{But}	%Ext _{Pro}
1	3	4.5	32.2	21.8	7	6	2.4	61.7	35.7
2	6	3.5	57.8	34.6	8	1	4.5	26.4	18.9
3	1	2.4	30.9	15.5	9	3	3.5	47.1	25.7
4	1	3.5	26.8	13.1	10	3	3.5	46.9	24.5
5	6	4.5	40.5	23.3	11	3	3.5	47.2	24.4
6	3	2.4	49.2	26.0	12	3	3.5	47.7	25.1

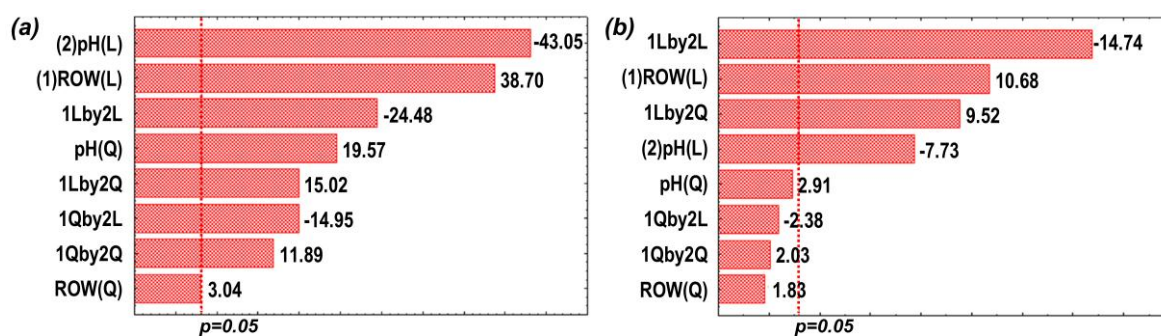


Figure 2. Pareto chart of significant factors for extraction of a) butyric acid and b) propionic acid using hexadecane at 95% confidence

Indeed, pH plays a crucial role in the extraction of carboxylic acids, controlling the equilibrium concentrations between the aqueous and organic phases (Reyhanitash et al., 2016). In the present study, it was observed that low pH values, around 2.5, favored acid recovery. This occurs because, at these values, the acids remain predominantly in their undissociated forms, facilitating their transfer to the organic phase due to affinity with the organic solvent. This behavior is consistent with extractions conducted under similar conditions (Chun et al., 2018; Vidal et al., 2024). The extraction efficiency was also influenced by the nature of the acid. Acids with greater hydrophobicity, such as butyric acid, showed higher affinity for the organic phase, resulting in higher recovery percentages compared to propionic acid. This behavior is consistent with the observation that longer-chain carboxylic acids exhibit greater hydrophobicity, favoring their extraction by non-polar solvents, such as hexadecane (Yang et al., 2024). It was evidenced that this difference directly reflects the distribution coefficients, which indicate higher efficiency in the transfer of butyric acid to the organic phase compared to propionic acid. The adequacy of the models for butyric and propionic acid extraction was evaluated through analysis of variance (ANOVA), as presented in Tables 3 and 4. The adjusted correlation coefficients (R^2) were 99.98% and 99.79%, respectively, indicating minimal unexplained variability. The adjusted (R^2_{ADJ}) and predicted (R^2_{PRED}) values—99.91% and 99.97% for butyric acid, and 99.22% and 99.79% for propionic acid—demonstrate strong agreement between experimental and predicted values. The calculated Fischer's values (750.03 and 57.22 for butyric and propionic acids, respectively) far exceeded the tabulated F-values, confirming statistical significance at a 95% confidence level. The tabulated F-value is determined from tables that relate the confidence interval and the degrees of freedom of the regression and residuals. For the verification of the tabulated F-values, the tables provided by Rodrigues and lemma (2014) were used.

Table 3 – ANOVA Table for Butyric Acid Extraction ($R^2 = 0.99976$).

Source of Variation	SS	df	MS	F	P-Value
ROW	179.63	2	89.81	750.02	0.000089
pH	363.67	2	181.83	1518.47	0.000031
(x ₁ x ₂)	110.92	4	27.73	231.57	0.000458
Pure Error	0.35	3	0.11	-	-
Total	1482.01	11	-	-	-

SS (Sum of Squares), df (Degrees of Freedom), MS (Mean Square), F (F-ratio).

Table 4 – ANOVA Table for Butyric Acid Extraction ($R^2 = 0.99789$).

Source of Variation	SS	df	MS	F	P-Value
ROW	38.77	2	19.38	57.22	0.004083
pH	38.64	2	19.32	57.03	0.004102
($x_1 \times x_2$)	91.55	4	22.88	67.56	0.002857
Pure Error	1.01	3	0.33	-	-
Total	481.72	11	-	-	-

SS (Sum of Squares), df (Degrees of Freedom), MS (Mean Square), F (F-ratio).

