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Studies on Reactive Extraction of Itaconic Acid from Fermentation Broths

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Abstract: Itaconic acid is a high-value organic acid that serves as a platform molecule in different industries. This research focuses on the separation of itaconic acid using reactive extraction as a sustainable and efficient method for acid recovery from fermentation broth. Itaconic acid was produced through fungal fermentation processes involving *Aspergillus terreus* ATCC® 32588™, obtaining a concentration of 47 g/L in the final broths. For the reactive extraction system, the organic phase included tri-n-octylamine as an extractant dissolved in dichloromethane or n-heptane and 1-octanol as a phase modifier. The effect of the main influencing factors (pH of the aqueous phase, extractant concentrations in the organic phase, and the addition of 1-octanol) on extraction efficiency was investigated. The highest extraction degree (97%) was achieved using an organic phase with tri-n-octylamine dissolved in dichloromethane and with 20% 1-octanol. Conversely, in the reactive extraction system with tri-n-octylamine dissolved in n-heptane and 20% 1-octanol, the extraction efficiency reached 67%. This finding suggests a promising separation system that is less toxic for microorganisms. The study results highlight the feasibility of employing reactive extraction systems for the direct separation of itaconic acid.

Keywords: itaconic acid; *Aspergillus terreus*; fermentation broths; tri-n-octylamine; reactive extraction

Citation: Blaga, A.C.; Kloetzer, L.; Cascaval, D.; Galaction, A.-I.; Tucaliuc, A. Studies on Reactive Extraction of Itaconic Acid from Fermentation Broths. *Processes* **2024**, *12*, 725. https://doi.org/10.3390/pr12040725

Academic Editors: Prashant K. Sarswat and Yanlin Zhang

Received: 3 March 2024
Revised: 31 March 2024
Accepted: 1 April 2024
Published: 3 April 2024

1. Introduction

In the context of sustainable development, many important chemicals are suitable to be replaced by natural alternatives through biobased processes. Itaconic acid (IA) is a dicarboxylic acid (Figure 1) with various applications across different industries. These include chemical applications (as a building block for products like resins, biodegradable and sustainable polymers, and coatings), cosmetic uses (as a thickening agent, emulsion stabilizer, and moisturizing agent), textile applications (as a dye-binding agent and as a modifier for enhancing the dye ability of fibers), and even in the food industry (as an acidulant and flavor enhancer) [1].

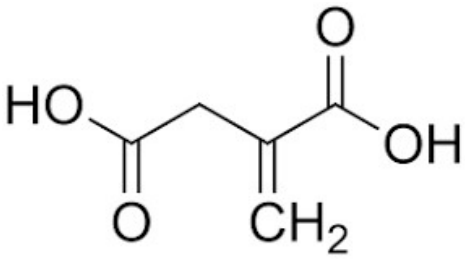

Figure 1. Itaconic acid chemical structure.

Itaconic acid has the advantage that it can be obtained by fermentation processes. Biotechnological production of itaconic acid involves various microorganisms, including filamentous fungi such as *Aspergillus itaconicus*, *Aspergillus terreus*, *Pseudozyma antarctica*, and *Ustilago maydis* [2–4], as well as yeasts like *Candida* sp., *Rhodotorula* sp. [5], and even bacteria such as engineered *E. coli* [1]. The fermentation process for itaconic acid production provides a sustainable, cost-effective, and environmentally friendly approach that aligns with the growing demand for biobased chemicals and renewable manufacturing processes. Furthermore, it generates fewer greenhouse gas emissions and requires less energy input, reducing the carbon footprint associated with itaconic acid production. Additionally, it can use renewable feedstocks such as sugars derived from biomass, agricultural waste, or even lignocellulosic materials as carbon sources [6].

Notably, commercial production of IA often relies on the fermentation of *A. terreus* using glucose as a substrate, resulting in a maximum concentration of 129 g/L in the fermentation broth [2].

Following fermentation, designing a cost-effective downstream process becomes a key step in biobased itaconic acid production. Several separation processes, including ion exchange, adsorption, evaporative crystallization, precipitation, electrodialysis, and extraction) have been used for IA recovery. Among these, crystallization is the most commonly employed recovery method, yielding approximately 75%. However, it requires further downstream steps to improve the purity of the final product. The choice of separation method for itaconic acid recovery from fermentation broth—whether through physical or chemical-based separation and whether it is coupled with fermentation—comes with its own set of advantages and disadvantages. As the demand for itaconic acid continues to grow in various industries, having a reliable and efficient separation method becomes crucial for meeting market requirements while promoting environmentally friendly products. Considering important criteria such as yield, equipment costs, product purity, energy consumption, and downstream complexity, the reactive extraction method emerges as a favorable choice for IA separation. Also, this method can be directly coupled with fermentation if the chosen solvents are non-toxic to microorganisms. By expanding the availability of premium-grade IA, manufacturers can enhance their competitiveness in the market. This approach not only meets customer demand but also positions them as reliable suppliers, ultimately driving business growth.

