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Integrated Recovery and Upgrading of Bio-based Dicarboxylates

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Integrated Recovery and Upgrading of Bio-based Dicarboxylates

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de TECHNISCHE UNIVERSITEIT DELFT,
op gezag van de Rector Magnificus PROF. IR. K.C.A.M. LUYBEN;
voorzitter van het College voor Promoties,
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door

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"Porque también somos lo que hemos perdido"

A mis padres, Gloria y Sixto
A mi hermano, Carlos
Porque la distancia es sólo física

À Amanda,
Por teu apoio e compreensão
Por decidir caminhares comigo
Não há palavras

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General introduction

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1.1 Renewable resources as feedstock for chemical building blocks

Our current society makes use of a great variety of goods to satisfy modern life standards, sustain high life expectancy and to allow the existence of an increasing world population on Earth. During the last century, notable developments in chemistry made viable the mass production of materials derived from non-renewable resources, which are now part of almost every aspect of such goods. Consequently, is unavoidable that alternatives to these current materials are required as the continued exploitation and imminent depletion of their forming constituents, oil and gas, have proven adverse social, environmental, political and economic consequences that threat the future of human culture.

The processing route to materials goes from feedstocks through chemical building blocks, which are used to build larger and more complex molecules that can be transformed in all the variety of finished goods known today. Renewable resources, such as biomass, carbon dioxide and waste streams (partially renewable) can be used as feedstocks for producing chemicals. Although the use of carbon dioxide for the production of chemicals could help to a cyclic use of fossil carbon sources, its efficient capture is cumbersome and further conversion into useful chemicals is highly unfavorable from kinetic and thermodynamic standpoints (Dibenedetto et al., 2014), limiting its exploitation as feedstock in the foreseeable future. Waste streams, and particularly wastewater can be transformed mostly through bioprocesses to simple chemicals that can transformed to chemical building blocks, particularly via methanogenic anaerobic digestion to syngas to chemicals (Angenent et al., 2004). However, wastewater supply has major challenges that compromise its use as a truly large scale feedstock.

Biomass, produced at a rate close to 77×10^9 tons/year of equivalent fixed carbon is a very large and available renewable resource (Bozell, 1999). Biomass comprises several fractions

including carbohydrates, oils and fats and proteins. The carbohydrate fraction is of particular interest as accounts for a great part of most crops, for instance, in corn and soybean crops about 45% corresponds to lignocellulosic residues and from the harvested fraction between 33-83% stands as carbohydrate (McLaren, 2001).

The use of biomass as a feedstock is empowered by its diversity, its use mitigates carbon dioxide build up and their known environmental consequences and by its flexibility as can be tailored using genetic modification to produce certain desired qualities. Yet, its adoption as feedstock also faces challenges such as existing production economics, unclear effect on availability as food source, land requirements and seasonal characteristics. Is fair to say, though, that biomass diversity is also disadvantageous as leads to undesired process diversification (Bozell, 1999).

In general, the conversion of biomass to chemicals is done following two approaches. A first route is thermochemical conversion, such as pyrolysis and gasification followed by chemical upgrading, typically requiring high temperatures and pressures to attain high conversions. In order to by-pass such conditions, biomass can be deconstructed to sugars and upgraded via catalytic or biological processes (Schwartz et al., 2014). The latter approach is described in more detail in the next section.

1.2 Bio-based dicarboxylates via sugars from biomass

The utilization of biomass through its deconstruction to sugars can follow a large array of processing routes, as depicted in Figure 1. Complex (lignocellulosic) biomass is pre-treated by different methods to open the biomass structure, increasing surface area and pore size to improve further hydrolysis and partially hydrolyzing hemicellulose. After pretreatment, hydrolysis of cellulose and hemicellulose to sugars is performed, which is the intermediate platform to chemical building blocks.

Sugars can be transformed to a wide range of chemical building blocks via chemical or bio-based (mostly fermentation) conversions. In recent years, discussions at different levels have been promoted to identify the most promising chemicals from biomass. Prominent studies led by the US Department of Energy suggested research focus on 12 building blocks (Werpy et al., 2004) which were later revisited and narrowed to 10 compounds (Bozell and Petersen, 2010). Interestingly, in both selections the majority of compounds corresponded to organic acids due to their strong potential as chemical platform, can be produced using few transformation steps and for which a similar production technology could be applied across molecules (Bozell and Petersen, 2010).

Among organic acids, dicarboxylic acids such as *succinic* and *2,5-furandicaboxylic acid* were highlighted as of great interest, given its technological maturity and its potential to become replacers of already existing non-renewable building blocks. Petrochemical succinic acid is an intermediate of the maleic acid/anhydride route and in that sense renewable succinic acid could act as a direct or drop-in replacer (Taylor et al., 2015). Similarly, 2,5-furandicarboxylic acid has the potential to become a functional replacer of terephthalic acid, an important commodity chemical and precursor (Schwartz, O'Neill, 2014).

