



HAL
open science

New insights in reactive extraction mechanisms of organic acids: An experimental approach for 3-hydroxypropionic acid extraction with tri-n-octylamine

Florian Chemarin, Marwen Moussa, Morad Chadni, Brigitte Pollet, Pascale Lieben, Florent Allais, Ioan-Cristian Trelea, Violaine Athes-Dutour

► To cite this version:

Florian Chemarin, Marwen Moussa, Morad Chadni, Brigitte Pollet, Pascale Lieben, et al.. New insights in reactive extraction mechanisms of organic acids: An experimental approach for 3-hydroxypropionic acid extraction with tri-n-octylamine. *Separation and Purification Technology*, 2017, 179, pp.523-532. 10.1016/j.seppur.2017.02.018 . hal-01530851

HAL Id: hal-01530851

<https://hal.science/hal-01530851v1>

Submitted on 4 Jul 2017

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - ShareAlike 4.0 International License

1 **New insights in reactive extraction mechanisms of organic acids: an experimental approach for 3-**
2 **hydroxypropionic acid extraction with tri-*n*-octylamine**

3 F. Chemarin^{a,b}, M. Moussa^b, M. Chadni^b, B. Pollet^b, P. Lieben^b, F. Allais^{a,b}, I.C. Trelea^b, V. Athès^{b*}

4 ^a Chaire Agro-Biotechnologies Industrielles (ABI), AgroParisTech, CEBB, 3 rue des Rouges Terres,
5 Route de Bazancourt, F-51110 Pomacle, France

6 ^b UMR GMPA, AgroParisTech, INRA, Université Paris-Saclay, F-78850, Thiverval-Grignon, France

7 * Corresponding author: UMR GMPA, AgroParisTech, INRA, Université Paris-Saclay, F-78850,
8 Thiverval-Grignon, France. E-mail address: violaine.athes-dutour@inra.fr

9 Abstract

10 A detailed study of 3-hydroxypropionic acid (3-HP) reactive extraction with tri-*n*-octylamine (TOA) is
11 proposed for the first time. It aims at uncovering some solvent-solutes interactions and providing
12 global mechanisms to better understand and design the reactive liquid-liquid extraction of 3-HP in a
13 biotechnological process. Eleven solvents of similar molecular sizes and several chemical types
14 (alcohols, esters and alkanes) were investigated to understand their role on the extraction ability.
15 Alcohols were found to be the best solvents thanks to their H-bond donor characteristic and water
16 loading that allowed good solvation of the acid-amine complexes. Further investigations were then
17 undertaken, for *n*-decanol and oleyl alcohol as solvents, varying acid (0.0028 – 0.56 mol/L
18 corresponding to 0.25 – 50 g/L) and amine (0 – 2.3 mol/L corresponding to 0 – 100% v/v)
19 concentrations. At 0.011 mol/L (1 g/L) of 3-HP, maximum extraction yields of 77% for *n*-decanol and
20 51% for oleyl alcohol were found for 0.46 mol/L TOA (20% v/v). The initial TOA purity proved to have
21 a major impact on the extraction yield at low initial acid concentration (<0.1 mol/L = 10 g/L).
22 Impurities from the TOA manufacturing process were identified as *n*-octylamine and di-*n*-octylamine
23 and quantified in the aqueous phase after extraction. Their major effect on the extraction yield has
24 been assessed (up to 86% decrease).

1 Keywords

2 Solvent extraction; organic acid; active solvent; tri-*n*-octylamine impurity; *In Situ* Product Recovery;

- 1 Abbreviations
- 2 3-HP: 3-hydroxypropionic acid
- 3 AH: acid under non-dissociated form
- 4 $[AH]_{HPLC}$: total concentration of 3-HP under all forms in water as determined using HPLC (mol/L)
- 5 ANOVA: analysis of variance
- 6 B: organic base
- 7 BH^+ : conjugate acid of B
- 8 H^+ : hydrogen ion
- 9 K_A^{AH} : dissociation constant of 3-HP in water
- 10 K_A^{OA} : dissociation constant of *n*-octylammonium in water
- 11 K_w : ionic product of water
- 12 $[OA]_{LCMS}$: total concentration of *n*-octylamine (and di-*n*-octylamine) in water under all forms as
- 13 determined using UHPLC-HRMS (mol/L)
- 14 p: stoichiometric coefficient
- 15 \bar{p} : p-value of ANOVA tests
- 16 pH_{th} : calculated pH according to $[AH]_{HPLC}$ only
- 17 $pH_{th,OA}$: calculated pH according to $[AH]_{HPLC}$ and $[OA]_{LCMS}$
- 18 q: stoichiometric coefficient
- 19 R_3N : tertiary amine
- 20 TOA: tri-*n*-octylamine
- 21 Y: extraction yield
- 22 *Subscript and superscript:*
- 23 aq: in the aqueous phase
- 24 eq: at the equilibrium
- 25 ini: initially, before extraction
- 26 org: in the organic phase

1. Introduction

It is now recognized that 3-hydroxypropionic acid (3-HP) is a valuable platform molecule that can be used as a building-block for the production of new polymers like polyesters and acrylic acid derivatives such as acrylates or acrylamides [1]. This molecule is currently not widely used due to its exclusive and difficult synthesis via chemical pathways including transformation of petrochemical products such as propylene but is expected to exhibit a significant growth in the next years. Indeed, it has been referred as one of the Top 10 “chemical opportunities from biorefinery carbohydrates” [2]. Lately, research has focused on the production of 3-HP using biotechnologies with natural [3,4] and modified [5,6] micro-organisms able to convert glycerol or glucose into 3-HP [7]. Other substrates like CO₂[8,9] and C5 sugars [10,11] are also currently studied. Biotechnology is considered as a promising route for the sustainable and efficient production of 3HP but one of the main drawbacks of this approach is the inhibitory effect the products can have on the micro-organisms, limiting productivity and final concentrations to quite low amounts, which is particularly true for the 3-HP production [7]. In this context, *in-situ* product recovery (ISPR) seems to be a promising approach because the simultaneous removal of the product from the bioconversion medium could favor higher yields, productivity and concentrations. Liquid-liquid reactive extraction for organic acids' recovery is known to be particularly efficient and selective for dilute media as an ISPR technique in biotechnological processes and can prevent toxic metabolites and/or products from accumulating [12,13].

Current concentrations of 3-HP in bioconversion media for the best current biotechnological performances found in the literature do not exceed about 70 g/L [6,14]. For short chain carboxylic acids in dilute streams such as 3-HP, their hydrophilic character prevents their direct recovery in an organic solvent and a reagent is needed in the organic phase to trap the compound in a rather hydrophobic complex. In the case of hydrophilic carboxylic acids, these reagents can be hydrophobic basic molecules being able to interact with acidic groups [15]. Among such molecules, long chained aliphatic amines have been widely studied for a large set of carboxylic acids, such as

1 hydroxycarboxylic acids like citric [16–18], glycolic [19,20], malic [21,22] and tartaric [23–26] acids,
2 C3 monocarboxylic acids like acrylic [27], propionic [28–31] and pyruvic [32–34] acids and even lactic
3 acid, an α -hydroxylated C3 monocarboxylic acid (positional isomer of 3-HP) [22,23,35].

4 Amine basicity, steric hindrance and water solubility are relevant parameters and have an influence
5 on extraction performances. For example, Kyuchoukov and Yankov [36] tested several amines for the
6 extraction of lactic acid and their results show that, when amines are used in oleyl alcohol, extraction
7 yields decrease when the steric hindrance of the amines increases. The contrary is observed when
8 amines are used without oleyl alcohol as the active diluent. Kaur and Elst [37] studied a wide range of
9 amines and diluents for the extraction of itaconic acid. For all the diluents tested, they found that
10 short chain amines (tributylamine, *N,N*-dimethyloctylamine) and primary amines (*n*-octylamine) had
11 the lowest performances. On the contrary, long chain secondary and tertiary amines proved to be
12 very efficient with very high extraction yields, probably due to their higher basicity and very low
13 solubility in water. Secondary amines (di-*n*-octylamine) had better performances than tertiary
14 amines (tri-*n*-octylamine) [37], and this could be explained by the lesser extent of the steric
15 hindrance around the reactive amine group, their higher basicity and higher density of amine groups.
16 Similar results were found in a previous study which also demonstrated that primary amines were
17 not to be used in reactive extraction due to moderate extraction efficiency, high water solubility and
18 high interfacial activity causing emulsion [38].

19 The structure of the aliphatic amines is crucial for their role as extractants, but they cannot be used
20 alone, due to their poor solvation ability and sometimes high viscosity [39]. Consequently, they are
21 often used in mixture with organic solvents to increase their extraction performances. Diluents can
22 be divided into two types: active and inert. Active diluents have chemically active functional groups
23 such as hydroxyl or carbonyl groups that are able to interact with the amphiphilic acid-base complex
24 through hydrogen bonds and dipole-dipole interactions [15,23]. Inert diluents such as hydrocarbons
25 that cannot interact with diluted molecules show lower efficiency since they are not able to stabilize

1 hydrophilic compounds in the organic phase. Solvent polarity decrease, *e.g.* when the length of the
2 alkyl chain increases, tends to reduce the extraction efficiency and so does the steric hindrance
3 around the active group. For example, secondary alcohols have lower solvation capacity than their
4 primary isomers [37,40].