As a promising method for IA separation, reactive extraction, derived from liquid-liquid separation methods, is a process that involves the transfer of a solute from one liquid phase to another immiscible phase, facilitated by an extractant. This method is especially useful for separating compounds with similar physical properties or when conventional separation techniques like distillation or crystallization are not effective or economically viable [7]. Reactive extraction is commonly employed for efficiently separating organic acids, leading to improved results [8–12]. Various types of extractants have been evaluated for IA separation [12–18]. These include organophosphates and aliphatic amines dissolved in different solvents [11–16]. Among them, tributyl phosphate (TBP) [13,16], trioctylmethylammonium chloride (Aliquat 336) [15,16], trioctylphosphineoxide [10], dioctylamine, N-methyldioctylamine [8], and tri-n-octylamine (TOA) [8,13,17,18] were used and the efficiency of reactive extraction from the aqueous phase followed the order of quaternary ammonium > tertiary > secondary > primary amines [11]. Different solvents, both active and inactive, were analyzed for extraction, including alcohols, esters, and alkanes. Aliquat 336 was dissolved in ethylacetate, hexane, toluene, and kerosene. The system containing ethyl acetate provided the highest extraction degree of 72.66% [15]. Considering the solvent phase toxicity, Aliquat 336 and TBP were compared by dissolving them in a biocompatible solvent, sunflower oil, and Aliquat 336 exhibited sustainable enhancement in itaconic acid extraction in the organic phase, surpassing TBP. The distribution coefficient was 0.95 for Aliquat 336 and 0.4 for TBP [16]. In a study by Pal et al. (2016) tributyl phosphate dissolved in alcohols (1-butanol, 1-octanol, 1-dodecanol) resulted in the highest extraction degree (82.47%) when 50% TBP was dissolved in 1-butanol [19]. Tri-n-octylamine, an aliphatic

amine with a long carbon chain, has been identified as an effective extractant for itaconic acid separation. Its advantages include low solubility in water and thermal stability, allowing for easy recycling through distillation [13]. The stoichiometry of reactive extraction for IA using TOA dissolved in various solvents, such as alkane, esters, ketone, and alcohols, was established by Uslu and Datta (2015). Among these systems, TOA combined with 1-octanol demonstrated the most favorable performance in terms of distribution coefficient (KD = 18.20) and extraction degree (% E = 94.80%) [18].

Considering that the applications of IA are constantly evolving as research and development efforts continue to explore its potential uses in various industries, and the fact that the downstream processing for IA manufacturing constitutes approximately 40% of overall production expenses, like for other important organic acids, ensuring the creation and implementation of effective recovery methods remains necessary [20,21]. The commercial production of IA continues to draw interest due to its high high-end application potential. However, the critical barrier to overcome is finding an efficient and environmentally friendly separation process. Taking into account the trends in direct separations of the carboxylic acids, the research objectives of this study focused on two distinct steps: (i) itaconic acid production by controlling the fungal fermentation of *Aspergillus terreus* (ATCC® 32588™), and (ii) the investigation of the efficiency of different reactive extraction systems for IA recovery from filtered fermentation broths; two solvents, n-heptane and dichloromethane, were employed for diluting the aminic extractant, TOA. The research aim of this study was to establish a suitable solvent system and investigate the impact of various factors on efficiency during the reactive extraction of IA, including aqueous phase pH (from the clear fermentation broth), TOA concentrations in the organic phase, and the addition of 1-octanol as a phase modifier.

2. Materials and Methods

2.1. Microorganism and Culture Media

For fungal fermentation processes, *Aspergillus terreus* (ATCC® 32588™, ATCC, Manassas, VA, USA) was cultivated on malt agar medium at 35 °C. To create spore suspension, the spores were washed twice with 10 mL of sterilized water each time. Conidiospores were then quantified using a hemocytometer (specifically, a CE Neubauer-improved Marienfeld hemocytometer, Fisher Scientific, Göteborg, Sweden) and an Optika microscope (manufactured by, OPTIKA S.r.l., Ponteranica (BG), Italy). The resulting spore suspensions, containing approximately 10^8 or 10^9 spores/mL, served as the inoculum for the fermentation process. The inoculum size was considered 10% of the fermentation culture medium volume. The inoculum medium used in the spore-germination experiment had the following composition (in grams per liter): glucose 45, KH_2PO_4 1, $MgSO_4$ 1, $ZnSO_4$ 0.05, NH_4SO_4 5, and $FeSO_4$ 0.04. The inoculum medium compounds were provided from Carl ROTH, Karlsruhe, Germany.