As seen in Figure 1, organic acids can be produced via selective catalytic dehydration of sugars or through bio-based transformation, preferably via microbial fermentation. Focus will be on fermentative routes to dicarboxylates as they have been broadly investigated in the recent years, resulting in processes to produce specific molecules arising high industrial interest and important production volumes (Straathof, 2014, Yang et al., 2006)

1.3 Technology opportunities on fermentative dicarboxylates

The bio-based processing route of dicarboxylates from sugars involves fermentative production, recovery and purification of the acid and downstream chemistry to derivatives (Figure 1, shaded box). Linear dicarboxylic acids are produced by *de novo* fermentation of sugars via citrate cycle and related pathways. Although a few wild type organisms are able to produce relevant titers, recombinant microorganisms (bacteria and yeast) have been successfully engineered to overproduce fumaric, malic, itaconic and succinic acids (Straathof, 2014). Differently, the furan derivative 2,5-furandicarboxylic acid can be produced by biotransformation of hydroxymethylfurfural (HMF), a dehydration derivative from fructose or glucose using a recombinant bacteria (Koopman et al., 2010).

The produced dicarboxylates undergo a series of downstream processing steps aimed mostly to remove water and major impurities (primary separation) then purified to the required quality for further transformation into secondary products. As will be discussed in Chapter 2, the pH of the bio-based production of dicarboxylates determines to a great extent its downstream process. Fermentations carried out at neutral pH may lead to recovery processes in which up to equimolar amounts of waste salt respective to dicarboxylates are produced, negatively impacting the implementation of these processing routes.

Until today, research gaps in production strains, downstream processing and catalysis have been studied and developed separately. However, recently the need for further integration across these processing steps has been highlighted, especially integration with chemical conversion steps to: efficiently produce upgraded products or monomers of sufficient purity; improve continuous processing to lever penetration of bio-based products as replacers; and i overcome limitations of a bio-based only route (Schwartz, O'Neill, 2014, Taylor, Nattrass, 2015). Moreover, the sustainability of these routes should be taken into consideration as for current bio-based production of carboxylic acids, such as lactic acid, is partly compromised due to waste generation (Álvarez-Chávez et al., 2012). The rational use of process design strategies such as bioprocessing integration and intensification (Kiss et al., 2015) could derive in new, better and potentially more sustainable processes for bio-based production and upgrading of dicarboxylates.