5 Apart from the effects of amines and diluents' characteristics, the acid hydrophobicity [19] and
6 acidity [39] are key factors to evaluate the degree of extraction: the more hydrophobic and acidic the
7 extracted acid, the higher the extraction yield [41]. Differences in extraction yields even exist when
8 comparing very similar acids like the positional isomers 3-hydroxypropionic acid and 2-
9 hydroxypropionic acid (lactic acid) [42]. Moreover, hydrophilic carboxylic acids being polar, they tend
10 to bind together in apolar environments such as organic phases. It is known for example that they
11 can form dimers, trimers, and so on [23,39]. This phenomenon is less likely to occur in more polar
12 active diluents. In particular, protic diluents like alcohols or chloroform are known to prevent such
13 phenomena because of their H-bond donor ability. The competition between the acid and the diluent
14 to form H-bonds with free or complexed acid molecules tends to favor acid extraction through free
15 and complexed acid monomers [39]. All the characteristics of the compounds found in these systems
16 play an important role on the mechanisms of extraction.

17 Considering all these aspects, should be preferred for 3-HP extraction a water insoluble amine (high
18 molecular weight) providing a high density of non-hindered reactive amine groups (low molecular
19 weight). The optimum stays in the tertiary C8-C10 amines range [43]. Therefore, we selected tri-*n*-
20 octylamine (TOA) to perform this study and investigated several diluents according to their
21 physicochemical properties.

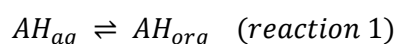
22 Since the purpose of an ISPR strategy is to keep the concentration of a product low to limit inhibition,
23 in this study, 3-HP concentration will cover a rather dilute range of 0.0028 to 0.56 mol/L (0.25 to 50
24 g/L). The study focuses on phenomena occurring at low concentrations and related mechanisms such
25 as the presence of impurities coming from the extractant phase.

1 2. Materials and methods

2 2.1. Theory

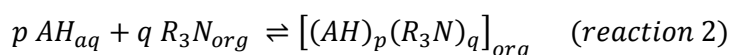
3 2.1.1. Physical extraction

4 Reaction 1 describes the acid partitioning between the aqueous and organic phases:



5 2.1.2. Reactive extraction

6 The formation of acid-amine complexes is the major reaction involved in acid recovery from aqueous
7 phase. In an aqueous phase, acids and bases can react together through reversible reactions to give
8 the corresponding solvated free conjugated bases and acids. In an organic phase, the weak polarity
9 and dielectric constant can prevent species from forming the corresponding solvated free ions and
10 the acidic and basic molecules can stay bound to each other through acid-base interactions. These
11 interactions can consist in H-bondings and/or ion pair formation according to the polarity of the
12 solvent and the strength of the acid-base interactions. As a general scheme, several acid and amine
13 molecules can form aggregates of different stoichiometries [23]. The acid-amine complex formation
14 can be described by the reaction 2:



15 2.2. Chemicals

16

17 2.2.1. Commercial products

18 Solutions of 3-hydroxypropionic acid were prepared by diluting a commercial product (28.1%wt
19 solution in water) from TCI Europe, Zwijndrecht, Belgium. The organic phase consisted in tri-*n*-
20 octylamine (TOA, 98% purity) diluted either in *n*-hexanol (99% purity), *n*-octanol (99% purity), *n*-
21 decanol (99% purity), *n*-dodecanol (98% purity), ethyl octanoate (98% purity), butyl hexanoate (98%
22 purity), *n*-hexane (99% purity), *n*-decane (99% purity), *n*-dodecane (99% purity) from Sigma-aldrich,

1 Saint-Louis, the USA, oleyl alcohol (82.4% purity, with impurities consisting mainly in other fatty
2 alcohols like linoleyl and stearyl alcohols [44,45]) from TCI Europe or refined sunflower oil (mainly
3 triglycerides of oleic and linoleic acids, Lesieur, France).

4 In order to further investigate the influence of TOA purity on extraction efficiency, three different
5 organic phases have been made with three different TOA purities. Two TOA solutions were
6 purchased from the same commercial reference but had two lot numbers with different purity levels
7 corresponding to the variations of production quality: 98.1%, solution 1, and 99.6%, solution 2,
8 according to the certificates of analysis given by the supplier (Sigma-Aldrich). Solution 3 was obtained
9 by purification of solution 2 as detailed below.

10 2.2.2. TOA purification

11 Purified TOA (solution 3) was produced as follows. The 99.6% TOA was washed using same volumes
12 of H_2SO_4 0.1 M. After centrifugation, 3 homogeneous phases were observed. Only the top phase was
13 recovered and further washed with NaOH 0.3 M. Indeed, the phase in-between is suspected to be
14 loaded in acid-amine complexes [23] and to be sure that a maximum of impurities were removed
15 from the TOA phase, only the top phase was recovered. These operations were repeated twice and
16 the last TOA top phase was finally washed with deionized water. The pH of the final aqueous phase
17 has been checked to be neutral, proving that no inorganic salts were left in purified TOA and this
18 latter is called solution 3.

19 2.3. Experiments

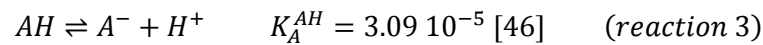
20 Several experiments of reactive liquid-liquid extractions were performed for different initial
21 concentrations of TOA and 3-HP. Unless specified, TOA of solution 2 (99.6% purity) was used. For
22 each extraction, 10 mL of the aqueous phase and 10 mL of the organic phase were mixed in
23 centrifuge tubes which were then manually shaken for 3 minutes. The stable emulsion formed was
24 then left at 25 °C for one day before being shaken again for 3 minutes and centrifuged at 15557 g for

1 1 hour. Once the aqueous phase recovered, it was analyzed using High Performance Liquid
 2 Chromatography (HPLC) and the extraction yield was calculated as follows:

$$Y = \frac{[AH]_{HPLC}^{ini} - [AH]_{HPLC}^{eq}}{[AH]_{HPLC}^{ini}} \quad (1)$$

3 with $[AH]_{HPLC}^{ini}$ being the total initial acid concentration in the aqueous phase according to HPLC
 4 analysis and $[AH]_{HPLC}^{eq}$ the same at equilibrium.

5 pH of the resulting aqueous phases was measured and compared to the theoretical pH value, based
 6 on the 3-HP dissociation (reaction 3) and calculated as follows:



$$pH_{th} = -\log \left(\frac{-K_A^{AH} + \sqrt{(K_A^{AH})^2 + 4K_A^{AH}[AH]_{HPLC}}}{2} \right) \quad (2)$$

7 All the extractions were made in duplicates. For some experiments, organic phases have been back
 8 extracted with NaOH solutions (0.1-0.5 M) in order to confirm that mass balance was satisfied within
 9 3% or less.

10 Experiments were centered on 0.011 mol/L (1 g/L) 3-HP and the optimal extractant concentration for
 11 this condition (0.46 mol/L = 20% v/v TOA) [42]. The initial acid concentration ranged from 0.0028
 12 mol/L to 0.56 mol/L (0.25 to 50 g/L) for 0.46 mol/L (20% v/v) TOA in order to clearly observe limiting
 13 factors at low concentrations while having good quantification results in HPLC. Concerning the initial
 14 concentrations of amines, the whole range from 0 to 100% v/v (0 – 2.3 mol/L) was studied with steps
 15 of 10%v/v (0.23 mol/L) for 0.011 mol/L (1 g/L) 3-HP.

16 **2.4. Analytical methods**

17 The 3-HP concentration in aqueous phases was determined using HPLC containing an Aminex HPX-
 18 87H H⁺ exchange column (300x7.8 mm, Biorad, USA) and an H⁺ Micro-Guard column (30mmx4.6mm,

1 Biorad, USA). The mobile phase consisted in a 0.01N H₂SO₄ solution circulating at a flowrate of 0.6
2 mL/min and the column temperature was set to 50 °C. An internal standard consisting in 10 g/L citric
3 acid in water was added (50% v/v) to the samples and 20 µL of each sample were used for injection.
4 Signals were obtained using a UV detector with the specific wavelength of 210 nm for acids. The
5 overall HPLC analysis uncertainty, defined as the coefficient of variation of the internal standard peak
6 area, was evaluated to 1%.

7 Impurities identification and titration in the aqueous phase were performed using Ultra High
8 Performance Liquid Chromatography coupled with High Resolution Mass Spectroscopy (UHPLC-
9 HRMS). Separations were performed using a Hypersil Gold C18 column (50X2.1 mM; 1.9 µm particle
10 size; Thermo Fisher Scientific, USA) with an Ultimate 3000 pump and Thermo 3000 RS autosampler
11 (Thermo Fisher Scientific, USA). The oven temperature was set to 25°C. The mobile phase was a
12 mixture of LC/MS grade acetonitrile and water loaded with 0.1% formic acid (Optima, Thermo Fischer
13 Scientific, USA) flowing at 250 µL/min. In a run, it first consisted in 2% acetonitrile for 4 minutes.
14 Then a gradient was applied to reach 98% acetonitrile in 6 minutes and the mixture was then
15 stabilized at 98% acetonitrile for 5 minutes. The mobile phase was finally set back to initial conditions
16 (2% acetonitrile) with a 2 minutes gradient.

17 Samples were diluted 100-fold in water (Optima LC/MS grade, Thermo Fischer Scientific, USA) and
18 filtered before assay with syringe filters (nylon membrane, 0.2 µm porosity and 13 mm diameter,
19 Merck Millipore, USA).