2.2. Fermentation Processes

The fermentation experiments were conducted in a 2 L laboratory stirred bioreactor (manufactured by Fermac, Electrolab, Mumbai, India), equipped with a computer system for controlling and recording key parameters such as temperature, pH, and dissolved oxygen. The working volume for all fermentation experiments was 1 L. *Aspergillus terreus* cultivation was carried out in a batch mode at 35 °C, a fermentation time of 190 h, an agitation rate of 200 rpm using a turbine-type impeller, and an aeration rate of 1 L/minute. The acid production medium contained (in grams per liter) glucose 180, KH_2PO_4 0.2, NH_4NO_3 2.5, $FeSO_4 \cdot 7H_2O$ 0.04, $MgSO_4 \cdot 7H_2O$ 1.9, and $ZnSO_4 \cdot 7H_2O$ 0.15, and the pH value was adjusted to 3.5 [22]. The culture medium compounds were provided from Carl ROTH, Karlsruhe, Germany. The pH value was set at the beginning of fermentation and was not controlled during the fermentation processes. The bioreactor and the culture medium were sterilized at 120 °C for 21 min in a vertical autoclave (type Raypa AES-110, Raypa, Terrassa (Barcelona), Spain). During the fermentation processes, samples were collected at 24-h

interval. Upon completion of the fermentation step, the broths underwent the procedure described in reference [22]. To determine the cell dry weight (d.w.) gravimetrically, 2 mL of the fermentation broth was centrifuged at 3000 rpm for 10 min at room temperature. The experimental conditions were similar to those reported in the literature [23,24]. After fermentation, the biomass was separated through filtration, and the supernatant was used for subsequent IA reactive extraction.

2.3. Reactive Extraction Procedure

For the reactive extraction experiments, we utilized a laboratory extraction column equipped with vibratory mixing. Detailed specifications of this equipment were previously documented [25]. The extraction column consists of a glass column with specific geometric characteristics: an internal diameter of 46 mm and a height of 250 mm. These dimensions ensure a high interfacial area and facilitate rapid attainment of the equilibrium state. For temperature control during experiments, a thermostatic jacket was employed, circulating a thermal agent (a mixture of water and ethylene glycol, SERVA Electrophoresis GmbH, Heidelberg, Germany) at 25 °C. To maintain the desired interfacial area, a perforated disk made of stainless steel, with a diameter of 45 mm and a 20% free section, was precisely positioned at the initial contact interface between the aqueous and organic phases. The vibration applied had a frequency of 50 s^{-1} and an amplitude of 5 mm. The extraction process was conducted at 25 °C, with an extraction time of 1 min. Following extraction, the resulting emulsion was separated using a centrifugal separator (a Biobase Laboratory Centrifuge high-speed TG16-WS, Biobase Biodustry Co., Ltd., Jinan, China) operating at 5000 rpm. The aqueous phase used for extraction originated from the fermentation broth, containing 50 g/L (0.384 M) IA. The IA separation by reactive extraction was performed using TOA (Merck KGaA Billerica, MA, USA) as an extractant, dissolved in two organic solvents with distinct dielectric constants: dichloromethane (DCM) (ε = 9.08) (Fluka, Paris, France, GC 99.9%) or n-heptane (ε = 1.90) (Merck, GC 99%) [9]. The concentration of extractant in the organic phase (either pure solvent or solvent mixed with 1-octanol) ranged between 0 and 240 g/L (0.678 M). Additionally, a phase modifier, 1-octanol with purity exceeding 98% (from Merck, ε = 10.3) was mixed into the aforementioned solvents. The volumetric ratio between the aqueous and organic phases was 1:1 (20 mL of each phase).

The pH range of the aqueous phase with itaconic acid was between 1 and 6 and was measured using a digital pH meter (type C836, Consort bvba, Turnhout, Belgium). To obtain the desired pH value of the aqueous phase, solutions of 3% H_2SO_4 or 3% NaOH (Merck KGaA, Darmstadt, Germany) were used. During the experiments, the pH of the aqueous phase was monitored, and no variations were observed.

The extraction results were analyzed based on the IA extraction degrees, calculated as the ratio of the itaconic acid concentration in the extracted phase ($[(H_2I)_{(org)}]$) to the initial acid concentration in the aqueous phase ($[(H_2I)_{(aq)i}]$) while assuming no change in volume at equilibrium (Equation (1)):

$$\eta = \frac{\left[(H_2I)_{(org)}\right]}{\left[(H_2I)_{(aq)_i}\right]} \times 100 \quad (1)$$

The equilibrium for the IA extraction process with TOA occurs when the rate of formation equals the rate of dissolution. It can be described as a series of reactions between m molecules of acid (H_2I), and n molecules of extractant (E), resulting in the formation of various acid:extractant complexes (m:n) (Equation (2)):

$$m\ H_2I + n\bar{E} \boxed{\leftrightarrow} \overline{(H_2I)_m(E)_n} \quad (2)$$

The studies on the stoichiometry (m, n) of the reactive extraction of IA with TOA suggested that different types of acid:amine complexes like [H_2I.E] (1:1), and [$(H_2I)_2.E_2$] (2:1) are formed [11].

