

Salting-out assisted solvent extraction of L (+) lactic acid obtained after fermentation of sugarcane bagasse hydrolysate

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Abstract

Lactic acid is among the twelve platform chemicals produced from inexpensive and renewable feedstocks such as lignocellulosic biomass. The present study illustrates salting-out assisted solvent extraction of L (+) lactic acid, derived from sugarcane bagasse hydrolysate. Screening studies with various extractants and diluents revealed that a combination of tri-*n*-butyl phosphate and ethyl acetate yielded the best results and extracted $59.63 \pm 1.28\%$ lactic acid from the dilute fermentation broth adjusted to a pH of 1.6 ± 0.2 . Various inorganic salts were screened to enhance extraction efficiency further. The addition of 60% (w/v) ammonium sulfate improved the lactic acid extraction by 36.17%. This salt concentration successfully extracted $85.95 \pm 0.44\%$ lactic acid to the organic phase under optimized conditions from the fermentation broth at a pH of ~ 2.5 containing 40-100 g L⁻¹ lactic acid. Recovery of >80% salt is also shown using chilled acetone, which upon reuse showed a nominal decline of 3.3% during extraction. Thus an eco-friendly approach using a green solvent like ethyl acetate with mild operating conditions is demonstrated in the present study.

Keywords: Lactic acid; sugarcane bagasse hydrolysate; extraction; ammonium sulphate; ethyl acetate; tri-*n*-butyl phosphate

1. Introduction

The demand for bio-based chemicals has shown an upsurge in recent years. One of the most crucial enablers contributing to their popularity is the low cost and sustainable supply of renewable or lignocellulosic feedstocks for their production. Besides growing awareness and consumer interest in green products, a significant reduction in the carbon footprint, ongoing technological developments, and back up against price volatility of fossil-based feedstock are some added advantages [1]. Based on applications, these bio-based chemicals are broadly divided into ten major categories in which platform chemicals have a substantial share [2]. As per the recent survey by “market research future”, the growth of bio-based platform chemicals is anticipated to expand at a compound annual growth rate (CAGR) of 12.5% by the year 2027 [3]. Among the several lignocellulosic or second-generation platform chemicals, whose technology readiness level (TRL) is eight and above, lactic acid represents one such essential commodity chemical [4]. Chemically also known as 2-hydroxy-propionic acid, this organic acid finds wide applications in diverse areas like food, pharmaceuticals, cosmetics, speciality chemicals, textiles, leather, biodegradable plastics/polymers [5-8].

Fermentative route of lactic acid production is more profitable and attractive as it generates optically pure lactic acid with high productivity and yields and no side product formation [5, 9]. However, different literature studies confirm that the downstream processing of lactic acid from the fermentation broth accounts for nearly 40-70% of the total production cost [6, 8-10]. “Sulfuric acid precipitation” is the most commonly employed technique used to recover lactic acid despite being multi-step and generating a considerable amount of effluent and low-cost calcium sulfate or gypsum, a burden in terms of environmental viewpoint [11, 12]. Other promising alternatives include solvent extraction (liquid-liquid, reactive, aqueous two phase), membrane-based processes (ultrafiltration, nanofiltration, reverse osmosis, electrodialysis), use of ionic liquids, distillation (reactive, vacuum), and adsorption [9, 13, 14]. Typically, solvent extraction is an equilibrium-driven process that relies on partitioning or distributing “product of interest” between the original aqueous and organic phases. It offers numerous advantages like low energy consumption, ease of operation, high selectivity, high extraction capacity of the solvents, ease of recovery and reuse etc. [15, 16]. This process involves using different diluents and extractants. The most commonly used extractants either tertiary or quaternary amine salts or organophosphorus compounds such as tripropylamine, trioctyl amine, triisooctylamine, Alamine 336 (tri-octyl/decyl amine), Aliquat 336 (tri(C₈C₁₀) methylammonium chloride), Tributyl phosphate, phosphine oxides (under the name Cyanex®). Diluents generally fall in two major categories, namely organic, polar but sparingly water-soluble or water-immiscible solvents such as methyl isobutyl ketone (MIBK), chloroform, ethyl acetate and non-polar organic solvents such as alkanes (C₅ and above), long-chain alcohols (C-5 and above), diethyl ether etc. [13,15]. The recent review by Fu et al., [17] showcases the increasing importance of sugaring or salting out in liquid-liquid extraction of bio-based chemicals and fuels obtained after microbial fermentations. They have exclusively discussed mechanism by which salting-out extraction works and how it has gained a status of “new double-layer extraction technology” by altering the surface properties of targetted compounds, electric charge distribution, hydrophobic bonds, ionic bonds and H-bonds [17]. Inspired by the recent outcomes, where salting out has proven to be a driving force for extraction of organic acids in organic phase, in the present study, we attempted to increase the partitioning ratio of lignocellulose derived L (+)

lactic acid in the organic phase by adding inorganic salts. Those salts which are inexpensive and have aqueous solubilities $> 700 \text{ g L}^{-1}$ were chosen. We applied a simple concept that upon ionization in water, these salts would reduce the availability of water molecules for lactic acid, thereby increasing its distribution ratio in the organic phase. Unlike the state of the art that either use model LA solution or simulated fermentation broths, the present study used real-time fermentation broth [18].