20 MS analysis was carried out with a Q-EXACTIVE Orbitrap mass spectrometer (Thermo Fisher
21 Scientific, USA) in positive mode with a Heated ElectroSpray Ionization (HESI) probe using a high
22 resolution full scan. The MS resolution was 70000 in a full MS mass range (m/z) from 50 to 700 AMU.
23 The ion transfer tube and vaporizer temperatures were set to 300°C. The electrospray voltage was
24 set to 3 kV and the sheath, auxiliary and sweep gas (nitrogen) flow rates were set to 35, 10 and 0
25 arbitrary units, respectively.

1 IR analyses were performed using a Cary 630 Fourier Transform InfraRed spectrometer of Agilent
2 Technologies (California, USA).

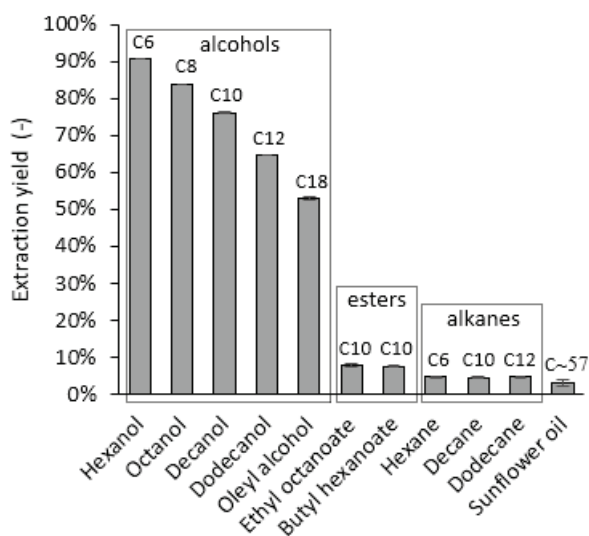
3 pH measurements were obtained from a SevenCompact pHmeter equipped with the Inlab Micro Pro-
4 ISM combined pH electrode with temperature probe (Mettler Toledo, Switzerland).

5 Water concentration in the organic phase was determined using Karl-Fischer method with a 756 KF
6 Coulometer (Metrohm, Switzerland). Methanol was the solvent for titration. Organic phase samples
7 were analyzed in triplicate.

8 Statistical significance of differences between data was assessed using ANOVA tests at 0.05 level.

9 3. Results and discussion

10 3.1. Solvents screening



11
12 *Figure 1: Extraction yields for different solvents with 0.46 mol/L of TOA (20% v/v) and 0.011 mol/L of 3-HP (1*
13 *g/L). Error bars represent standard deviations (n=2).*

14 First, a set of 11 solvents were selected for a screening regarding the reactive extraction of 0.011
15 mol/L (1 g/L) of 3-HP in aqueous phase using 0.46 mol/L of purified TOA (20% v/v) in the organic
16 phase. Figure 1 provides the extraction yields for these solvents.

1 Three kinds of solvents were tested: alcohols, esters and alkanes with different lengths of alkyl chain
 2 and numbers of carbon atoms. They can be compared according to their extraction ability and
 3 physicochemical properties like polarity (dipole moment and relative permittivity) and H-bond ability.

4

5 *Table 1: Physicochemical properties of the studied solvents [47-50]*

Solvent	Dipole moment (Debye)	Relative permittivity (25°C)	H-bond ability	Water solubility (mg/L, 25°C)	Number of carbon atoms	Molecular weight (g/mol)
Hexane	0	1.9	no	9.5	6	86
Decane	0	2.0	no	0.052	10	142
Dodecane	0	2.0	no	0.0037	12	170
Butylhexanoate	~ 1.8 ^a	~ 4.1 ^a	Acceptor	~ 40 ^c	10	172
Ethyl octanoate	~ 1.8 ^b	~ 4.2 ^b	Acceptor	70.1	10	172
Oleyl alcohol		3.8	Donor and acceptor	~ 0.02 ^c	18	268
Dodecanol	1.70	5.7	Donor and acceptor	4	12	186
Decanol	1.62	8.1	Donor and acceptor	37	10	158
Octanol	1.76	10.3	Donor and acceptor	540	8	130
Hexanol	1.55	13.0	Donor and acceptor	5 900	6	102
Water	1.85	78.4	Donor and acceptor		0	18
Trioctylamine	0.8	~ 2.1	Acceptor	0.05 mg/L	24	354
Sunflower oil		~ 3.1	Acceptor		~ 57	~ 882

6 ^a approximate values from pentylpentanoate

7 ^b approximate values from octylacetate

8 ^c estimated values from EPI Suite™ estimation program (Syracuse Research Corporation and the U.S. Environmental
 9 Protection Agency; copyright 2000).

10
 11 The immediate conclusion from the observation of Figure 1 is that alcohols have the highest
 12 extraction yields. Among alcohols, the shorter the chain, the higher the extraction yield: 91% for the
 13 C₆ hexanol, 53% for the C₁₈ oleyl alcohol. For alkanes and esters, the yield is independent of the chain
 14 length (ANOVA test with $p=0.6$ for both), which means that the chain length cannot be used as a
 15 good parameter to discriminate solvents. If all the *n*-alcohols have approximately the same dipole
 16 moment (*i.e.* “molecular polarity”) and H-bond ability due to the same primary hydroxyl groups (Table
 17 1), they differ in their relative permittivity that is more related to the “bulk polarity” and hence to the
 18 molecular size and organization. In this case, the difference in solvent activity is given by their
 19 relative permittivity so that the smaller the *n*-alcohol the higher the yield. It is obvious that totally
 20 inert solvents like alkanes (apolar and without H-bond ability) are unable to extract the acid

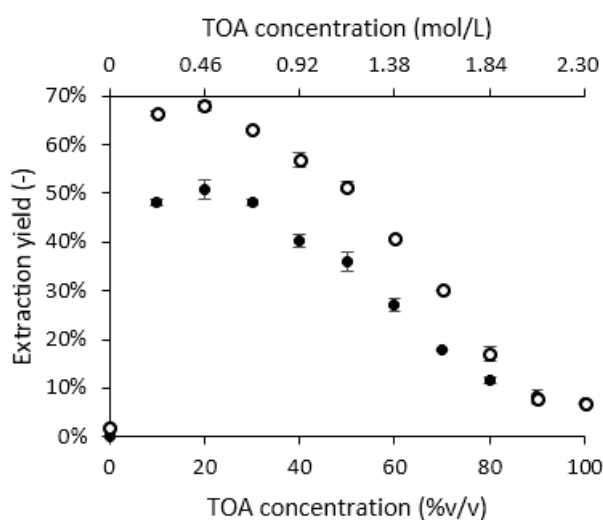
1 efficiently. *n*-hexane, *n*-decane and *n*-dodecane having the same values of relevant parameters
2 (dipole moment, relative permittivity and H-bond ability), all provide the same efficiency as expected
3 (5% yield). The same observations apply to the esters: both esters having the same relevant
4 properties, they show equivalent performances (around 8% yield). This slightly, but significantly
5 higher yield (ANOVA test with $p < 0.02$), can be explained by the slightly higher polarity of the two
6 esters compared to the three alkanes, which makes them a bit more active diluents. It is also
7 interesting to compare esters and alcohols with similar polarities but different H-bond abilities like
8 butylhexanoate and ethyloctanoate vs. oleyl alcohol and *n*-dodecanol. A much higher yield is
9 obtained for both alcohols (7 times higher for oleyl alcohol than for the esters), which leads to the
10 conclusion that the H-bond donor character of the solvent plays a key role, implying that alcohols
11 stabilize the reactive system through H-bonding interactions. Ricker [43] suggests that the acidic
12 hydrogen of the carboxylic acid being involved in the acid-amine bond, carboxyl becomes a strictly
13 acceptor group. This would explain such a difference between H-bond donors and acceptors as
14 solvents of otherwise similar polarities. Similar results in the literature confirm the importance of the
15 solvent polarity and specific H-bond donor character to favor the complex formation and increase
16 the extraction yields [23,31,37,39].

17 Based on these results, *n*-decanol and oleyl alcohol were chosen for further investigations. *n*-hexanol
18 and octanol were ruled out due to their excessive solubility in water that is expected to be toxic for
19 microorganisms under integrated extractive bioconversion (ISPR) [51,52]. According to the literature
20 review, some biocompatible vegetable oils, thanks to their low polarity and solubility in water, have
21 been shown to be quite efficient for the removal of similar acids (propionic, pyruvic, lactic) from
22 aqueous phases [47–49]. But in our present results, sunflower oil was found to be inefficient for 3-HP
23 extraction with TOA (less than 7% yield) whatever the proportions used because of its weak solvation
24 properties.

25 **3.2. Physical extraction**

1 For physical extraction experiments, concentration of 3-HP were varied from 0.0028 to 0.56 mol/L
2 (0.25 to 50 g/L) with no TOA in the organic phase. In *n*-decanol, a linear dependence ($R^2=0.96$) of the
3 equilibrium acid concentration in the organic phase with respect to that in the aqueous phase was
4 found (results not shown). The slope, i.e. the partition coefficient (defined as the ratio of the molar
5 concentrations at equilibrium), was evaluated to 0.020 ± 0.001 by linear regression, meaning that
6 physical extraction yield was around 2%. This is in line with Kertes and King's statement that the acid
7 partition coefficient is independent on its concentration in the case of extraction using alcohols [15].
8 In oleyl alcohol, 3-HP partitioning was not detectable. Physical extraction yields were thus too low for
9 practical purposes, confirming that the efficient liquid-liquid extraction of 3-HP needs to be driven by
10 a chemical reaction.