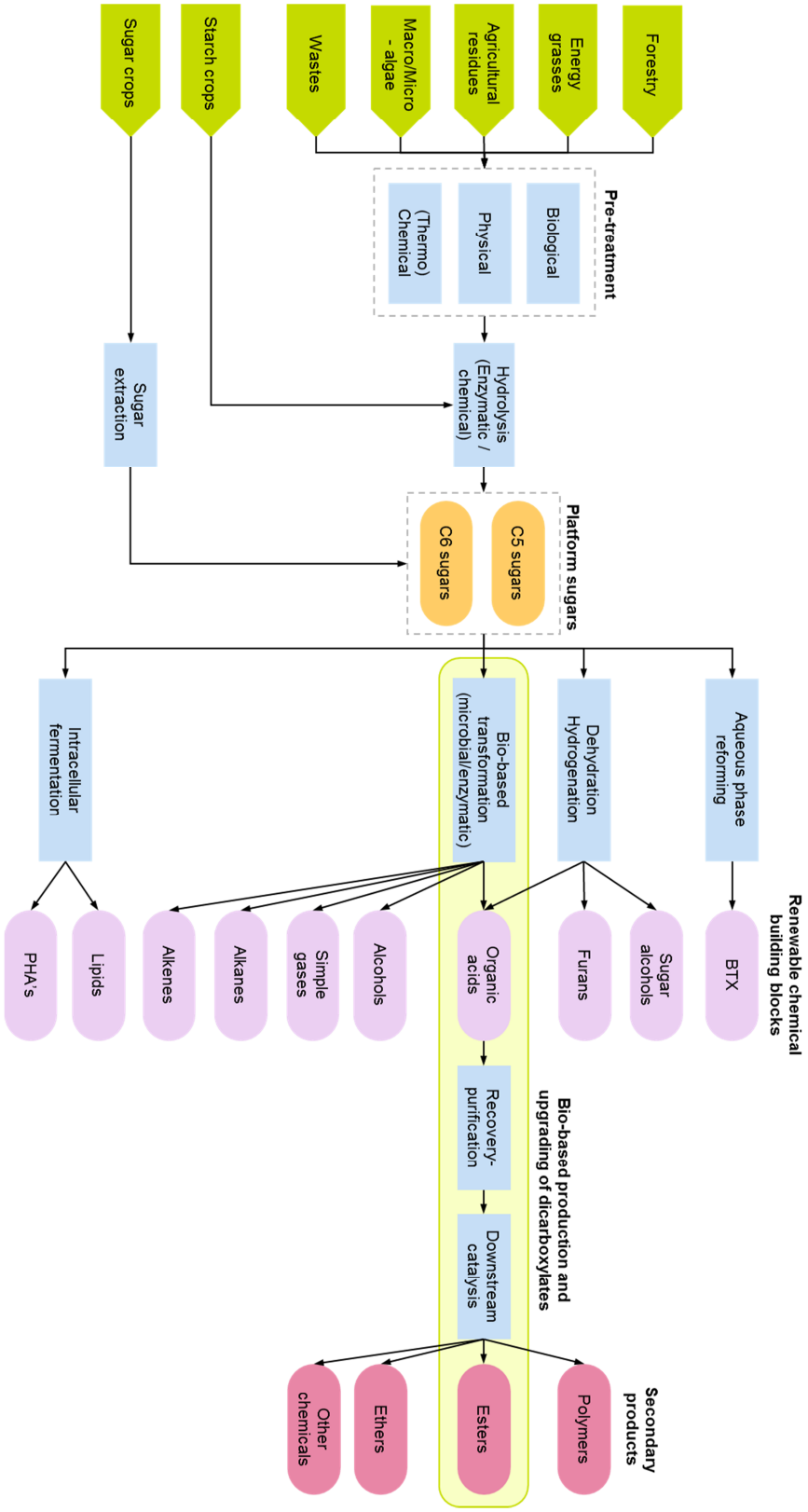

Figure 1. Pathways to renewable chemical building blocks via sugars from biomass. Adapted from Taylor et al., (2015)

1.4 Scope and thesis outline

The aim of this thesis is to develop new options to recover bio-based dicarboxylates, allowing the use of a high productivity microbial fermentation at neutral pH and minimizing the production of waste salt. Succinate and 2,5-furandicarboxylate are used as model molecules for development.

In **chapter 2**, the current literature on recovery of fermentative carboxylic acids is reviewed. Special attention is made on how these processes differ depending on fermentation pH which determines the degree of acid dissociation and thus the applicable recovery principles. All the processing steps, their combination and industrial application are discussed within that scope. Primary recovery is identified as the defining step in the process for which adsorption and extraction are the most suitable operations for soluble carboxylic acids or carboxylates, however is noted that a comparison between them is very limited and often lacking.

Therefore, **chapter 3** compares strong anion exchange extraction and sorption equilibrium for itaconate, fumarate, malate and succinate. Aliquat 336, an ionic liquid, and Dowex Marathon A, a type I strong anion exchange resin, are used as extractant and sorbent, respectively, and the influence of the counterion type (Cl^- and OH^-) in both capture systems is studied. All dicarboxylates showed similar equilibrium parameters, being better captured by the sorbent in OH^- form. As a consequence, challenges in desorption and regeneration for this counterion without salt as byproduct were identified.

In **chapter 4,** a direct downstream catalysis route is devised to explore the transformative potential of strong ion exchange sorbents within downstream processing of dicarboxylates towards further derivatives. An experimental proof of concept is achieved by capturing succinate from aqueous solution and upgrading it to diethyl succinate via O-alkylation with chloroethane, while regenerating the resin for a subsequent capture cycle. In this chapter, two main research leads are identified, the impact of the alkylating agent leaving group type in the overall process stoichiometry and the influence of fermentation impurities in the catalytic performance of the resin, that are respectively addressed in the last chapters of this thesis.

Chapter 5 presents an improved route for succinate to dimethyl succinate using dimethyl carbonate (DMC) as alkylating agent. The DMC alkylation chemistry allows the regeneration of the resin to bicarbonate form, which presents better sorption equilibrium and leads to a process without waste salt production if integrated with a succinate fermentation.

Lastly, in **chapter 6** the green upgrading route presented in the previous chapter is validated for bio-based succinate and furandicarboxylate. Fermentation by-products decrease sorption capacity towards the target dicarboxylate, but good dimethyl ester yields are obtained for both model molecules. Interestingly, the captured carboxylate impurities are also converted into their respective esters, opening opportunities to extend the applicability of the proposed downstream route to other bio-based carboxylates.

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2

Recovery of carboxylic acids produced by fermentation

Carboxylic acids such as citric, lactic, succinic and itaconic acid are useful products and are obtained on large scale by fermentation. This review describes the options for recovering these and other fermentative carboxylic acids. After cell removal, often a primary recovery step is performed, using liquid-liquid extraction, adsorption, precipitation or conventional electrodialysis. If the carboxylate is formed rather than the carboxylic acid, the recovery process involves a step for removing the cation of the formed carboxylate. Then, bipolar electrodialysis and thermal methods for salt splitting can prevent that waste inorganic salts are co-produced. Final carboxylic acid purification requires either distillation or crystallization, usually involving evaporation of water.

Process steps can often be combined synergistically. In-situ removal of carboxylic acid by extraction during fermentation is the most popular approach. Recovery of the extractant can easily lead to waste inorganic salt formation, which counteracts the advantage of the in-situ removal.

For industrial production, various recovery principles and configurations are used, because the fermentation conditions and physical properties of specific carboxylic acids differ.

Keywords: Organic acids, succinic acid, lactic acid, citric acid, reactive extraction, adsorption, electrodialysis, precipitation, ion exchange, recovery.