The IA concentration in the aqueous phase was determined by the HPLC method. For the acid concentration in the solvent phase, the mass balance calculation has been done. The HPLC equipment used was the UltiMate 3000 Dionex system, equipped with an Acclaim™ OA column (4 mm diameter, 150 mm length, 5 µm porous particle size, Dionex—Thermo Fisher Scientific, Sunnyvale, CA, USA) and a UV detector set at 210 nm [12]. The mobile phase consisted of a 100 mM Na_2SO_4 solution with a pH of 2.65 (adjusted using methanesulfonic acid). The flow rate of the mobile phase was 0.6 mL/min. The analysis has been carried out at 30 °C. Each experiment was repeated three times under identical conditions, and the average value of the considered parameters was used. The maximum experimental error observed was 6.1%. The methods and equipment used in this study were chosen based on the accuracy of results and the advantages offered, as mentioned in previously published papers [9,12,25].

3. Results and Discussion

3.1. Fermentation Process

Aspergillus terreus (ATCC® 32588™) stands out as a highly promising strain for itaconic production through aerobic cultivation. This strain leads to high concentrations of itaconic acid in fermentation broths. The production of itaconic acid was significantly influenced by several factors:

(i) Fungi morphology and growth form: In this study, the dominant growth form of *A. terreus* was filamentous, characterized by short hyphae. This morphology was advantageous because it avoids high viscosities that could otherwise influence the oxygen transfer rate from the gas phase to the cells;

(ii) Bioreactor design and operational parameters: The successful production of itaconic acid hinges on a well-designed bioreactor and carefully controlled parameters during the fermentation process.

From the experimental data depicted in Figure 2, it can be observed that itaconic acid production started at 45 h of *A. terreus* cultivation. As the glucose concentration decreased, itaconic acid accumulated in the broth gradually. However, beyond 120 h from the start of fermentation, the itaconic acid concentration stagnates and reaches a maximum value of 47 g/L.

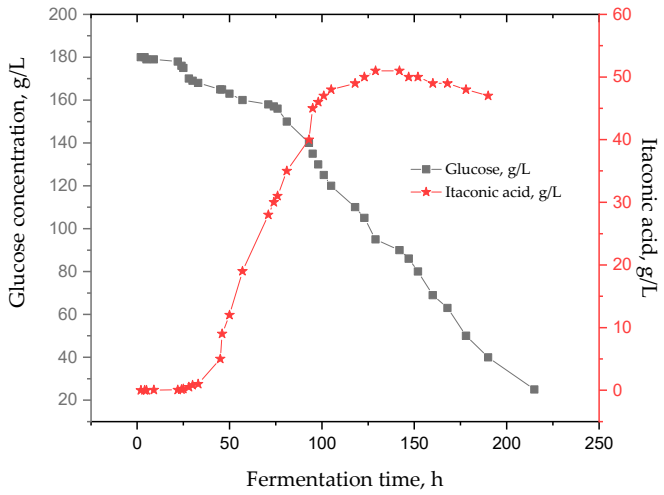

Figure 2. Glucose consumption and itaconic acid accumulation during *A. terreus* fermentation.

Regarding glucose consumption and itaconic acid accumulation, Figure 3 indicated the following presumptions: (i) *A. terreus* might be sensitive to acidic conditions, leading to

reduced glucose utilization [26], (ii) the low pH affects the enzymatic reactions involved in glucose metabolism, and, additionally, the accumulation of itaconic acid could further impact the microorganism's metabolism due to the product inhibition mechanism [27,28]. According to the experimental results, the glucose consumption gradually decreased during the first 80 h of fermentation, while there was an accumulation of itaconic acid in the fermentation broth. At the end of fermentation, the residual glucose concentration was 19 g/L, which indicated that the metabolic activity of the microorganism *A. terreus* was influenced by the presence of IA [26–28]. The initial pH value was set at 3.5, and by the end of fermentation, the pH value dropped below 2. This initial pH value was adopted according to the literature to achieve IA production due to an improved performance of *A. terreus* [29–31]. Such a significant decrease in pH can have several implications for microbial metabolism and enzyme activity [28].