It was obtained by fermenting sugar-rich hydrolysates produced during high-solids hydrolysis of alkali pretreated sugarcane bagasse with thermophilic *Bacillus coagulans* NCIM 5648 as described previously [19]. In the first phase, screening various diluent and extractant combinations was done, followed by shortlisting the best salt. Later different parameters like the effect of temperature, agitation, initial pH of the fermentation broth and initial concentration of lactic acid were studied using one variable (OVAT) approach. In this approach, one parameter is varied at a single instance, and the following experiment was conducted by incorporating the optimized parameter.

2. Experimental Section

2.1. Microorganism and *L*(+) lactic acid fermentation

A homofermentative and thermophilic *Bacillus coagulans* NCIM 5648 was obtained from National Collection of Industrial Microorganisms, Pune, India. The culture was routinely maintained on nutrient agar medium and preserved at -20°C as 25% glycerol stocks. The sugar-rich wash ($\geq 55\text{g/L}$ glucose and xylose) and filtrate ($\geq 150\text{g/L}$ glucose and xylose) fractions obtained after fed-batch hydrolysis of 18% alkali pretreated sugarcane bagasse served as carbon source for the seed and fermentation medium respectively, as described previously [19]. A high-density inoculum was developed in modified CM5 medium, by inoculating 3 ml glycerol stock in 50 ml of seed-medium and incubating at 50°C , 180 RPM for 8h (Stackable Orbitek Shaker, Model: LE 4676 H). The cell pellet of the actively growing bacterial cells was obtained by centrifuging at 5752 g for 10 min at 4°C and resuspended in physiological saline. Lactic acid production was initiated by adding 2.5 ml inoculum (equivalent to 70 mg of cells on dry weight basis) to 100 ml of fermentation medium and maintaining pH-stat of 7.0 ± 0.2 , by intermittent addition of 8N NaOH. After 30h, when the production of lactic acid attained saturation, the fermentation was terminated. The cells were harvested by repeating the centrifugation step and the clarified real-time fermentation broth was used for extraction studies.

2.1. Materials

All the diluents, extractants and salts used for the present study were either purchased from Sigma Aldrich or Merck. The concentration of the *L* (+) lactic acid in the real-time fermentation broth was found to be $105.32 \pm 3.85 \text{ g L}^{-1}$. Since the pK_a of “L” form of lactic acid is 3.79, all the studies were conducted below the said value so that it remained mostly in its undissociated form [6]. The pH of the cell-free fermentation broth was adjusted below pK_a using concentrated hydrochloric acid. For the initial studies, the fermentation broth was diluted so that the lactic acid concentration was $20 \pm 2 \text{ g L}^{-1}$ and pH being 1.6 ± 0.2 .

2.2. Screening of extractant

Five different extractants, namely tripropylamine (TPA), trihexylamine (THA), trioctylamine (TOA), triisootylamine (TIOA) and tributylphosphate (TBP), were screened in the said study. The lactic acid extraction efficiency with these extractants was evaluated with and without 1-octanol, used as the diluent. The ratio of

fermentation broth and extractant was 1:1:1 and 1:1 on volume basis, in presence and absence of diluents, respectively. During the extraction step, the conditions were 25°C, 50 rpm and one hour. Once the extraction was done, two phases required effective separation from each other. Therefore, during the settling step agitation step was eliminated and the temperature was reduced to 4°C. After an hour, the upper organic phase was separated from the aqueous phase. Thereafter, the volume of the aqueous phase was measured, and residual lactic acid was quantified using high-performance liquid chromatography (HPLC).

2.3. Screening of diluent with the best extractant

Once the best extractant was selected, different diluents such as chloroform, dodecane, ethyl acetate, MIBK, Hexane and 1-octanol were screened to identify the best extractant-diluent combination which facilitated the lactic acid extraction. All the conditions were identical, as mentioned in section 2.2.