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2.1 Introduction

Carboxylic acids are the best known type of organic acids. They are used on large scale in the chemical and food industry. Many carboxylic acids can be conveniently produced from carbohydrates or other renewable raw materials by fermentation or whole-cell biotransformation using pure cultures (Goldberg et al., 2006, Sauer et al., 2008, Straathof, 2014, Yang et al., 2007). Anaerobic degradation of waste streams by mixed cultures is a source of so-called volatile fatty acids (such as acetic, propionic and butyric acid). The current interest in a bio-based economy triggers the improvement of existing processes and, for carboxylic acids not previously available by fermentation, the development of completely new processes. Dedicated production of carboxylic acids is already performed on large scale for citric acid, lactic acid, D-gluconic acid, itaconic acid, and 2-keto-L-gulonic acid. Succinic acid production is currently being commercialized by several companies (Mc Coy, 2009), while other acids, not previously available by fermentation, such as 3-hydroxypropionic acid (Della Pina et al., 2011), acrylic acid (BASF, 2013) and adipic acid (Beardslee and Picataggio, 2012) are in advanced stages of industrial development and will probably be commercialized in the coming years. The status of the carboxylic acids is given in Table 1**1**, which includes a number of carboxylic acids for which process development is still in an early phase.

The production costs of carboxylic acids will generally be dominated by feedstock costs and some other upstream costs, but the contribution of downstream processing (DSP) costs is also large, typically 30-40% of the total production costs (Straathof, 2011). Thus, development of a competitive recovery process is critical to enable bio-based production of a carboxylic acid. This has led to a high need for a good overview of recovery alternatives that might be used.

Most of the existing reviews in this field focus on a single, well-known carboxylic acid, and discuss how it has been recovered so far. There are reviews dealing with lactic acid (Datta and Henry, 2006, Joglekar et al., 2006, Wasewar et al., 2004b), citric acid (Dhillon et al., 2011, Pazouki and Panda, 1998, Soccol et al., 2006), succinic acid (Cheng et al., 2012a, Cheng et al., 2012b, Kurzrock and Weuster-Botz, 2010), and itaconic acid (Klement and Büchs, 2013), amongst others. Some other reviews (Milsom and Meers, 1985b, Rogers et al., 2006) treat several carboxylic acids sequentially, and include their recovery. There are also reviews that focus on a particular recovery method, and discuss its application in the field of carboxylic acids. For example, there are reviews focusing on solvent extraction (Hong et al., 2001a, Kertes and King, 1986, Schügerl, 2005, Yang et al., 2007), adsorption (Garcia, 1991), or electrodialyis (Bailly, 2002, Huang et al., 2007) of carboxylic acids. Moreover, there are books that treat all relevant recovery methods in the area of fermentation (Belter et al., 1988, Harrison et al., 2003, Prasad, 2010, Wesselingh and Krijgsman, 2013), but these do not focus on carboxylic acids, in contrast to the current review. We aim to treat the recovery principles that are relevant in the context of a generic process configuration for producing fermentative carboxylic acids. Fermentation pH and type of inorganic acid or base that might be used in the process will also be treated, because these may determine which recovery methods can actually be used, how process steps can be integrated, and how side streams may be reused in the process.

Per recovery principle that will be described, the number of relevant scientific publications and patents is sometimes very large, and in such cases only a selection will be cited in this

review. Concerning recovery of some specific carboxylic acids or concerning some specific recovery methods, the aforementioned reviews provide more detail.

For simplicity, we confine our analysis to recovery of carboxylic acids containing C, H, and O atoms, thus excluding amino acids and penicillin, for example. Also, poly-3-hydroxybuyrate and related fermentation products are left out of the scope.

Not all carboxylic acids need to be recovered from their fermentation broth. For example, the acetic acid that is used in food industry is traditionally produced by biological oxidation of ethanol, and is not isolated. Sometimes a salt of a carboxylic acid rather than the carboxylic acid itself is the desired products and may be isolated, but in this review we focus on recovery of undissociated carboxylic acids.

Table 1. Carboxylic acids of commercial interest for production by fermentation or biotransformation of renewable resources**.** See also (Straathof, 2014).

Molecular formula	Carboxylic acid	Status biochemical production	Main application	Literature entry
$C_2H_4O_2$	Acetic	Industrial	Vinegar	(Xu et al., 2011)
$C_3H_4O_2$	Acrylic	Research	Polymers	(Straathof et al., 2005)
$C_3H_6O_2$	Pyruvic	Research	Chemicals	(van Maris et al., 2004)
$C_3H_6O_2$	Propionic	Design stage	Chemicals	(Liu et al., 2012)
$C_3H_6O_3$	D/L-Lactic	Industrial	Food, polymers	(Miller et al., 2011)
$C_3H_6O_3$	3-Hydroxy-propionic	Research	Polymers	(Jiang et al., 2009)
$C_4H_4O_4$	Fumaric	Formerly industrial	Food, polymers	(Straathof and Van Gulik, 2012)
$C_4H_6O_4$	Succinic	Industrial	Polymers, chemicals	(McKinlay et al., 2007)
$C_4H_6O_5$	L-Malic	Research	Chemicals	(Zelle et al., 2008)
$C_4H_8O_2$	Butyric	Design stage	Chemicals	(Dwidar et al., 2012)
$C_5H_6O_4$	Itaconic	Industrial	Polymers	(Klement and Büchs, 2013)
$C_5H_8O_4$	Glutaric	Research	Polymers	(Otto et al., 2011)
$C_6H_4O_5$	2,5-Furan-dicarboxylic	Research	Polymers	(Koopman et al., 2010)
$C_6H_8O_7$	Citric	Industrial	Food	(Soccol et al., 2006)
$C_6H_{10}O_4$	Adipic	Design stage	Polymers	(Polen et al., 2013)
$C_6H_{10}O_7$	2-Keto-L-gulonic	Industrial	Vitamin C precursor	(Cui et al., 2012)
$C_6H_{12}O_7$	D-Gluconic	Industrial	Food	(Rogers et al., 2006)