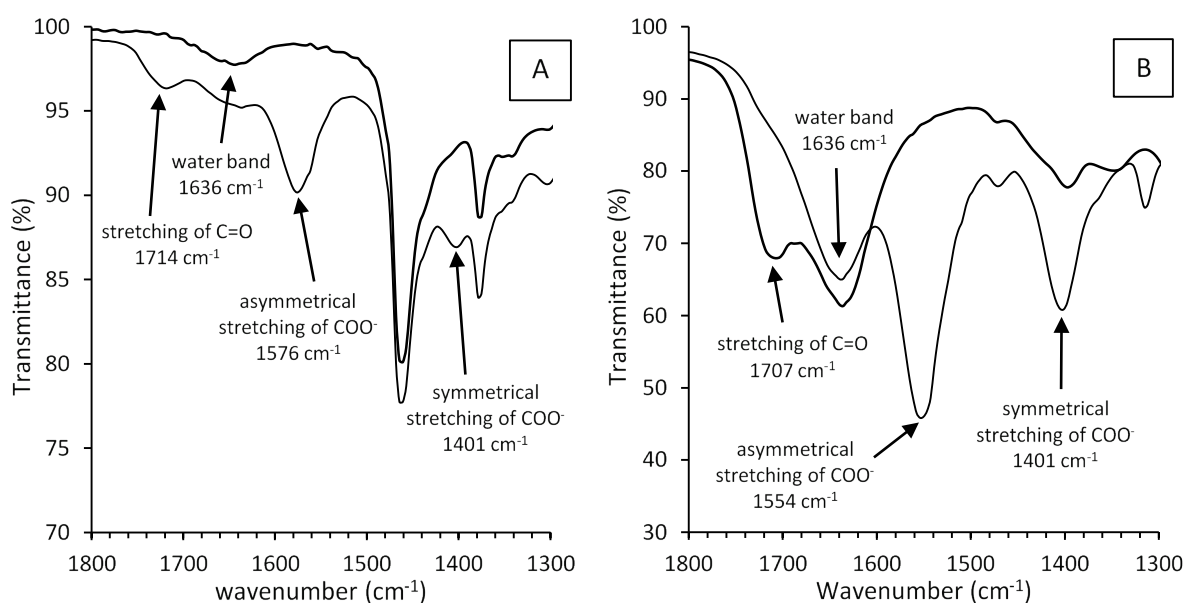
11 3.3. Effects of initial amine concentration



12
13 *Figure 2: Extractions yields as a function of TOA concentration in decanol (open circles) and oleyl alcohol (filled*
14 *circles), with 0.011 mol/L (1g/L) of 3-HP initially. Error bars represent standard deviations (n=2).*
15 Figure 2 shows the extraction yield as a function of initial TOA concentration in the organic phase for
16 0.011 mol/L (1 g/L) of 3-HP initially in water, with *n*-decanol and oleyl alcohol as solvents. For both
17 solvents, a bell-shaped profile can be observed. This profile illustrates the synergistic effect between
18 TOA and these active diluents and thus the major role of the solvent. When used alone, neither TOA
19 nor the solvents are able to extract 3-HP efficiently (less than 10% yield). However, a strong

1 synergistic effect was observed when TOA was mixed with an active diluent. Indeed, TOA has poor
2 solvation abilities to accumulate complexes whereas *n*-decanol and oleyl alcohol are not hydrophilic
3 enough to extract such a hydrophilic compound in substantial proportions. When the amine and the
4 solvent are used together, new hydrophobic properties of the complexed acid and new solvation
5 abilities of the organic phase improve the extraction efficiency.

6



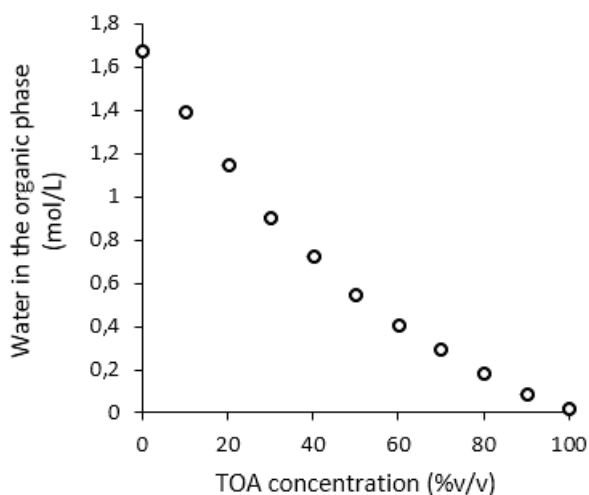
7

8 *Figure 3: IR spectra of organic phases after 3-HP extraction (53g/L in the organic phase, bold line) and after*
9 *simple water contact (thin line) (A) and IR spectra of commercial aqueous phases of 3-HP before (bold line) and*
10 *after (thin line) neutralization with NaOH (B)*

11 Figure 3 provides IR spectra obtained from two different solutions: Figure 3A shows organic phases
12 consisting in 20% v/v TOA in *n*-decanol and Figure 3B shows aqueous phases of the 3-HP commercial
13 solution for comparison. When TOA is present in the organic phase (Figure 7A), characteristic
14 infrared bands between 1300 and 1800 cm⁻¹ prove the existence of a delocalized carboxylate group
15 in the organic phase demonstrating strong interactions between the acidic proton of 3-HP and the
16 amine leading to the formation of an ion pair. This observation is related to the formation of
17 complexes involving 3-HP bonded to TOA. The fatty amine provides hydrophobic properties to the
18 species formed, enabling a favorable distribution of the acid towards the organic phase. However,
19 the anion, being such a polar part, needs a polar counterparts in the medium to be stabilized.

1 Alcohols, with polar and H-bond donor hydroxyl groups, are thus able to stabilize the hydrophilic
2 anionic conjugate in the organic phase.

3 As a consequence, the extraction yield of 3-HP decreases at high amine concentrations, a behavior
4 that aligns well with reported data for acetic [43], lactic [36,56], malonic [57], propionic [30], succinic
5 [23,57] and tartaric [25] acids in active diluents. This behavior is not observed in the case of inert
6 diluents in which the yield increases along with the amines concentration [23]. The greatest extent of
7 extraction of 3-HP is reached around 0.46 mol/L (20% v/v) TOA in both active diluents at 0.011 mol/L
8 (1 g/L) of 3-HP initially with 68% and 51% yields for *n*-decanol and oleyl alcohol respectively. The
9 maximum of the extraction yield corresponds to the optimal composition of the extracting phase.



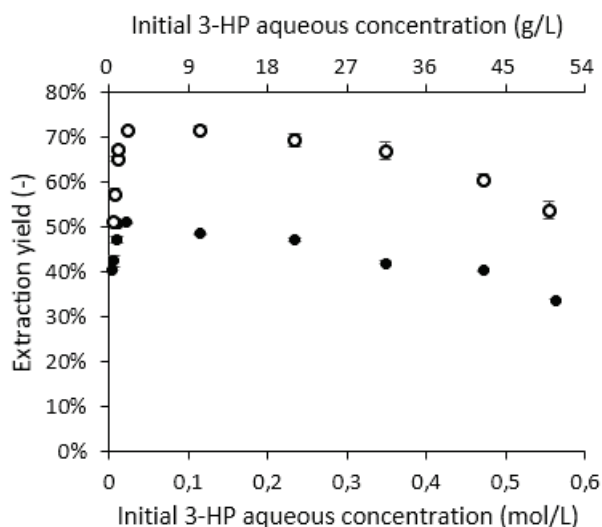
10

11 *Figure 4: Water solubility in the organic phase as a function of TOA concentration (0 – 2.3 mol/L) in decanol at*
12 *25°C. Error bars represent standard deviations (n=3) and are masked by the symbols.*

13 Moreover, water solubility appears to play a crucial role. As shown in Figure 4, the more the organic
14 phase is loaded with TOA, the less water is soluble, water being practically insoluble in TOA.
15 Increasing the amount of TOA leads to a decrease of water content in the organic phase which could
16 lead to a lesser stabilization of the complex and hence a lesser extraction efficiency. Tamada et al
17 [57] reported that the amount of acid extracted and the stoichiometry of the complex in the organic
18 phase depended on the water content in the organic phase. The amount of water soluble in the
19 organic phase is supposed to stabilize the hydrophilic part of the complex in a rather apolar

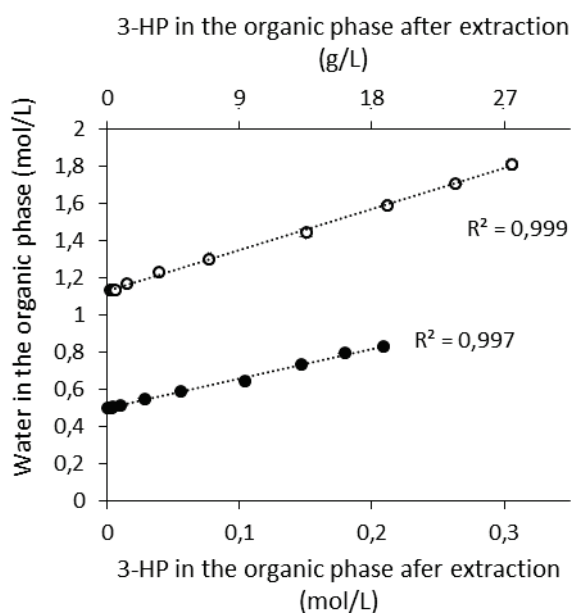
1 environment. Increasing the concentration of alcohol in the organic phase increases the water
2 solubility and therefore the solvation effect of alcohols could be in fact partially due to the solvation
3 of water. The balance between the respective contributions in complex stabilisation cannot be
4 assessed based on the present results because the amounts of water and alcohol are correlated.