2.4. Evaluating the addition of inorganic salts for enhanced solvent extraction

Four different salts, namely ammonium sulfate, sodium dihydrogen orthophosphate, di-potassium hydrogen orthophosphate and calcium chloride, were used for the present study. The solubility of $(\text{NH}_4)_2\text{SO}_4$, NaH_2PO_4 , K_2HPO_4 , CaCl_2 in the water at 25°C are 767, 949, 1700 and 745 g L⁻¹, respectively. These salts were initially added at a concentration of 10% (w/v) to the diluted fermentation broth. A shift in the pH was noted down after their addition using pH meter (Eutech make 510), reassuring that the final pH of the solution did not exceed 3.5. The inorganic salt, which led to significant improvement in extraction efficiency, was used for all the other experiments at its optimum concentration.

2.5. Effect of physicochemical parameters during the extraction step.

Effect of various physicochemical parameters such as the effect of temperature (25-55°C at an interval of 10°C), duration, initial pH (1.5, 2.0, 2.5, 3.0, 3.5) of the fermentation broth and initial concentration of lactic acid (20, 40, 60, 80 and 100 g L⁻¹) was studied using OVAT approach. Temperature studies were necessary as the solubility of the salts increased with temperature. The initial pH of the fermentation broth was crucial to identify its effect on the partitioning of lactic acid. It was essential to understand whether the extraction ability of extractant-diluent combination was dependent or independent of initial lactic acid concentrations. Hence extraction was carried out at varying concentrations of initial lactic acid.

2.6. Quantification of LA and measurement of extraction efficiency

The lactic acid in the aqueous phase was quantified by making appropriate dilution in MilliQ water and filtering it through 0.22 µm syringe filters before injecting in the HPLC. A Shimadzu make HPLC system was used equipped with Aminex HPX-87H (7.8 × 300 mm) coupled with refractive index detector (RID-10A; Shimadzu Corporation, Japan) using 5 mM H₂SO₄ as mobile phase at a flow rate of 0.55 mL/min. The lactic acid extraction efficiency was calculated as described by Uslu et al. [20]:

$$\text{Extraction Efficiency (\%)} = ((1 - (\text{Residual}_{\text{LA}}/\text{Initial}_{\text{LA}})) * 100$$

Where the $\text{Residual}_{\text{LA}}$ represents the residual lactic acid concentration in the aqueous phase after solvent extraction and $\text{Initial}_{\text{LA}}$ represents the initial lactic acid concentration in the aqueous phase before solvent extraction.

2.7. Fourier-transform infra-red (FTIR) spectroscopy

The shortlisted inorganic salt was later recovered using chilled acetone. FTIR spectroscopic analysis of the standard samples and the recovered salt was carried out to assure that both the compounds bear similar chemical structure. The data was acquired in the mid IR region i.e., 4000-400 cm^{-1} . FTIR spectrometer equipped with deuterated triglycine sulfate (DTGS) detector (Perkin Elmer Spectrum Two) was used. Samples for FTIR analysis were ground with moisture free KBr and then KBr disc was formed by using hydraulic press. Data acquisition was performed with 4 cm^{-1} resolution by fixing eight scans in every analysis.

3. Results and discussion

3.1. Screening of extractant

Table 1 summarizes the lactic acid extraction efficiency during the screening of various extractants with and without the addition of 1-octanol as diluent. A significant improvement in extraction efficiency was noticed when 1-octanol was used as diluent and TOA and TBP as extractants. TBP emerged as the best extractant for lactic acid recovery. However, TPA without a diluent failed to extract any lactic acid from the aqueous phase among all the alkylamines. Without the use of diluent, the extraction efficiency was $\text{TBP} > \text{TIOA} > \text{THA} > \text{TOA}$. However, when 1-octanol was added to the fermentation broth, the extraction efficiencies were in the $\text{TBP} > \text{THA} = \text{TIOA} > \text{TOA} > \text{TPA}$.