At industrial scale, the key requirements of a good recovery process are:

- Purity according to specification, which might be >99.5% for dicarboxylic acids that need to be used for polymerization (Musser, 2005). Then, monocarboxylic acid impurities might terminate polymerization and should be at very low levels.
- High extent of recovery, i.e. 90-100% yield in the DSP part of the process.

- Low chemicals and energy consumption and low waste production during product recovery.
- Modest investment costs, due to efficient mass and heat transfer in the recovery equipment.

To achieve the required purification, DSP typically has to fulfill the following functions (see Figure 1):

1. **Clarification.** Removal of large particles, mostly cells and their debris.
2. **Primary recovery.** Removal of product from bulk aqueous solution and major impurities.
3. **Counterion removal.** Replacing the cation of a carboxylate by H^+ to get carboxylic acid (if required).
4. **Concentration / purification.** Removal of the bulk solvent or capture of the carboxylic acid, achieving concentration. Removal of remaining impurities.
5. **Upgrading.** Transformation to chemical derivatives (if required).
6. **Formulation.** Adaptation of product to storage and customer needs.

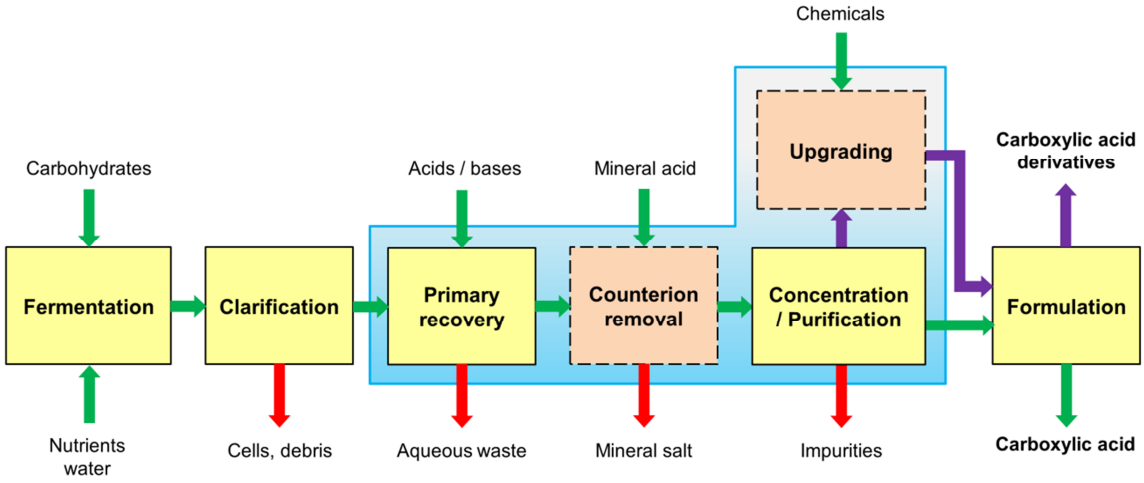

Figure 1. General downstream processing sequence for the recovery, purification and upgrading of fermentative carboxylic acids. Dashed boxes are optional steps. Within the blue region the steps are covered in this review.

This review will treat mainly steps 2 to 4 and discuss relevant integrated alternatives involving further chemical upgrading. In practice not all steps are always necessary, often they may be combined (integrated), and their order may be different than in Figure 1.

Cell removal is usually the first DSP step and done by filtration or centrifugation, like for other types of fermentation products. Cell retention may be favorable (Meynial-Salles et al., 2008), but will be complicated if the fermentation would lead to poorly soluble products such as fumaric acid or calcium citrate. Cell removal may be combined with primary recovery, for example if liquid-liquid extraction is used (Wennersten, 1983). In some other cases in which recovery via adsorption is carried out in expanded bed mode, cell removal is not required (Li et al., 2011). However, cell removal or retention will not be treated in this review. The focus will be on removal of (cationic) counterions, water, and impurities. Since the physical

properties of the target carboxylic acids are widely different (see Table 2), there is not a single recovery process that can be used for all.

Yet, some carboxylic acids may be grouped on the basis of physical properties and they should be recoverable in a similar way, for example acetic, propionic, and butyric acid; and succinic and itaconic acid.