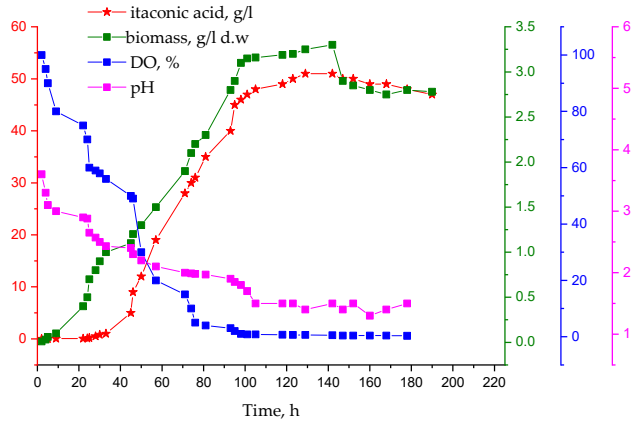

Figure 3. Evolution of parameters (itaconic acid, cells dry weight, dissolved oxygen, pH) during aerobic cultivation of *A. terreus*.

A key parameter with a strong impact on *A. terreus* growth and itaconic acid production was represented by dissolved oxygen (DO). Dissolved oxygen directly influences itaconic acid biosynthesis. In the first stage of fermentation, DO decreased rapidly to a minimum value due to fungus growth and biomass accumulation in the broth. However, in the late stage of fermentation, the mycelial growth slowed down, and itaconic acid production increased. Thus, the significant decrease in DO can be correlated with itaconic acid accumulation in the broth (Figure 3).

Additionally, this effect is related to the possibility that molecular oxygen may serve as a co-substrate for IA synthesis, representing a key element for the growth and metabolism of aerobic fungi. The final biomass concentration of 3.3 g/L d.w was attained after 142 h of fermentation. Moreover, the DO level of 15% was related to the maximum biomass concentration after 80 h of fermentation. This result also highlights the limitation of IA production due to oxygen supply during fermentation. A critical DO value was observed to be 20%. Therefore, during the IA production phase in *A. terreus* fermentation, the concentration of DO significantly affected the production of IA. Interestingly, the variation in the dissolved oxygen level between 20% and 60% did not significantly impact IA accumulation in the broth. However, a slight increase in IA production was observed at a low level of DO [32]. Also, the literature indicated that a dissolved oxygen concentration of 2% saturation was sufficient for maximal biomass formation. Increasing it to 30% saturation had no effect on IA production [33].

Following the maximum accumulation of itaconic acid in the broth (47 g/L) and with the pH value remaining constant, the fermentation broths were subject to separation. Consequently, the filtered fermentation broths were subsequently utilized for IA separation by reactive extraction.

3.2. Reactive Extraction without Phase Modifier

The initial step was to investigate the effectiveness of reactive extraction without the addition of a phase modifier in the organic phase. The filtered fermentation broths were then brought into contact with the organic phase containing varying concentrations of extractant.

The efficiency of reactive extraction for carboxylic acid is highly influenced by the pH value of the aqueous solutions. Two traditional mechanisms—ion-pair formation and hydrogen bonding—have been mentioned in the literature as responsible for the formation of interfacial hydrophobic complexes [34].

Itaconic acid has two dissociation steps with pKa values of 3.84 and 5.55 at 25 °C [35], which significantly impacts the extraction mechanisms at various pH values of the aqueous phase. The undissociated acid molecule and the aminic extractant form a hydrogen bond only at aqueous solution pH values lower than pKa (below pH 3, with the undissociated form of itaconic acid, H_2I). On the other hand, ion-pair formation can occur independent of the pH value. This is due to proton transfer at pH values below the acid's pKa value or ionic interactions between charged species above the pKa value [34]. In systems without the addition of a phase modifier (as shown in Figure 4), an increase in the aqueous phase's pH value led to a lower value of the itaconic acid's extraction degree. This result is attributed to increased itaconic acid dissociation and weaker interactions between the extractant and dissociated IA. Moreover, organic solvents like n-heptane and dichloromethane are hydrophobic, and dissociated IA is less soluble in these, an aspect that contributes to a lower extraction degree and weaker interaction.

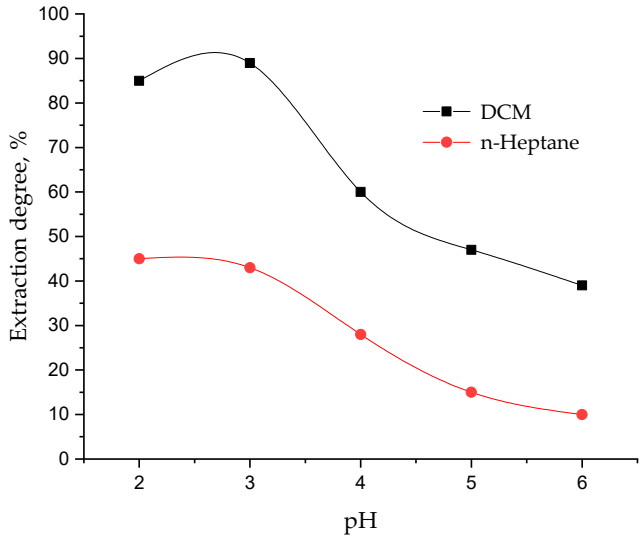

Figure 4. Influence of aqueous phase's pH value on itaconic acid extraction degree (extractant concentration, TOA = 120 g/L).