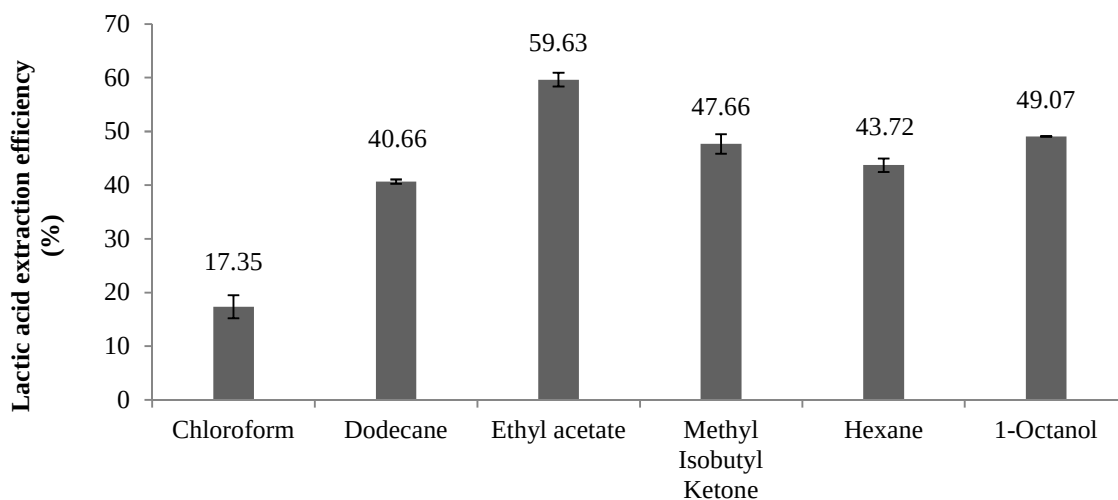
Table 1: Effect of various extractants with and without diluent addition on the extraction efficiency of lignocellulose-derived lactic acid diluted to $20 \pm 2 \text{ g L}^{-1}$

Extractants Used	Extraction Efficiency (%)		Process improvement (%) by adding 1-octanol
	Without Diluent	With Diluent	
TPA	0	4.27 ± 0.09	-NA-
THA	21.86 ± 0.03	29.47 ± 0.09	34.8
TOA	17.75 ± 0.10	26.78 ± 0.06	50.64
TIOA	27.49 ± 0.10	28.77 ± 0.04	4.77
TBP	32.65 ± 0.01	49.07 ± 0.01	50.29

Generally, all the alkylamines interact with lactic acid via ionic interactions. In such case, lactic acid in its fully ionized form and the relative basicity of the alkylamines can further enhance these interactions besides solvation and ion-pair stability [20]. When no diluent was added, the relative basicity of TPA was not enough to form a stable ion-pair, unlike long-chain alkylamines (C6 and above). However, when 1-octanol was added as a diluent, it facilitated higher solvation of all the extractants, thereby promoting the improved distribution of lactic acid in the organic phase [22]. Datta et al. [15], in their recent review, have confirmed that unlike alkylamines, TBP forms a coordinate bond with the carboxylic acid, including lactic acid. Since the bond strength of the coordinate bond is higher than ionic interactions and TBP is a strong Lewis base [23], they collectively contributed towards its high extraction ability in the absence of diluent. It was apparent that TBP should give better results than alkylamines and our results were congruent with the hypothesis. Moreover, when 1-octanol was added, owing to its inherent characteristic to form hydrogen bonds with Lewis base, it improved the extraction efficiency of TBP by favouring the complex to move in the organic phase.

3.2. Screening of diluent with the best extractant

When different diluents, including polar, non-polar, polar aprotic solvents, were tested to decipher the best counterpart for TBP, ethyl acetate emerged as the best organic solvent, as shown in Figure 1.



Types of Diluents used during L (+) lactic acid extraction from fermentation broth

Figure1: Screening of various diluents for enhanced extraction of lignocellulose-derived lactic acid using TBP as an extractant.

Ethyl acetate, a polar but aprotic solvent, emerged as the best diluent. Its lactic acid extraction efficiency combined with TBP (mixed in ratio of 1:1) was $59.63 \pm 1.28\%$. The order of diluents which showed high compatibility with TBP as extractant was ethyl acetate > 1-octanol > MIBK > hexane > dodecane > chloroform. The exceptionally high performance of the ethyl acetate may be attributed to high relatively solubility of lactic acid in the said solvent (60g/100 ml at 20°C) as reported earlier [24]. Even Hu et al. [25] claimed 33.3% lactic acid extraction yields with ethyl acetate during ultrasonic-mediated solvent extraction. Ethyl acetate, in combination with TBP, likely facilitated better extraction of lactic acid.

Thus at the end of this experimental study, we were able to find the right combination of extractant and diluent. However, it was essential to improvise the LA extraction efficiency further. Therefore, cheap and highly water-soluble inorganic salts were looked upon, which can synergistically enhance the distribution of lactic acid in the ethyl acetate phase by reducing water availability for its dissolution.

3.3. Evaluating the addition of inorganic salts for enhanced solvent extraction

The following results were obtained when different salts were evaluated for phasing out lactic acid into the organic phase, as shown in Table 2.