Table 2. Physical properties of carboxylic acids (Weast, 1979, Windholz, 1976).

Acid name	pK_a values	Solubility in water (g/L)	Melting point (°C)	Boiling point (°C)
Acetic	4.75	Miscible	17	118
Butyric	4.81	Miscible	-8	163
Citric	3.14; 4.77; 6.39	~600	153	decomposes
Fumaric	3.03; 4.44	6.3	sublimes	200
Gluconic	3.60	Good	131	decomposes
3-Hydroxypropionic	4.51	High	<25	decomposes
Itaconic	3.85; 5.45	80-95	165 (decomp.)	
Lactic (D or L)	3.86	High	53 (18 for DL)	decomposes
Malic	3.40; 5.11	558	130	decomp. >140
Propionic	4.87	Miscible	-21	141
Pyruvic	2.50	Miscible	12	decomp. 165
Succinic	4.16; 5.61	77	185-187	235

2.2 Acids and bases used for carboxylic acid recovery

Typical for carboxylic acid processes are switches between uncharged carboxylic acid and anionic carboxylate. This involves the use of several types of bases and (other) acids. Because the choice of the types is important for the process structure and economics, the most common types will be treated before discussing specific parts of the recovery processes.

2.2.1 Inorganic acids used

Table 3 Table 3 indicates inorganic acids that are considered for converting carboxylate into carboxylic acids and also for other acidification steps in the recovery process. With respect to costs per equivalent H^+ and effectiveness, sulfuric acid is usually superior, but in specific cases other acids may be more useful.

CO_2 deserves a special discussion because of recycling and integration options (see sections 2.7.1: Thermal cracking of inorganic salt and 2.8: Integration with esterification and other reactions), its use as co-substrate in some carboxylic acid fermentations, its nontoxicity, its low price, and its availability at production sites. The pK_a values of the actual acidic species (H_2CO_3) are 3.6 and 10.3. The apparent pK_a of CO_2 depends on the equilibrium of the reaction $H_2O + CO_2 \rightarrow H_2CO_3$. The achievable pH depends on the partial pressure of CO_2. The pH value is 3.72 for 1 bar CO_2 and 3.42 for 10 bar CO_2. This allows some extent of protonation of carboxylic acids, if their pK_a is high. Acidification by CO_2 can be favorably combined with

liquid-liquid extraction and precipitation options (van Halsema et al., 1998) such as discussed elsewhere in this review. Designing such systems requires models that can simultaneously solve dissociation, partitioning and precipitation equilibrium.

Table 3. Inorganic acids considered for converting carboxylate into carboxylic acid.

Acid	pK_a [a]	Approximate price ($/kg)	Approximate price ($/kmol H^+ equivalent)	Remarks
HCl	<0	0.23 [c]	8	Corrosive
H_2SO_4	<0; 1.92	0.08 [c]	4	Sulfate salts may precipitate
NH_4HSO_4	1.92	N/A	N/A	Used in recycling option (section 2.7.1)
H_3PO_4	2.12; 7.21; 12.67	1.0 [c]	100	Often only one useful H^+ equivalent
CO_2	6.27[b]; 10.25	0.05 [d]	2	Often too weak; not very soluble in water

[a] (Weast, 1979).
[b] pK_a accounting for the coexistence of dissolved CO_2 and H_2CO_3.
[c] The source (Anonymous, 2006) gives price ranges and various qualities, so the actual values may deviate considerably.
[d] Wide price ranges (IAE/UNIDO, 2011).

2.2.2 Inorganic bases used

Table 4 indicates inorganic bases that are considered for converting carboxylic acid into carboxylate and also for other alkalinization steps in the recovery process. With respect to costs per equivalent OH^-, there are several good options with calcium hydroxide giving the lowest costs. Although not an inorganic base, trimethylamine has been included because it may be used as alternative in some recycling options (see sections 2.4.1, 2.4.2 and 2.5.1).

Table 4. Examples of bases used or considered for controlling fermentation pH

Base	pK_a [a]	Approximate price[b] ($/kg)	Approximate price ($/kmol OH^- equivalent)	Aqueous solubility at 25 °C (g/L)[a]
NaOH	>14	0.62	25	~1000
KOH	>14	0.72	40	~1000
$Ca(OH)_2$	11.6; 12.6	0.077	3	~ 1.5
$Mg(OH)_2$	cf. $Ca(OH)_2$	0.24	7	~0.012
$NaHCO_3$	6.37	0.54	45	~90
Na_2CO_3	6.37; 10.25	0.15	8	~100
K_2CO_3	6.37; 10.25	0.87	60	~1000
$CaCO_3$	6.37; 10.25	0.1	5	~0.015
$MgCO_3$	6.37; 10.25	N/A	N/A	~2
NH_3 (NH_4OH)	9.25	0.31	5	Miscible
Trimethylamine	9.81	N/A	N/A	Miscible

[a] (Weast, 1979). [b] The source (Anonymous, 2006) gives price ranges and various qualities, so the actual values may deviate considerably.