5 3.4. Effects of initial acid concentration



6
7 Figure 5: Extraction yields in decanol (open circles) and oleyl alcohol (filled circles) with 0.46 mol/L TOA (20%
8 v/v) as a function of initial acid concentration. Error bars represent standard deviations ($n=2$).

9 Concerning the influence of the acid concentration, Figure 5 shows that the plot of the extraction
10 yield against initial acid concentration is not monotonic. If we consider only the partitioning of the
11 acid and the complex formation, such a profile is not expected. Indeed, the more diluted is the acid
12 and the more in excess are the amines; this should lead to a higher extent of reaction 2 (acid-amine
13 complex formation) and therefore the increase of extraction yield with the decrease of 3-HP
14 concentration. This behavior was indeed observed for initial acid concentration levels between 0.11
15 and 0.56 mol/L (10 – 50 g/L). But for the weakest concentration levels, a sharp decrease is observed
16 and other phenomena are needed to explain such results. For example, in oleyl alcohol the
17 extraction yield decreased from 51% to 40% and in *n*-decanol from 72% to 51% between 0.023 and
18 0.0041 mol/L (2 – 0.4 g/L) of 3-HP initially. Deeper investigations on pH effects were undertaken in
19 the case of *n*-decanol to understand these phenomena (section 3.5.).



1
 2 *Figure 6: water co-extraction in decanol (open circles) and oleyl alcohol (filled circles) with 0.46 mol/L TOA (20%*
 3 *v/v) as a function of the acid concentration in the organic phase after extraction. Error bars represent standard*
 4 *deviations (n=3) and are masked by the symbols.*

5 A further look at the water content of the organic phase after extraction proves that water is
 6 extracted in the organic phase jointly with the acid (Figure 6). Indeed, the more acid extracted, the
 7 more water in the organic phase. Indeed, water concentration reached higher amounts than its
 8 solubility in *n*-decanol with 20%v/v TOA (1.14 mol H₂O/L, Figure 4) and in oleyl alcohol with 20%v/v
 9 TOA (0.50 mol H₂O/L). Figure 6 shows that the amount of water in the organic phase varies linearly
 10 with the acid concentration. This tends to prove that, in the range studied, each 3-HP molecule
 11 carries the same average number of water molecules in the organic phase. This hydration number
 12 (given by the slopes of the regression lines in Figure 6) is around 2 for *n*-decanol and oleyl alcohol as
 13 solvents. This tendency of 3-HP to carry water molecules while being extracted in the organic phase
 14 could also partly explain why the extraction yield was very low with alkanes (section 3.1).

15
 16
 17
 18

1 3.5. Influence of TOA impurities on extraction performances

2 3.5.1. pH at equilibrium

3 *Table 2: amount of amines (OA+DOA) found in the aqueous phase after extraction and associated measured*
 4 *(pH_{eq}) and calculated (pH_{th} and $pH_{th,OA}$) pH, as a function of initial 3-HP and TOA concentrations, for*
 5 *solutions 1 (98.1% of TOA purity), 2 (99.6% of TOA purity) and 3 (>99.6%: purified TOA)*

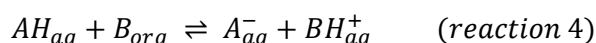
Solution 1 (TOA 98.1%)					
$[AH]_{ini}$ (mol/L)	$[TOA]_{ini}$ (mol/L)	$[OA]_{LCMS}$ (mmol/L)	pH_{eq}	pH_{th}	$pH_{th,OA}$
0.0028	0.46	2.0	5.4	3.6	5.1
0.011	0.46	2.6	4.6	3.4	4.5
0.029	0.46	2.8	4.1	3.3	4.1
0.058	0.46	2.7	3.8	3.1	3.8
0.10	0.46	2.5	3.5	3.0	3.5
0.21	0.46	2.9	3.2	2.9	3.3
0.31	0.46	2.8	3.0	2.8	3.1
0.50	0.46	2.8	2.8	2.6	2.8
0.011	0.23	1.4	4.2	3.4	4.2
0.011	0.69	3.6	4.9	3.4	4.6
0.011	0.91	4.6	5.0	3.3	4.7
0.011	1.4	6.4	5.2	3.3	4.9
0.011	1.8	8.5	5.4	3.3	5.3
0.011	2.1	8.7	5.4	3.3	5.3
Solution 2 (TOA 99.6%)					
0.0041	0.46	1.0	4.8	3.6	4.5
0.011	0.46	1.2	4.3	3.5	4.2
0.023	0.46	1.2	4.1	3.4	3.9
0.11	0.46	1.2	3.4	3.0	3.3
0.23	0.46	1.2	3.2	2.8	3.0
0.35	0.46	1.2	3.2	2.7	2.9
0.55	0.46	1.3	2.9	2.6	2.7
0.011	0.23	0.54	4.1	3.5	3.8
0.011	0.69	1.5	4.5	3.5	4.3
0.011	0.91	2.3	4.6	3.4	4.5
0.011	1.4	3.4	4.5	3.4	4.5
0.011	1.8	4.9	4.5	3.3	4.5
0.011	2.1	5.4	4.5	3.3	4.5
Solution 3 (purified TOA)					
0.0027	0.46	0.065	4.1	3.9	4.0
0.011	0.46	0.039	3.7	3.6	3.6
0.021	0.46	0.048	3.5	3.4	3.5
0.053	0.46	0.040	3.2	3.2	3.2
0.11	0.46	0.039	3.0	3.0	3.0
0.21	0.46	0.029	2.8	2.9	2.9
0.32	0.46	0.034	2.7	2.7	2.7
0.53	0.46	0.031	2.5	2.6	2.6
0.011	0.23	0.053	3.5	3.5	3.6
0.011	0.69	0.062	3.6	3.6	3.6
0.011	0.91	0.134	3.6	3.6	3.6
0.011	1.4	0.191	3.6	3.5	3.6
0.011	1.8	0.319	3.4	3.4	3.5
0.011	2.1	0.318	3.4	3.3	3.4

6
 7 pH of aqueous solutions were measured for after extraction and compared to the calculated values.

8 Results are shown in Table 2 (solution 2). Considering the pH at the equilibrium in the aqueous phase

1 (pH_{eq}), it appears that for the lowest acid concentrations the experimental data are above the
2 calculated values from acid concentrations at equilibrium alone (pH_{th} , reaction 3, equation 2). This
3 means that higher amounts of 3-HP molecules are deprotonated compared to what is expected with
4 only 3-HP in water. When performing pH titrations of aqueous phases after acetic acid extraction by
5 TOA in chloroform, Wardell and King [58] also reported pH deviations. They attributed this
6 phenomenon to the transfer of tri-*n*-octylamine in the aqueous phase in the presence of acid. The
7 same was found for lactic acid extraction at low concentration with Alamine 336 [59]. However,
8 Ricker [43] demonstrated that, even in 10% wt acetic acid in water, TOA solubility would not exceed
9 10 ppm (*i.e.* $< 3 \cdot 10^{-5}$ mol/L) and suggested that pH deviation may have been due to impurities in
10 commercial products like primary and secondary amines, lower-molecular-weight tertiary amines
11 and starting materials for the amine synthesis.

12 When sodium hydroxide was added to our aqueous samples after extraction, a condensation of
13 organic species occurred and the solution became cloudy as already reported by some authors
14 [57,58]. On the contrary, pure distilled water contacted with commercial TOA solution resulted in a
15 neutral pH without any noticeable visual change when NaOH was added. This illustrates the fact that
16 impurities are extracted in the aqueous phase under protonated form through reactive extraction
17 with acidic species (see reaction 4) and that their partitioning as neutral forms can be neglected:



18 3.5.2. Identification of TOA impurities

19 Aqueous samples after extraction were analyzed with mass spectroscopy. Two main peaks appeared
20 at $m/z=130$ and $m/z=242$ corresponding to both peaks of protonated *n*-octylamine ($129 + 1 = 130$
21 g/mol) and protonated di-*n*-octylamine ($241 + 1 = 242$ g/mol). Commercial samples of *n*-octylamine
22 (OA) and di-*n*-octylamine (DOA) were also analyzed to confirm species identification. Several
23 manufacturing processes exist for the production of fatty amines like nitrile hydrogenation or
24 alcohols amination. For example, TOA has been produced by BASF using *n*-octanol amination with

1 ammonia and OA leading to impurities of around 2% DOA and 1% OA [60]. This tends to confirm the
2 presence of these impurities in the products in our study.

3 When acidic species are present in the aqueous phase, these TOA impurities (OA and DOA) undergo
4 protonation and both deprotonated acid molecules and protonated amine molecules are released in
5 the aqueous phase increasing the pH (Table 2, solutions 1 and 2). This phenomenon can be neglected
6 at high acid concentration because impurities amount remains relatively low and the protonated
7 form of TOA is practically insoluble in water [43] so that relatively low amount of acid is neutralized
8 in the aqueous phase. Consequently, the increase of the dissociated part of 3-HP is significant only
9 for low concentrations and this phenomenon significantly decreases the extraction yield only for
10 concentrations under 0.1 mol/L (10 g/L, Figure 5).