Table 2: Screening of inorganic salts (10% w/v) on the extraction efficiency of lignocellulose-derived lactic acid diluted to $20 \pm 2 \text{ g L}^{-1}$ with TBP and ethyl acetate as extractant and diluent, respectively

Type of salt	pH shift	Extraction Efficiency (%)	Improvement in extraction efficiency (%)
No Salt	1.64	58.8 ± 1.0	-
$(\text{NH}_4)_2\text{SO}_4$	1.64 $\rightarrow$ 1.90	63.6 ± 0.1	8.14
CaCl_2	1.64 $\rightarrow$ 1.65	62.4 ± 0.6	6.1
NaH_2PO_4	1.64 $\rightarrow$ 1.90	62.7 ± 1.2	6.6
K_2HPO_4	1.64 $\rightarrow$ 2.25	65.2 ± 1.4	11.0

An addition of 10% K_2HPO_4 enhanced LA's extraction efficiency by 11%, followed by $(\text{NH}_4)_2\text{SO}_4$. However, owing to mildly alkaline nature, there was a significant shift in the pH of the fermentation broth. Calcium chloride did not alter the pH of the fermentation broth, and for the rest of the salts, the pH remained below 2.0. Despite being the best inorganic salt, K_2HPO_4 could not be pursued further at its higher concentration; it crossed the pKa value of lactic acid, which is 3.86 and drifted the pH of the fermentation broth towards neutrality. Hence, ammonium sulfate was selected for further studies, and its different concentrations were added to evaluate its maximum extraction efficiency. Following results were obtained as observed in Figure 2.

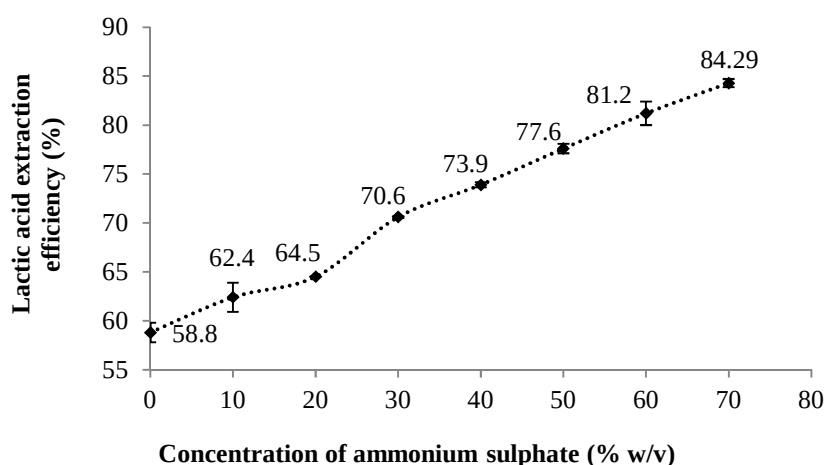


Figure 2: Effect of increasing concentration of ammonium sulfate on the extraction efficiency of lignocellulose derived lactic acid diluted to $20 \pm 2 \text{ g L}^{-1}$ with TBP and ethyl acetate as extractant and diluent, respectively.

With increasing concentration of ammonium sulfate, the extraction efficiency improved significantly. However, at 70% salt concentration, a fraction of ammonium sulfate could not dissolve in the fermentation broth and settled as crystals at 25°C , exceeding the limit of salt saturation. Therefore, 60% (w/v) of salt was chosen for further studies. Thus, using the right combination of extractant, diluents and salt concentration, we were able to phase out the lactic acid in the organic fraction by $>80\%$. During these studies, the maximum pH drift did not exceed one unit. Interestingly, $>80\%$ salt could be recovered for the next round of precipitation, when ten volumes of chilled acetone were added to the aqueous fraction. When the extraction was performed using the recovered ammonium sulfate, the

extraction efficiency reduced nominally by 3.3% ($81.6 \pm 0.26\% \rightarrow 78.91 \pm 0.26\%$). The plausible explanation for lower extraction could be the purity of the $(\text{NH}_4)_2\text{SO}_4$, as the chances of other salts present in the fermentation broth getting co-precipitated are likely. Later, the recovered salt was subjected to FTIR spectroscopic analysis to compare it with the pure ammonium sulphate. Figure 3 shows the FT-IR spectra was pure and recovered ammonium sulphate in which the X and Y axis represents the wavenumber (cm^{-1}) and transmittance (%T) respectively .