The pH directly impacted the dissociation behavior of itaconic acid. Thus, for pH values before 4, itaconic acid undergoes partial dissociation into two forms (HI^- and I^{2-}) and the interfacial reaction of the partially ionized acid with TOA influenced the extraction efficiency. For pH values above 4, itaconic acid continues to dissociate but its ability to form intermolecular hydrogen bonds decreases. This effect was more pronounced for solvents with a lower dielectric constant, such as n-heptane. The extraction degree of itaconic acid reached low values and was almost impractical for the alkaline pH domain. In this reactive extraction system with TOA and no addition of 1-octanol, the superior dielectric constant of dichloromethane offers the highest value of itaconic acid's extraction degree (88%), the lowest value being reached for n-heptane (43%). For pH values higher than 5, the extraction degree decreased for the system with dichloromethane and became very low for extraction with n-heptane due to the complete dissociation of itaconic acid at

higher pH values. Therefore, the optimum pH value for the extraction was selected as 3, when only 11% of itaconic acid was dissociated to a single functional group and 89% was in the undissociated form, as calculated by the measured pKa values [36]. This pH value corresponds to the initial stage of itaconic acid formation during fermentation, making it appropriate as a direct extraction method [37].

The increase of amine extractant concentration in the organic phase had a positive impact on the extraction degree due to the increased interfacial amount of one of the reactants. In Figure 5, it becomes evident that the impact of TOA concentration is significant only when it varies below a specific threshold (120 g/L). Beyond this threshold, its effect becomes negligible.

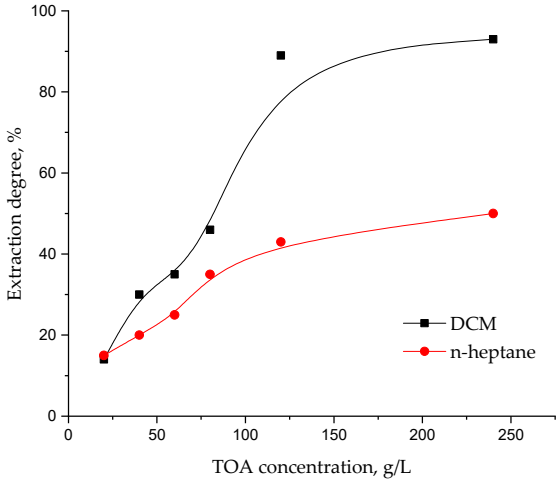

Figure 5. Influence of amine extractant concentration on itaconic acid extraction degree (aqueous phase pH = 3).

The magnitude of this effect was influenced by the polarity of the organic phase. Thus, the amine concentration threshold is 120 g/L for the system with dichloromethane, while for n-heptane, it is 80 g/L. This is the result of the modification of the number of TOA molecules involved in the formation of the complex with itaconic acid.

By using non-polar solvents such as n-heptane, for the separation of carboxylic acid, a stable emulsion and dimer might be formed in the organic phase [11]. To counteract this effect and ensure an increase in the solubility of the formed itaconic acid–TOA complex, a phase modifier, 1-octanol, was added to the organic phase.

3.3. Reactive Extraction with Phase Modifier

The addition of 1-octanol in the organic phase does not change the shapes of the dependence between reactive extraction efficiency and the aqueous phase's pH values. However, the addition of the phase modifier increased the extraction degree, an effect that was more pronounced for n-heptane, the solvent with lower polarity (Figure 6).

At pH 2, the elevated extraction degrees for both solvents primarily result from the increased solubility of the interfacial compound. This effect is further amplified by the additional solubilization of ionized molecules facilitated by 1-octanol. Notably, when 1-octanol was added to n-heptane, it significantly intensified the formation of an amine:acid complex, surpassing the effect observed in dichloromethane. Also, the positive effect of adding 1-octanol on the reactive extraction system with n-heptane was accentuated by using different volume fractions of phase modifier and TOA concentrations (Figure 7).