3.2 Experimental vs. Simulation Results

Alongside the experimental results, a simulation was performed in Aspen Plus® to evaluate the accuracy of the Dortmund-UNIFAC model in the extraction of volatile fatty acids (VFAs) with hexadecane. The input values are described in Table 1, and the results, comparing both analyses, are presented in Figure 3. It is worth noting that ROW and pH were varied during the experiments, with the best results observed at a 6:1 ratio and pH 2.4. Under these conditions, the extraction efficiency reached 61.7% for butyric acid and 35.7% for propionic acid. The simulation yielded similar extraction values, with 64.3% for butyric acid and 36.1% for propionic acid, demonstrating good agreement between the experimental and simulated data.

Conventional solvents, such as hexadecane, are not as efficient when applied to dilute propionic acid solutions due to the low aqueous activity of carboxylic acids, resulting in low distribution coefficients, which explains the lower recovery compared to butyric acid (Kumar et al., 2006).

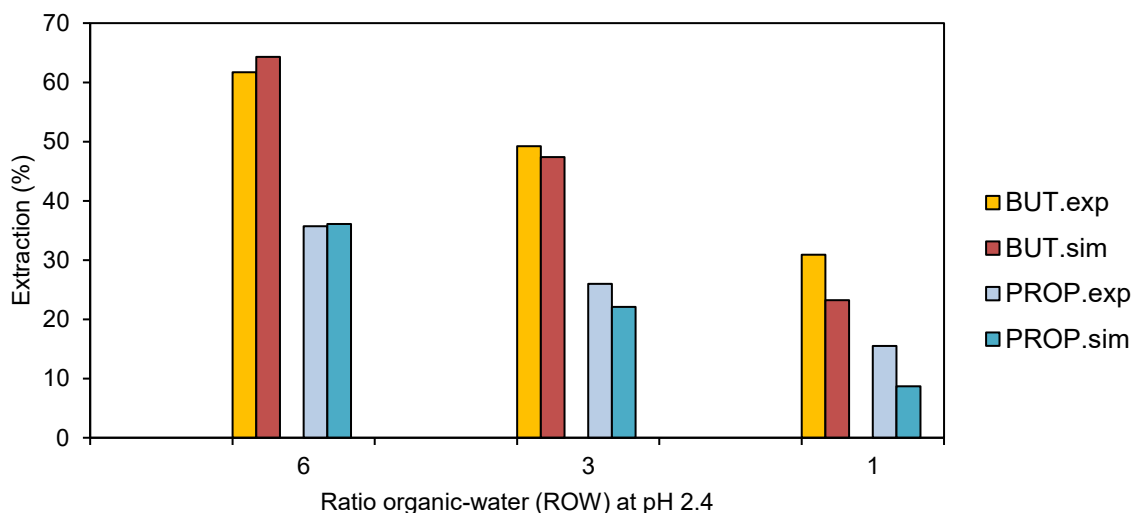


Figure 3 - Experimental vs. Simulated VFA Extraction at Different ROW Ratios

The choice of the Dortmund-UNIFAC model for analyzing the experiments involving the extraction of volatile fatty acids (VFAs) with hexadecane is justified by its ability to handle complex systems that involve specific interactions between functional groups. Unlike the original UNIFAC model, the Dortmund-UNIFAC introduces modifications to the residual term, adjusting group interaction parameters based on a broader and more refined database (Kuramochi et al., 2009). This is essential to accurately represent the interactions between butyric and propionic acids, which have polar carboxylic groups, and hexadecane, a non-polar solvent. Additionally, the Dortmund-UNIFAC model includes adjustments to the combinatorial term, which enhances its representation of asymmetric systems, such as the mixture of large, non-polar molecules (hexadecane) with smaller, polar organic acids (Kikic et al., 1980). This feature is crucial for describing solubility differences and interactions between the aqueous and organic phases in the experiments. Therefore, the model developed has a good representation of reality and it can be used for further process design and optimization.

4. Conclusion

This study demonstrated that liquid-liquid extraction of VFAs from synthetic media using hexadecane as a solvent is an effective technique, especially for butyric and propionic acids when high solvent-to-feed ratios are employed. The Dortmund-UNIFAC model proved to be suitable for describing the equilibrium between the phases, showing good agreement between experimental and simulated results. Statistical analysis further

supported this agreement, with average deviations of less than 5%, validating the model's accuracy in predicting experimental data. These findings highlight the potential of hexadecane as a solvent in extraction processes, reinforcing its applicability in real fermentation systems. However, to optimize recovery efficiency, it is necessary to test other solvents that may show better affinity for carboxylic acids. Thus, with the use of improved organic acid recovery techniques, renewable and low-cost biomass, along advancements in cell engineering, bio-based butyric acid has the potential to become more competitive with its fossil-derived counterpart.

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