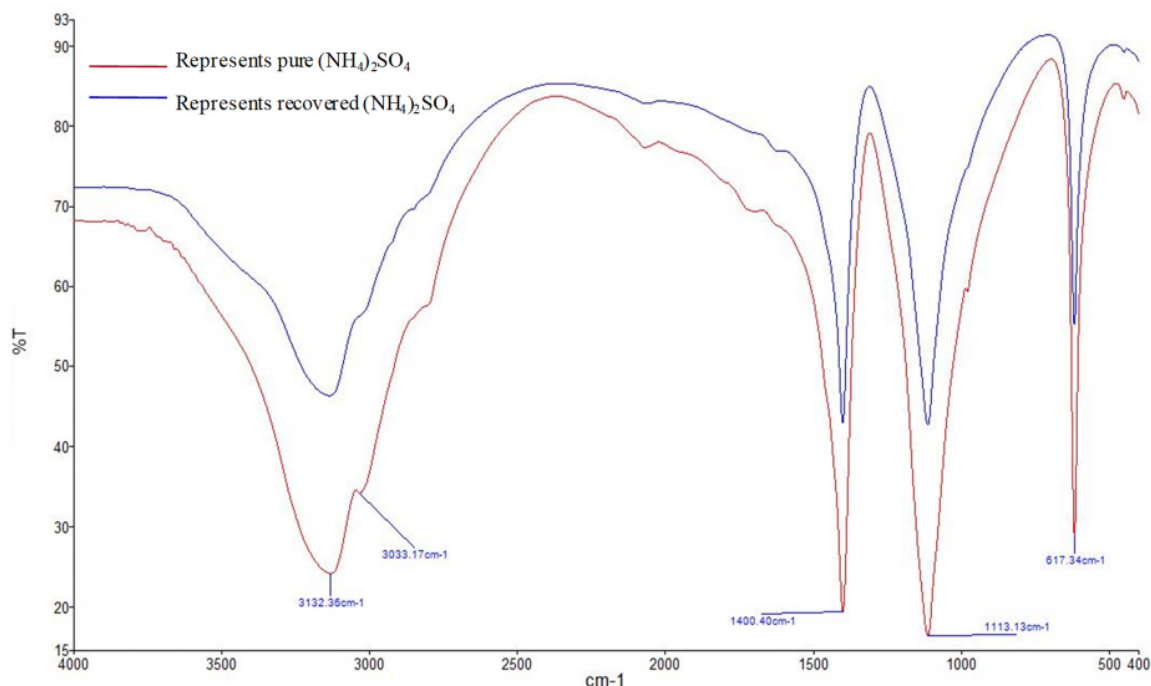


Figure 3: FT-IR Spectra of the pure and recovered ammonium sulfate

As observed in figure 3, the obtained spectra of both the samples appeared similar. A broad and strong vibration in the region of 3121-3033 cm^{-1} may be assigned for N-H stretching vibration of ammonium ion whereas a peak at 1400 cm^{-1} corresponds to N-H bending vibration. S=O bond of the sulphate ion showed its signature peak at 1113 cm^{-1} in the spectra while vibration signature at 617 cm^{-1} corresponded to S-O bond. On the basis of the above observation, it was concluded that the recovered salt was chemically identical to the pure and standard ammonium sulphate salt.

The high dielectric constant of acetone ($\kappa=77$) probably engaged the water molecules, reducing its affinity for ammonium sulfate/other salts and leading to precipitation. The acetone from the aqueous fraction was quickly recovered back using a rotary evaporator assembly.

3.4. Effect of physicochemical parameters during the extraction phase.

The effect of temperature and time during the extraction step led to no significant improvement in the extraction efficiency of lactic acid. When temperature studies were conducted to decipher the impact of increasing solubility of ammonium sulfate on lactic acid recovery, we found increasing temperature from 25°C to 55°C reduced lactic acid

recovery by ~8% (Table S1). The experiment suggested that a temperature between 5-25°C was conducive for maximum extraction, and since 25°C represented room temperature, it was selected for further experiments.

Even when the duration of the extraction step was increased from one hour to three hours, the improvement in extraction efficiency was insignificant. When the effect of pH was studied on extraction efficiency following results were obtained, as shown in Table 3.

Table 3: Effect of initial pH of the fermentation broth on the extraction efficiency of lignocellulose-derived lactic acid during salt aided (60% ammonium sulphate) extraction with TBP and ethyl acetate

Initial pH of the medium	pH shift	Extraction Efficiency (%)
1.64	1.64 → 2.45	81.22 ± 1.2
2.06	2.06 → 3.12	75.7 ± 1.15
2.54	2.54 → 3.91	74.29 ± 1.11
3.05	3.05 → 4.17	75.63 ± 0.18

Earlier studies of Kumar et al. [26] also claim that a pH of 2.5 was best for the recovery of lactic acid when DeMan, Rogosa, and Sharpe (MRS) broth the fermented using *Lactobacillus pentosus* SKL-18. Addition of 60% (w/v) ammonium sulfate at even an initial pH of 2.06 shifted the final pH above 3. As a result, the extraction efficiency reduced even when the initial pH of the medium was 2.06.