The 1-octanol addition improved the extraction efficiency regardless of the extractant concentration. This effect was more significant in the n-heptane system due to the supplementary solubilization of itaconic acid dissociated molecules by increasing this solvent polarity. The substantial enhancement in the reactive extraction degree of itaconic acid, achieved by adding 1-octanol in n-heptane, stems from the reduction in the formation of

intermolecular hydrogen bonds between carboxylic groups of the acid. Consequently, this facilitates the reaction of carboxylic groups with TOA, leading to the formation of compounds with greater solubility in the organic phase [9]. The most important increase (67%) was recorded for 20% 1-octanol dissolved in n-heptane at a 120 g/L TOA concentration.

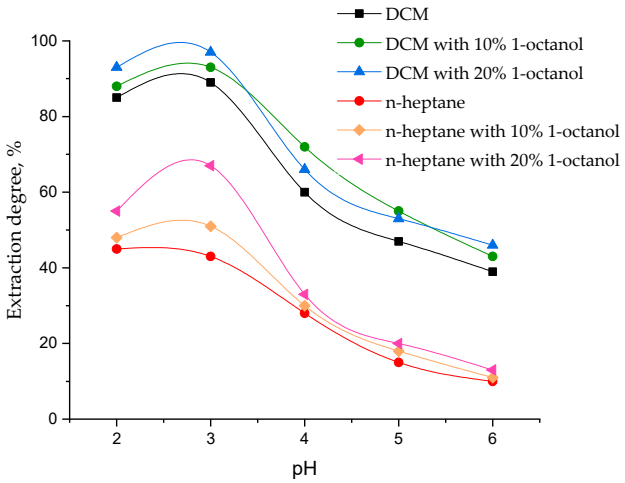

Figure 6. Influence of pH values of the aqueous phase on itaconic acid extraction degree in the presence of a phase modifier (TOA = 120 g/L).

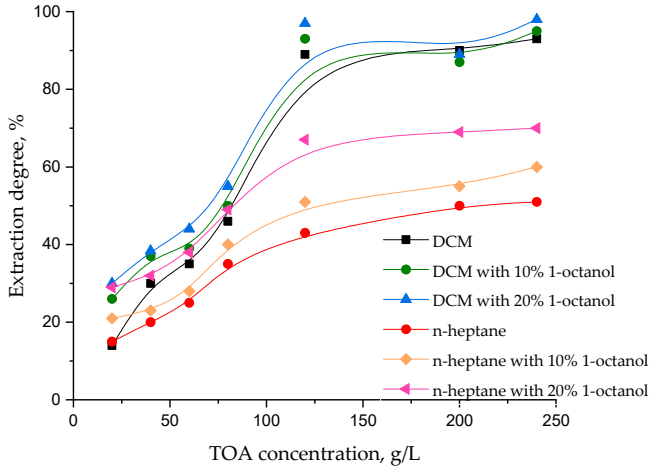

Figure 7. The influence of TOA concentration on the extraction degree of itaconic acid in the presence of phase modifier (aqueous phase's pH = 3).

To underline the beneficial effect of 1-octanol addition, the variation of amplification factor with pH and extractant concentration was graphically represented in Figures 8 and 9 (the amplification factor represents the ratio between the extraction degrees of itaconic acid with and without 1-octanol in the organic phase, respectively, obtained under identical experimental conditions).

Therefore, according to Figures 8 and 9, the increase of 1-octanol concentration determines the increase of the amplification factor. The amplification factor values were higher in the presence of phase modifier, especially for n-heptane, indicating an improved reactive extraction degree for itaconic acid especially in systems with a volumetric 1-octanol fraction of 0.2. At higher 1-octanol concentrations, the increased effect of the amplification factor can be attributed to ester formation at the interfacial reaction between itaconic acid and alcohol. Thus, the reaction rate was positively influenced by the high-phase modifier concentration in the extraction system with low polarity, which considerably improved the solubilization capacity of n-heptane. In the case of using DCM, the presence of 1-octanol in

high concentration also induced the increase of the amplification factor, but the obtained values were lower than in the organic phase with high polarity.

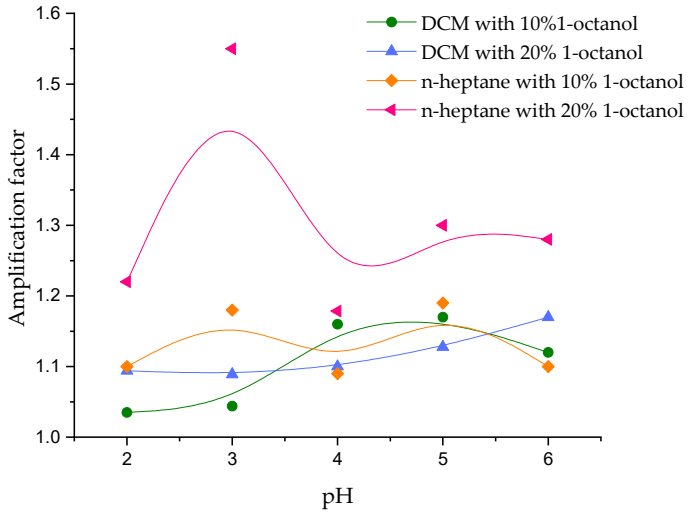

Figure 8. The variation of amplification factor with pH value of aqueous phase (TOA concentration = 120 g/L).