11 Potentiometric titration with colorimetry is often used to determine extraction yields in reactive
12 extraction with phenolphthalein as a pH indicator [18,20,21,23,29,34]. However, the usual color
13 turning point of phenolphthalein is at pH=8.2 [46] which means that acidic species at pH > 8.2 are not
14 titrated. The pKa of *n*-octylammonium is 10.65 [46] which means that OA remains under its acidic
15 form (*n*-octylammonium) above pH=8.2 and is not titrated. It implies that acidic species neutralized
16 by *n*-octylamine are not taken into account in this titration method. From our results, it can be
17 concluded that pH colorimetric titration is not appropriate to determine extraction yields when
18 amines are used as extractants because it could lead to underestimated acid concentration in the
19 aqueous phase and hence overestimating the yields, especially for low acid concentration.

20 3.5.3. Influence of TOA impurities on the extraction mechanisms

21 Three TOA solutions with different purity levels have been used in order to evaluate the impact of
22 TOA impurities on the extraction process.

23 Analyses of aqueous phases after extraction proved that no significant TOA were released in the
24 aqueous phases. However, OA and DOA were identified and quantified, with the former as the main

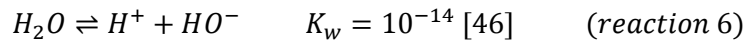
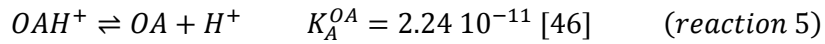
1 amine in the aqueous phase (more than 90% of total amines concentration). Hence, total amount of
 2 amines (OA+DOA) in the aqueous phase is assimilated to OA to simplify further interpretation. The
 3 amount of OA varied between 0.031 and 9.1 mmol/L (Table 2), depending on the following operating
 4 conditions: TOA initial concentration in the organic phase, purity of TOA and 3-HP initial
 5 concentration in the aqueous phase.

6 Amine transfer into the aqueous phase increases the pH. Accordingly, an amended formula should be
 7 used to calculate the pH by taking into account the amines. Knowing the total concentration of
 8 amines in the aqueous phase and their dissociation constants, the equilibrium pH of 3-HP solutions
 9 can then be calculated. If we consider that amines in the aqueous phase mainly consist in OA, the

10 theoretical pH calculation becomes:

$$pH_{th,OA} = -\log([H^+]) \quad (3)$$

11 $[H^+]$ being the physical solution of the following equation (4), based on 3-HP dissociation (reaction 3),
 12 *n*-octylammonium dissociation (reaction 5), water autoprotolysis (reaction 6) and the
 13 electroneutrality principle:

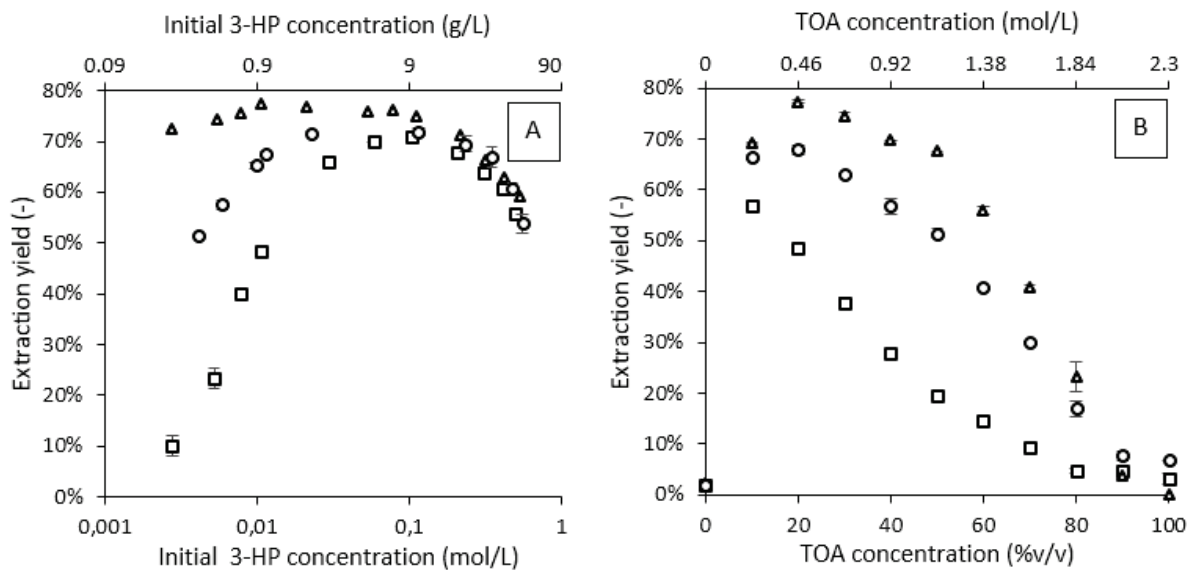


$$\frac{K_A^{AH} [AH]_{HPLC}}{[H^+] + K_A^{AH}} + \frac{K_w}{[H^+]} - \frac{[H^+][OA]_{LCMS}}{K_A^{OA} + [H^+]} - [H^+] = 0 \quad (4)$$

14 As can be seen in table 2, values of $pH_{th,OA}$ are much closer to the pH measured experimentally.
 15 Accordingly, the deviations observed between pH_{eq} and pH_{th} are directly related to the impurities in
 16 commercial TOA. Moreover, pH measurement can give, as a first approximation, reliable information
 17 about the amount of impurities (OA) transferred into the aqueous phase.

18 Since only non-dissociated 3-HP can be extracted by TOA in the organic phase, amines transfer into
 19 the aqueous phase and the consequent increase in pH_{eq} reduces the amount of protonated 3-HP

1 available for extraction. The final outcome of this phenomenon is the decrease of the extraction
2 yield.



3
4 *Figure 7: Extraction yields for different TOA solutions (squares: solution 1 (98.1% purity); circles: solution 2*
5 *(99.6% purity); triangles: solution 3 (purified TOA)) in decanol as a function of initial acid concentration at 0.46*
6 *mol/L TOA (20% v/v) (A) and initial TOA concentration at 0.011 mol/L 3-HP (1 g/L) (B). Error bars represent*
7 *standard deviations (n=2).*

8 Yield reduction caused by TOA impurities is illustrated in figures 5 and 7A for low acid concentrations.

9 In Figure 7A, the plot is given in logarithmic scale for initial 3-HP concentration in order to clearly
10 observe the discrepancies for the small acid concentrations and the similarities for high acid
11 concentrations. For a given TOA concentration in the organic phase (0.46 mol/L = 20% v/v), the three
12 solutions provide equivalent yields at high acid concentration (>0.11 mol/L = 10 g/L). This was
13 expected since the amount of amines released in the aqueous phase is small compared to the acid
14 concentration, and its influence on the yield becomes negligible. However, for low acid
15 concentrations, OA and DOA impurities present in TOA solutions have a strong impact. For example,
16 at around 5mmol/L (0.5 g/L) 3-HP initially, the extraction yield is 23% for solution 1 (2.5 mmol/L OA),
17 58% for solution 2 (1.0 mmol/L OA) and 74% for solution 3 (0.0058 mmol/L OA). The yield decrease
18 at low acid concentration with commercial TOA (solutions 1 and 2) is dramatic whereas with purified
19 TOA (solution 3) the decrease is much smaller. Nevertheless, it still denotes the release of weak
20 amounts of impurities, but with much lower importance than from unpurified TOA. In figure 7B, one

1 can see a much smaller yield decrease for purified TOA (solution 3) than for solution 1 along with the
2 increase of amine concentration, up to about 50% v/v TOA. This steeper decrease for unpurified TOA
3 is due to impurities release, whereas for purified TOA only the solvating effect is noticeable.

4 In the case of purified TOA, results shown in Table 2 confirm that the purification of TOA was
5 effective, dividing the amount of amines released in the aqueous phase by a factor 30 when
6 compared to solution 2 and a factor 65 when compared to solution 1. This decrease makes the
7 amount of amines practically negligible in the aqueous phase, as confirmed by pH measurements.

8 Besides the effects described above on the extraction chemistry, the diminution of the released
9 amines by means of the purification step is particularly recommended if microorganisms are used in
10 a biological process due to expected amine toxicity. For example, a similar lactate – long chains alkyl
11 ammonium salt in an aqueous phase proved to be very toxic for several kinds of microorganisms
12 [61]. Purification of fatty tertiary amines can be reached for example with an adsorption process as
13 described in a US patent [62]. In continuous chemical processes, however, the presence of impurities
14 would have less impact because the recycled organic phase would be washed by the continuous
15 fresh aqueous flow and small amines would be readily depleted [43].