A study by increasing the volume of diluent and changing the ratio of TBP: ethyl acetate had no stimulatory effect on the extraction efficiency of lactic acid. In the last part of the study, when the impact of varying lactic acid concentration was studied on extraction efficiency following results were obtained, as shown in Table 4.

Table 4: Effect of lactic acid concentration in fermentation broth on its extraction [Conditions: fermentation broth: TBP: Ethyl acetate → 1:1:1; Initial pH of the broth- 1.6 ± 0.2; Ammonium sulfate: 60% (w/v); Extraction step: 25°C for 1h; Settling step: 4°C for 1h]

Initial Concentration of Lactic acid (g L ⁻¹)	The residual lactic acid in aqueous fraction (g L ⁻¹)	Extraction Efficiency (%)
22.6 ± 0.45	4.24 ± 0.27	81.22 ± 1.2
39.6 ± 1.07	5.64 ± 0.43	85.76 ± 1.09
60.0 ± 1.45	8.50 ± 0.05	85.84 ± 0.11
82.7 ± 2.05	11.1 ± 0.57	86.59 ± 0.71
98.4 ± 2.21	14.2 ± 0.17	85.61 ± 0.17

The TBP and ethyl acetate extraction efficiency in the presence of 60% (w/v) ammonium sulfate was independent of lactic acid concentration present in the fermentation broth. The average extraction efficiency with the optimized protocol was 85.95 ± 0.44% with initial lactic acid concentration in the fermentation broth varying between 40 to 100 g L⁻¹. Following schematic gives a complete process description of lactic acid extraction after optimization through OVAT approach.

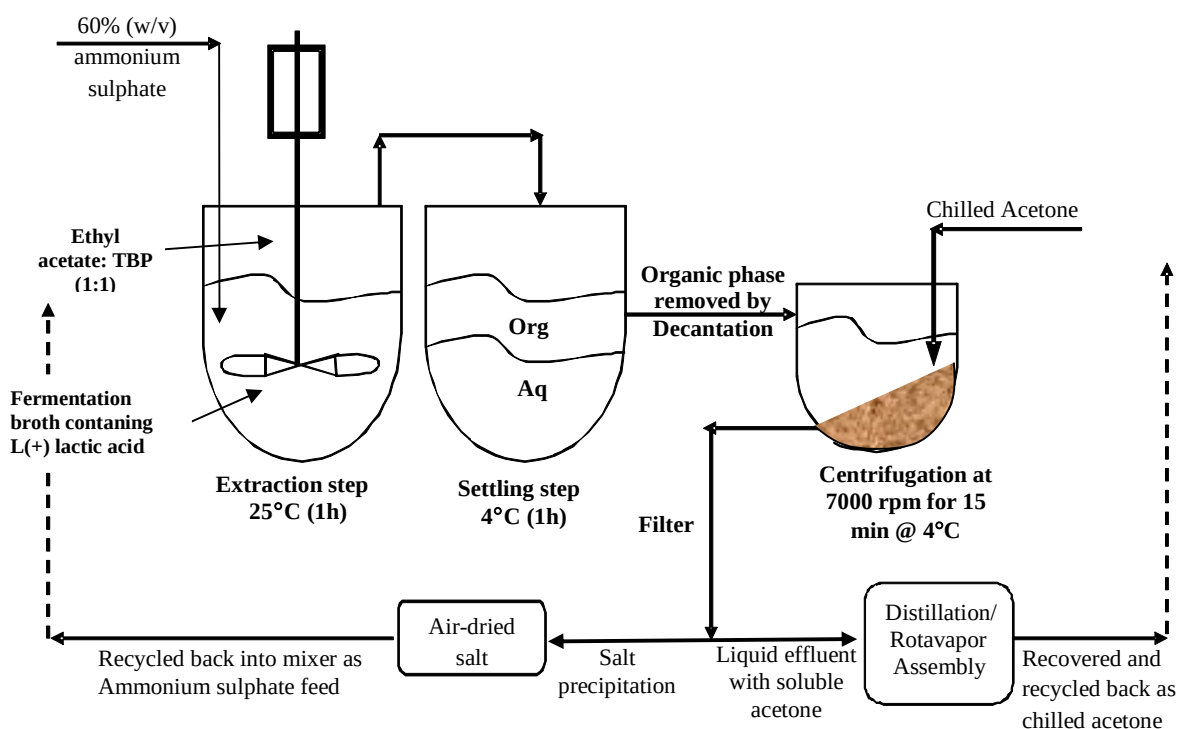


Figure 4: Extraction of lignocellulose-derived L (+) lactic acid by coupling ammonium sulfate (60% w/v) and liquid-liquid extraction with TBP and ethyl acetate as extractant and diluents, respectively.