2.2.3 Water-insoluble amine, ammonium and related bases used

The literature contains many descriptions of recovery operations involving interaction of carboxylic acid or carboxylate with bases containing amine or ammonium groups, for example Wasewar (2005). Often water-insoluble solid materials or liquids contain such groups. These are used as adsorbents and extractants, respectively. Their interactions with carboxylic acids are discussed here collectively, whereas adsorption and extraction processes are discussed later in this review.

Amine functionality has been explored extensively aiming for recovery process tailored to the target carboxylic acid. Long-chain alkyl-substituted primary, secondary and tertiary amines are used in extraction processes and short-chain (usually methyl) alkyl-substituted homologues introduced in solid matrices comprise functionalized adsorbents. Similarly, quaternary ammonium salts are also employed in both processes, see for example Wasewar (2005). Most of the studied extractants and sorbents are commercially available under registered names, thus trade names rather than a systematic chemical name are commonly found in the field. In the particular case of adsorbents, the exact nature of their chemical structures is typically unknown, which in certain cases might impede a deep analysis of their interaction mechanisms with carboxylic acids.

Table 55 summarizes the key aspects of selected amines used in extraction and adsorption applications. The basicity of amines, which can be discussed indirectly based on the acidity of their conjugate acids (pK_a), depends mainly on their degree of substitution, the nature and length of the substituent chains and the degree of solvation. The former two aspects influence directly the electronic properties of the amine and the availability of the ion pair of electrons from nitrogen. As a result of these features and for the compounds relevant to this review, i.e. long chain alkyl and polymer bound amines, it has been observed that for each type of amine, a broad range of basicity is possible. Quaternary amines (pK_a values > 10) are the strongest bases followed in order of decreasing basicity by primary and secondary (pK_a values between 5 and 10) and tertiary amines (pK_a values from 3 up to 10) (Cichy et al., 2005, Evangelista et al., 1994, Eyal and Canari, 1995, Garcia and King, 1989, Shan et al., 2006, Syzova et al., 2004). It should be noted that liquid alkyl amines are less basic than their polymer supported counterparts for the same amine type, as a consequence of the alkyl substituents length required for the liquid amines to be reasonable water insoluble. Moreover, the basicity of these amines is usually determined as an apparent basicity, meaning that the measured pK_a corresponds to the basicity of the separation system including organic solvents or polymer backbones. Interestingly, the liquid systems appear to influence the amine basicity to a larger extent than the polymer structures do (Evangelista et al., 1994, Kertes and King, 1986, Shan et al., 2006).

The different types of water-insoluble bases mentioned in this section can have very different types of interactions with carboxylic acids. In general, three interaction mechanisms are present: hydrogen bonding, ion pairing and ion exchange. Moreover, in the case of extraction, solvation should also be considered. The reader is referred to the comprehensive publications of Canari and Eyal for a deep understanding of the characteristics of such interactions (Canari and Eyal, 2003, Eyal and Canari, 1995).

Table 5 5 shows that it matters if the amine is primary/secondary, tertiary or quaternary, and that the ammonium counterion can also determine the interactions. In many cases, there will be a strong electrostatic interaction, sometimes supported by H-bonding. In other cases, only this weaker H-bonding occurs. In case of tertiary amine with carboxylate, only hydrophobic interactions can occur, so then there is no clear incentive to use ammonium compounds. The extent of occurrence of such interactions depends on the amine basicity, pK_a of HA, HX and X^- and aqueous pH. It has been observed that a more complicated interaction scheme is found at pH < pK_a, where generally more than one interaction occurs simultaneously, as reported in literature.

Recently, the application of a new generation of bases containing multiple nitrogen groups in recovery processes was reported (Krzyżaniak et al., 2013). Guanidines and piperazines might behave similarly to amine bases providing much stronger acid-base interactions. Contrary to amines, guanidinium groups present higher temperature stability and therefore their use in integrated transformation options has been suggested (López-Garzón et al., 2014).

The design of recovery systems for carboxylic acids in which these auxiliary materials are used requires the understanding of such interactions. Their type and strength will ultimately determine the possibility of back-extraction or desorption of the target molecule compromising economic and environmental aspects of the overall process. Sections 2.4.1, 2.4.2 and 0 will describe the application of these different types of interaction in adsorption, extraction and upgrading processes.

2.3 Fermentation pH

Carboxylic acids produced by fermentation typically have pK_a values in the range 2.5 to 6.5 (see Table **2** 2). In case of multiple carboxylic acid groups per molecule, the lowest pK_a value is below 5. Therefore, their production will acidify the medium. For example, the pH of an aqueous solution of 0.01 mol/L acetic acid (0.6 g/L) will be 3.4, which might easily be detrimental to neutrophilic production organisms. Production might stop at very low levels.