16 **4. Conclusion**

17 This paper reports a comprehensive study of the reactive extraction of 3-HP, a valuable platform
18 molecule. The experimental approach was designed in line with a potential application of reactive
19 extraction for the *in situ* product recovery of this acid. It appeared from the solvent screening
20 experiments, performed for the first time on 3-HP, that the type of solvent has a great influence on
21 the extraction yield, with H-bond donor characteristic and polarity being the most important
22 properties to increase the yield. This highlights strong interactions between the solvent and the
23 complexes formed between acids and amines. Water solubility in the organic phase also seems to be
24 a major factor in complex stabilization. In active diluents like *n*-decanol and oleyl alcohol, a bell-
25 shaped profile of the extraction yield as a function of amine concentration is observed with very low

1 extraction yields at high amine concentrations. This proves the major role of the solvent in the
2 extraction process. In these solvents, the increase in initial acid concentration from 0.003 up to 0.1
3 mol/L (0.25 – 10 g/L) led to an increase in extraction yield while a further increase from 0.1 to 0.56
4 mol/L (10 – 50 g/L) led to the reduction of the extraction yield. This phenomenon has been shown to
5 be due to amines transfer in the aqueous phase, with significant impact for low acid concentrations.
6 Deviation of equilibrium pH from what should be expected with the remaining acid in the aqueous
7 phase demonstrated the presence of impurities. We identified the release of *n*-octylamine and di-*n*-
8 octylamine coming from commercial TOA into the aqueous phase even with 99.6% purity. This
9 transfer reduces extraction yields at low initial acid concentrations and increases equilibrium pH
10 which is, accordingly, a reliable indicator of amines concentration in water. Given the extraction yield
11 reduction and the suspected toxicity for microorganisms performing bioconversions, purification of
12 TOA before use could be necessary. These results give insights in extraction mechanisms of organic
13 acids, further clarifying the role of the organic solvent and water in the stabilization of the acid-amine
14 complex and pointing out the role of the amine impurities in extraction yield reduction and
15 biocompatibility of the extraction process. All this understanding is essential for further mechanistic
16 and predictive modeling.

17

1 **Acknowledgements**

- 2 The authors would like to thank AgroParisTech students of the 189th promotion and Dr Claire Saulou-
- 3 Bérion for having provided valuable experimental assistance, and the Région Champagne-Ardenne,
- 4 the Conseil départemental de la Marne and Reims Métropole for financial support.

1 References

- 2 [1] T. Werpy, G. Petersen, A. Aden, J. Bozell, J. Holladay, J. White, A. Manheim, D. Elliot, L. Lasure,
3 S. Jones, M. Gerber, K. Ibsen, L. Lumberg, S. Kelley, Top Value Added Chemicals from Biomass,
4 Volume 1—Results of Screening for Potential Candidates from Sugars and Synthesis Gas, U.S.
5 Department of Energy, Oak Ridge, TN, 2004.
- 6 [2] J.J. Bozell, G.R. Petersen, Technology development for the production of biobased products
7 from biorefinery carbohydrates—the US Department of Energy’s “Top 10” revisited, *Green*
8 *Chemistry*. 12 (2010) 539.
- 9 [3] T. Dishisha, L.P. Pereyra, S.-H. Pyo, R.A. Britton, R. Hatti-Kaul, Flux analysis of the *Lactobacillus*
10 *reuteri* propanediol-utilization pathway for production of 3-hydroxypropionaldehyde, 3-
11 hydroxypropionic acid and 1,3-propanediol from glycerol, *Microbial Cell Factories*. 13 (2014) 1–
12 11.
- 13 [4] G. Burgé, C. Saulou-Bérion, M. Moussa, B. Pollet, A. Flourat, F. Allais, V. Athès, H.E. Spinnler,
14 Diversity of *Lactobacillus reuteri* Strains in Converting Glycerol into 3-Hydroxypropionic Acid,
15 *Applied Biochemistry and Biotechnology*. 177 (2015) 923–939.
- 16 [5] K. Kim, S.-K. Kim, Y.-C. Park, J.-H. Seo, Enhanced production of 3-hydroxypropionic acid from
17 glycerol by modulation of glycerol metabolism in recombinant *Escherichia coli*, *Bioresource*
18 *Technology*. 156 (2014) 170–175.
- 19 [6] H.S. Chu, Y.S. Kim, C.M. Lee, J.H. Lee, W.S. Jung, J.-H. Ahn, S.H. Song, I.S. Choi, K.M. Cho,
20 Metabolic engineering of 3-hydroxypropionic acid biosynthesis in *Escherichia coli*,
21 *Biotechnology and Bioengineering*. 112 (2015) 356–364.
- 22 [7] V. Kumar, S. Ashok, S. Park, Recent advances in biological production of 3-hydroxypropionic
23 acid, *Biotechnology Advances*. 31 (2013).
- 24 [8] E.I. Lan, D.S. Chuang, C.R. Shen, A.M. Lee, S.Y. Ro, J.C. Liao, Metabolic engineering of
25 cyanobacteria for photosynthetic 3-hydroxypropionic acid production from CO₂ using
26 *Synechococcus elongatus* PCC 7942, *Metabolic Engineering*. 31 (2015) 163–170.
- 27 [9] Y. Wang, T. Sun, X. Gao, M. Shi, L. Wu, L. Chen, W. Zhang, Biosynthesis of platform chemical 3-
28 hydroxypropionic acid (3-HP) directly from CO₂ in cyanobacterium *Synechocystis* sp. PCC 6803,
29 *Metabolic Engineering*. 34 (2016) 60–70.
- 30 [10] I.-Y. Jung, J.-W. Lee, W.-K. Min, Y.-C. Park, J.-H. Seo, Simultaneous conversion of glucose and
31 xylose to 3-hydroxypropionic acid in engineered *Escherichia coli* by modulation of sugar
32 transport and glycerol synthesis, *Bioresource Technology*. 198 (2015) 709–716.
- 33 [11] K.R. Kildegaard, Z. Wang, Y. Chen, J. Nielsen, I. Borodina, Production of 3-hydroxypropionic acid
34 from glucose and xylose by metabolically engineered *Saccharomyces cerevisiae*, *Metabolic*
35 *Engineering Communications*. 2 (2015) 132–136.
- 36 [12] Z. Jin, S.-T. Yang, Extractive fermentation for enhanced propionic acid production from lactose
37 by *Propionibacterium acidipropionici*, *Biotechnology Progress*. 14 (1998) 457–465.
- 38 [13] Z. Wu, S.-T. Yang, Extractive fermentation for butyric acid production from glucose **by**
39 *Clostridium tyrobutyricum*, *Biotechnology and Bioengineering*. 82 (2003) 93–102.
- 40 [14] L. Zhao, J. Lin, H. Wang, J. Xie, D. Wei, Development of a two-step process for production of 3-
41 hydroxypropionic acid from glycerol using *Klebsiella pneumoniae* and *Gluconobacter oxydans*,
42 *Bioprocess and Biosystems Engineering*. 38 (2015) 2487–2495.
- 43 [15] A.S. Kertes, C.J. King, Extraction Chemistry of Fermentation Product Carboxylic Acids,
44 *Biotechnology and Bioengineering*. 28 (1986) 269–282.
- 45 [16] V. Bízek, J. Horáček, M. Koušová, A. Heyberger, J. Procházka, Mathematical model of extraction
46 of citric acid with amine, *Chemical Engineering Science*. 47 (1992) 1433–1440.
- 47 [17] V. Bízek, J. Horáček, M. Koušová, Amine extraction of citric acid: effect of diluent, *Chemical*
48 *Engineering Science*. 48 (1993) 1447–1457.