Salting-out assisted liquid-liquid extraction (SALLE) is a relatively new technique in which water-miscible solvents like acetonitrile are used to recover biological molecules. Later, salt is added to phase out the material of intent in the organic phase [27]. Earlier, similar to this technique, an aqueous two phase extraction (ATPS) of lactic acid was successfully demonstrated by Aydoğan et al. They attained 80% extraction yield from fermentation broth rich in lactic acid (70g/L), using a combination of ethanol (30.23% w/w) and dipotassium phosphate (18.4% w/w) optimized through response surface methodology [28]. Similar to SALLE, sugaring out carboxylic acid and use of ionic liquids is an emerging trend. Recently, 12% (w/w) glucose was used for sugaring out lactic acid in 40% (w/w) isopropanol, leading to its 84.27% recovery [29]. An eco-friendly extraction method for lactic acid has been shown in two separate studies wherein two ionic liquids namely phosphonium ionic liquids and 3-alkyl-1-methylimidazolium saccharinates were used [30,31]. Particularly, in the latter study, use of magnesium sulphate alongwith imidazolium saccharinate below the pK_a of lactic acid, led to its 81% recovery [30].

However, the present study was conducted with ethyl acetate as an organic solvent whose water miscibility is merely 8.3% at 20°C. To the best of our knowledge, this is the first report where salting-out phenomenon exhibited by ammonium sulfate has been successfully demonstrated to enhance the extraction efficiency of lignocellulose derived lactic acid in TBP and ethyl acetate system. In future studies, we would focus on recovering L (+) lactic acid from the organic phase containing ethyl acetate and TBP. We would also evaluate different methods like ion-

exchange and nano-filtration with the developed method at a later stage. We would decipher the techno-economic associated with these downstream processing methods of lactic acid.

4. Conclusion

This short communication successfully demonstrates the ability of ammonium sulfate to improvise lactic acid extraction efficiency of TBP-ethyl acetate system. When 60% (w/v) of this salt was added to fermentation broth containing 40-100 g L⁻¹ of lactic acid derived from sugarcane bagasse hydrolysates, 85.95 ± 0.44% lactic acid was extracted in the organic phase (Ethyl acetate: TBP → 1:1) at 25°C and pH 2.5. The ability to recover the salt using chilled acetone and its subsequent reuse is also proven.

Acknowledgements

We acknowledge the Department of Biotechnology (DBT, India), BBSRC and Innovate UK under the Indo-UK Industrial Waste Challenge 2017 project for their financial support, with grant number being GAP 3513. The authors are thankful to Dr Anjan Ray, Director CSIR-Indian Institute of Petroleum, for his valuable inputs and guidance.

Declaration of competing interests

The authors declare that they have no known competing financial or personal interests that can influence the investigation reported in this study.

CRedit author statement

Pratibha Baral: Investigation, Methodology, Writing - Original Draft; **Anushka Pundir:** Investigation, Methodology and Visualization; **Akhilesh Kumar Kurmi:** Validation and Supervision; **Raghuvir Singh:** Investigation, Methodology; **Vinod Kumar:** Data Curation and Reviewing and Editing; **Deepthi Agrawal:** Funding acquisition, Conceptualization, Supervision, Validation and Writing- Reviewing and Editing

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Table S1: Effect of temperature during extraction of lignocellulose-derived lactic acid using TBP and ethyl acetate

Temperature (°C)	Extraction Efficiency (%)
5	80.76 ± 0.63
15	81.10 ± 1.40
25	81.22 ± 1.20
35	76.89 ± 0.33
45	76.95 ± 0.31
55	74.78 ± 0.13

Highlights

- Salting-out assisted solvent extraction performed for 2G lactic acid (LA)
- Tri-*n*-butyl phosphate and ethyl acetate emerged as best combination
- 60% (w/v) ammonium sulfate enhanced lactic acid extraction significantly
- >85% extraction achieved from fermentation broth containing 40-100 g/L LA at pH 2.5
- Ammonium sulfate recovered by chilled acetone and reused in 2nd round of extraction



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Salting-out assisted solvent extraction of L (+) lactic acid obtained after fermentation of sugarcane bagasse hydrolysate

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2021-04-18

Elsevier

<https://doi.org/10.1016/j.seppur.2021.118788>

Baral P, Pundi A, Kumar A, et al., (2021) Salting-out assisted solvent extraction of L (+) lactic acid obtained after fermentation of sugarcane bagasse hydrolysate. Separation and Purification Technology, Volume 269, August 2021, Article number 118788