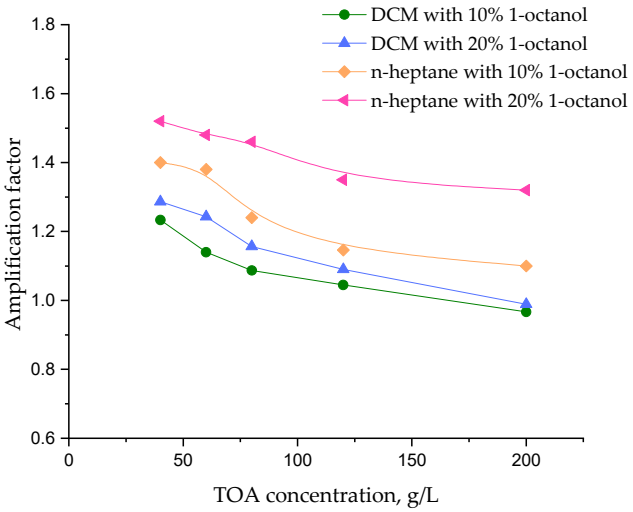

Figure 9. The variation of amplification factor with extractant concentration (pH of aqueous phase = 3).

The extraction of partially or totally dissociated forms of IA is closely related to the pH of the aqueous phase and the polarity of the organic phase. In the system without 1-octanol, the extraction degrees decrease with increasing pH. However, the addition of a phase modifier allows for high extraction degrees at pH values above 4. The maximum value of the amplification factor was reached for pH 5 of clarified broth. Contrarily, the minimum value of the amplification factor at pH = 3 confirmed the enhancement of the positive influence of a phase modifier on IA reactive extraction for solvents with lower polarity.

The graphical representation in Figure 9 underscores the positive impact of a phase modifier—specifically, 1-octanol—on IA reactive extraction. When compared to extraction systems without 1-octanol, the addition of this modifier significantly enhanced the extraction process. At a volumetric fraction of 1-octanol equal to 0.2, this effect was most pronounced. For dichloromethane, the extraction degree of itaconic acid increased by approximately 1.28 times, while for n-heptane, the enhancement was even more substantial, reaching 1.55 times.

Remarkably, regardless of solvent type and alcohol concentration, the highest amplification factor occurs in extraction systems containing 40 g/L TOA. This highlights the critical role of the phase modifier in improving the extraction process, particularly concerning solvent phase polarity. For the analyzed extraction system, the n-heptane solvent phase combined with 40 g/L yields the maximum amplification factor.

4. Conclusions

Considering the current trends in developing efficient separation processes for biobased products, the current study analyzed the possibility of obtaining a biocompatible separation system of itaconic acid from fermentation media. According to the analyzed extraction systems, the experimental conditions led to remarkable results. The highest extraction degree, reaching an impressive 97%, was achieved using TOA dissolved in dichloromethane with 20% 1-octanol. This combination proved exceptionally effective for extracting IA.

An alternative system involving TOA with n-heptane and 20% 1-octanol yielded a lower extraction degree (67%). However, this system has distinct advantages. Particularly, n-heptane exhibits low toxicity to microorganisms, making it suitable for direct IA extraction from fermentation broth.

The positive effect of the phase modifier (1-octanol) was further emphasized by the amplification factor variation with extractant concentration. The maximum amplification factor occurred in the system with 20% 1-octanol and n-heptane at 40 g/L TOA concentration in the solvent phase. Interestingly, the system with DCM required a higher TOA concentration (80 g/L) to achieve similar results.

This study highlights the possibility of IA production through fungal fermentation of *A. terreus* and underscores the importance of optimizing both fermentation and downstream extraction processes. The results of this study offer a viable pathway for in situ reactive extraction using a non-polar solvent and a phase modifier system. Upcoming research will explore the feasibility of combining fermentation and extraction steps for further process improvement. Additionally, scalability, cost-effectiveness, and environmental impact should be investigated to advance IA production and separation processes.

Author Contributions: Conceptualization, A.C.B. and A.T.; data curation, L.K.; formal analysis, A.C.B. and A.T.; investigation, A.T.; methodology, L.K. and A.T.; supervision, A.-I.G.; validation, A.C.B., L.K. and A.T.; visualization, A.C.B. and D.C.; Writing—original draft, A.T.; Writing—review and editing, A.C.B., L.K. and D.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The authors declare no conflicts of interest.

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