- 1 [18] A. Keshav, P. Norge, K.L. Wasewar, Reactive Extraction of Citric Acid Using Tri-n-octylamine in
2 Nontoxic Natural Diluents: Part 1—Equilibrium Studies from Aqueous Solutions, *Applied*
3 *Biochemistry and Biotechnology*. 167 (2012) 197–213.
- 4 [19] M. Matsumoto, T. Otono, K. Kondo, Synergistic extraction of organic acids with tri-n-octylamine
5 and tri-n-butylphosphate, *Separation and Purification Technology*. 24 (2001) 337–342.
- 6 [20] D. Datta, S. Kumar, Reactive Extraction of Glycolic Acid Using Tri-n-Butyl Phosphate and Tri-n-
7 Octylamine in Six Different Diluents: Experimental Data and Theoretical Predictions, *Industrial*
8 *& Engineering Chemistry Research*. 50 (2011) 3041–3048.
- 9 [21] H. Uslu, Ş.İ. Kirbaşlar, Extraction of aqueous of malic acid by trioctylamine extractant in various
10 diluents, *Fluid Phase Equilibria*. 287 (2010) 134–140.
- 11 [22] J. Prochazka, A. Heyberger, V. Bizek, M. Kousova, E. Volaufova, Amine extraction of
12 hydroxycarboxylic acids. 2. Comparison of equilibria for lactic, malic, and citric acids, *Industrial*
13 *& Engineering Chemistry Research*. 33 (1994) 1565–1573.
- 14 [23] J.A. Tamada, A.S. Kertes, C.J. King, Extraction of Carboxylic Acids with Amine Extractants. 1.
15 Equilibria and Law of Mass Action Modeling, *Industrial & Engineering Chemistry Research*. 29
16 (1990) 1319–1326.
- 17 [24] R.-S. Juang, R.-H. Huang, Comparison of extraction equilibria of succinic and tartaric acids from
18 aqueous solutions with tri-n-octylamine, *Industrial & Engineering Chemistry Research*. 35
19 (1996) 1944–1950.
- 20 [25] M. Marinova, G. Kyuchoukov, J. Albet, J. Molinier, G. Malmary, Separation of tartaric and lactic
21 acids by means of solvent extraction, *Separation and Purification Technology*. 37 (2004) 199–
22 207.
- 23 [26] N. Marchitan, C. Cojocaru, A. Mereuta, G. Duca, I. Cretescu, M. Gonta, Modeling and
24 optimization of tartaric acid reactive extraction from aqueous solutions: A comparison between
25 response surface methodology and artificial neural network, *Separation and Purification*
26 *Technology*. 75 (2010) 273–285.
- 27 [27] A.F. Tuyun, H. Uslu, Reactive extraction of acrylic acid using trioctylamine (TOA) in versatile
28 diluents, *Desalination and Water Treatment*. 55 (2015) 193–198.
- 29 [28] K. Wang, Z. Chang, Y. Ma, C. Lei, S. Jin, Y. Wu, I. Mahmood, C. Hua, H. Liu, Equilibrium study on
30 reactive extraction of propionic acid with N1923 in different diluents, *Fluid Phase Equilibria*.
31 278 (2009) 103–108.
- 32 [29] S. Kumar, D. Datta, B.V. Babu, Experimental Data and Theoretical (Chemodel Using the
33 Differential Evolution Approach and Linear Solvation Energy Relationship Model) Predictions on
34 Reactive Extraction of Monocarboxylic Acids Using Tri-n-octylamine, *Journal of Chemical &*
35 *Engineering Data*. 55 (2010) 4290–4300.
- 36 [30] A. Keshav, K.L. Wasewar, S. Chand, Reactive extraction of propionic acid using tri-n-octylamine,
37 *Chemical Engineering Communications*. 197 (2009) 606–626.
- 38 [31] Y.S. Aşçı, İ. İnci, Extraction equilibria of propionic acid from aqueous solutions by Amberlite LA-2
39 in diluent solvents, *Chemical Engineering Journal*. 155 (2009) 784–788.
- 40 [32] A. Senol, Influence of diluent on amine extraction of pyruvic acid using Alamine system,
41 *Chemical Engineering and Processing: Process Intensification*. 45 (2006) 755–763.
- 42 [33] M.E. Marti, T. Gurkan, L.K. Doraiswamy, Equilibrium and Kinetic Studies on Reactive Extraction
43 of Pyruvic Acid with Trioctylamine in 1-Octanol, *Industrial & Engineering Chemistry Research*.
44 50 (2011) 13518–13525.
- 45 [34] D. Pal, A. Tripathi, A. Shukla, K.R. Gupta, A. Keshav, Reactive Extraction of Pyruvic Acid Using
46 Tri-n-octylamine Diluted in Decanol/Kerosene: Equilibrium and Effect of Temperature, *Journal*
47 *of Chemical & Engineering Data*. 60 (2015) 860–869.
- 48 [35] R.-S. Juang, R.-H. Huang, Equilibrium studies on reactive extraction of lactic acid with an amine
49 extractant, *Chemical Engineering Journal*. 65 (1997) 47–53.
- 50 [36] G. Kyuchoukov, D. Yankov, Lactic Acid Extraction by Means of Long Chain Tertiary Amines: A
51 Comparative Theoretical and Experimental Study, *Industrial & Engineering Chemistry Research*.
52 51 (2012) 9117–9122.

- 1 [37] G. Kaur, K. Elst, Development of reactive extraction systems for itaconic acid: a step towards in
2 situ product recovery for itaconic acid fermentation, *RSC Advances*. 4 (2014) 45029–45039.
- 3 [38] M. Reschke, K. Schügerl, Reactive Extraction of Penicillin II: Distribution Coefficients and
4 Degrees of Extraction, *Chemical Engineering Journal*. 28 (1984) B11–B20.
- 5 [39] J.A. Tamada, C.J. King, Extraction of Carboxylic Acids with Amine Extractants. 2. Chemical
6 Interactions and Interpretations of Data, *Industrial & Engineering Chemistry Research*. 29
7 (1990) 1327–1333.
- 8 [40] H. Uslu, Ş. İsmail Kirbaşlar, K.L. Wasewar, Reactive Extraction of Levulinic Acid by Amberlite LA-
9 2 Extractant, *Journal of Chemical & Engineering Data*. 54 (2009) 712–718.
- 10 [41] A. Senol, Extraction equilibria of nicotinic acid using Alamine 336 and conventional solvents:
11 effect of diluent, *Chemical Engineering Journal*. 83 (2001) 155–163.
- 12 [42] M. Moussa, G. Burgé, F. Chemarin, R. Bounader, C. Saulou-Bérion, F. Allais, H.-E. Spinnler, V.
13 Athès, Reactive extraction of 3-hydroxypropionic acid from model aqueous solutions and real
14 bioconversion media. Comparison with its isomer 2-hydroxypropionic (lactic) acid, **Journal of**
15 **Chemical Technology and Biotechnology**. (2015)
- 16 [43] N.L. Ricker, C.J. King, Solvent Extraction of Wastewaters from Acetic-Acid Manufacture, Robert
17 S. Kerr Environmental Research Laboratory (U.S. Environmental Protection Agency), Ada, OK,
18 United States, 1980.
- 19 [44] D. Swern, H.B. Knight, T.W. Findley, Purification of oleic acid, methyl oleate, and oleyl alcohol
20 for use as chemical intermediates, *Oil & Soap*. 21 (1944) 133–139.
- 21 [45] M.A. Liebert, Final Report on the Safety Assessment of Stearyl Alcohol, oleyl Alcohol, and Octyl
22 Dodecanol, *Journal of the American College of Toxicology*. 4 (1985).
- 23 [46] D.L. Lide, *CRC Handbook of Chemistry and Physics*, 84th ed., CRC Press, Boca Raton, FL, 2003.
- 24 [47] J. Śliwiok, T. Kowalska, Spectroscopic and dielectric examination of the oleyl alcohol-oleic acid
25 system, *Microchemical Journal*. 18 (1973) 605–612.
- 26 [48] J.P. Hawranek, A.S. Muszyński, J.Z. Flejszar-Olszewska, Infra-red dispersion of liquid
27 trioctylamine, *Journal of Molecular Structure*. 436 (1997) 605–612.
- 28 [49] C.L. Yaws, P.K. Narasimhan, *Thermophysical Properties of Chemicals and Hydrocarbons*,
29 Chapter 19 Dipole moments - Organic compounds, William Andrew Publishing, Norwich, NY,
30 2009.
- 31 [50] Y. Marcus, *Wiley series in solution chemistry: volume 4, The properties of solvents*, Wiley,
32 Chichester ; New York, 1998.
- 33 [51] M. Matsumoto, K. Mochiduki, K. Kondo, Toxicity of ionic liquids and organic solvents to lactic
34 acid-producing bacteria, *Journal of Bioscience and Bioengineering*. 98 (2004) 344–347.
- 35 [52] M. Vermüë, J. Sikkema, A. Verheul, R. Bakker, J. Tramper, Toxicity of homologous series of
36 organic solvents for the gram-positive bacteria *Arthrobacter* and *Nocardia* Sp. and the gram-
37 negative bacteria *Acinetobacter* and *Pseudomonas* Sp., *Biotechnology and Bioengineering*. 42
38 (1993) 747–758.
- 39 [53] D. Pal, A. Keshav, Recovery of Pyruvic Acid using Tri-n-butylamine Dissolved in Non-Toxic
40 Diluent (Rice Bran Oil), *Journal of The Institution of Engineers (India): Series E*. (2015).
- 41 [54] T. Harington, M.M. Hossain, Extraction of lactic acid into sunflower oil and its recovery into an
42 aqueous solution, *Desalination*. 218 (2008) 287–296.
- 43 [55] A. Keshav, K.L. Wasewar, S. Chand, Reactive extraction of propionic acid using tri-n-octylamine,
44 tri-n-butyl phosphate and aliquat 336 in sunflower oil as diluent, *Journal of Chemical*
45 *Technology and Biotechnology*. 84 (2009) 484–489.
- 46 [56] B. Choudhury, A. Basha, T. Swaminathan, Study of lactic acid extraction with higher molecular
47 weight aliphatic amines, *Journal of Chemical Technology and Biotechnology*. 72 (1998) 111–
48 116.
- 49 [57] J.A. Tamada, C.J. King, Extraction of Carboxylic Acids by Amines Extractant, Lawrence Berkeley
50 Laboratory, CA (USA), United States, 1989.
- 51 [58] J.M. Wardell, C.J. King, Solvent equilibria for extraction of carboxylic acids from water,
52 *Journal of Chemical and Engineering Data*. 23 (1978) 144–148.

- 1 [59] L.-K. Ju, A. Verma, Characteristics of lactic acid transport in supported liquid membranes,
2 Separation Science and Technology. 29 (1994) 2299–2315.
- 3 [60] Encyclopedia of Chemical Technology, Alkanolamines to Antibiotics, 4th edition, Kirk-Othmer,
4 1993.
- 5 [61] J. Cybulski, A. Wiśniewska, A. Kulig-Adamiak, L. Lewicka, A. Cieniecka-Rostonkiewicz, K. Kita, A.
6 Fojutowski, J. Nawrot, K. Materna, J. Pernak, Long-Alkyl-Chain Quaternary Ammonium Lactate
7 Based Ionic Liquids, Chemistry - A European Journal. 14 (2008) 9305–9311.
- 8 [62] A. Coenen, K. Kosswig, F. Von Praun, H.-P. Schüller, Purification of tertiary amines using an
9 adsorbent, 4255356, 1981.
- 10