To achieve a reasonable production, e.g. 50 g of carboxylic acid per liter fermentation broth, different strategies can be pursued:

1. Use an acid-tolerant production organism and then continue the fermentation until the microorganism stops producing due to acid stress.
2. Remove the carboxylic acid *in-situ* by a chemical reaction with the result that acid stress diminishes and fermentation can continue. Usually this reaction is conversion of carboxylic acid into carboxylate salt by titration with a base at controlled, neutral pH.
3. Remove the carboxylic acid *in-situ* by a physical method with the result that acid stress diminishes and that fermentation can continue. This option becomes more attractive if higher carboxylic acid concentrations are tolerated by the microorganism, such that a higher driving force is available for in-situ product removal (ISPR).

Table 5. Electrostatic and H-bonding interactions of carboxylic acids (HA) and carboxylates (A^-) with amine bases (B) or ammonium compounds (Q). X^- is an inorganic anion, such as Cl^-.

Fermentation pH	Main carboxylate species	Amine base Q or ammonium compound B	Interaction mechanism	Remarks
pH < pK_a	HA	Primary, secondary, or tertiary amine (B)	• Acid-base reaction leading to BH^+ A^- ion pairing • H-bonding B:HA	• Ion pairing prevails over H-bonding for strong basic B • H-bonding between the amine and hydroxyl moiety of the acid
		Primary, secondary or tertiary ammonium salt[b] (BH^+X^-)	• H-bonding BH^+X^-:HA • Anion exchange reaction leading to BH^+A^-	Anion exchange will occur only for A^- being weaker base than X^-
		Quaternary ammonium hydroxide (Q^+OH^-)	Anion exchange reaction leading to Q^+A^-	
		Quaternary ammonium salt[b] (Q^+X^-)	• H-bonding Q^+X^-:HA • Anion exchange reaction leading to Q^+A^-	Anion exchange will occur only for A^- being weaker base than X^-
pH > pK_a	A^-	Primary or secondary amine (B)	H-bonding B:A	H-bonding between the amine and the carbonyl moiety of the acid
		Tertiary amine (B)	None[a]	
		Primary, secondary or tertiary ammonium salt[b] (BH^+X^-)	Anion exchange reaction leading to BH^+A^-	
		Quaternary ammonium hydroxide (Q^+OH^-)	Anion exchange reaction leading to Q^+A^-	
		Quaternary ammonium salt[b] (Q^+X^-)	Anion exchange reaction leading to Q^+A^-	

[a] Hydrophobic interactions occur in all cases and are not indicated in this table. [b] Several inorganic anions may be used.

These three strategies can be combined. For example, lactic acid might be produced by a strain that can produce at pH values as low as 3. In the used fermentor the pH value might be controlled at 3 using NaOH (such that lactic acid partly becomes sodium lactate). Simultaneously, the undissociated lactic acid may be removed from the solution by addition of a lactic acid-specific adsorbent. This reduces the amount of NaOH to be added for pH control and is already a first step in product recovery. The options for the three strategies and their combinations will be treated in the remainder of this review.

2.3.1 Fermentation at low pH

For several carboxylic acids, fermentation has been pursued at low pH (i.e. below or close to the lowest pK_a of the produced carboxylic acid) to minimize the consumption of bases for pH control and the subsequent re-conversion of carboxylate into carboxylic acid. Table 6 shows that usually higher concentrations have been achieved at neutral pH (cf. (Yang et al., 2007)), but for acetic and citric acid the highest values have been found at low pH.

A 'weak-acid uncoupling' mechanism is usually cited as the major mechanism underlying carboxylic acid toxicity to microbial cells (Abbott et al., 2007). At low extracellular pH, the carboxylic acids occur predominantly in the undissociated form, which have relatively high membrane permeability and may enter the cell via passive diffusion. This acidifies the cell and triggers ATP-dependent efflux of protons. At high extracellular carboxylic acid concentrations, ATP exhaustion, acidification of the cytoplasm and dissipation of the proton-motive force may occur. The anion of the weak acid is much less membrane-permeable than the undissociated acid, and may accumulate intracellularly, to reach concentrations that are toxic due to mechanisms such as membrane disruption and enzyme inhibition.

On the basis of these mechanisms, the tolerance of microorganisms toward undissociated carboxylic acids should be higher if the carboxylic acid diffuses slower through the cell membrane because of higher molar mass and lower hydrophobicity. The capacity of the cell to export the carboxylic acid sufficiently rapidly is another variable, which will depend on the cell type and its metabolic state (Rogers et al., 2006, Sauer et al., 2008). The cell may generate sufficient ATP from the production of the carboxylic acid for export of the acid and for cell maintenance, but growth might not be possible anymore at an inhibiting carboxylic acid concentration. Depending on the fermentor configuration, lower cell mass concentrations and consequently lower volume-specific productivities in the fermentor may be achieved at lower pH, in turn leading to lower achievable carboxylic acid concentrations.

The data shown in Table 6 are in qualitative agreement with these mechanisms, but quantitative understanding of the achievable concentrations of carboxylic acids is still absent due to the interplay of mechanisms. With the exception of *Acetobacter*, Table 6 contains eukaryotic microorganisms at low pH. Often these are more resistant to carboxylic acids than prokaryotes are.

Table 6. High carboxylic acid titers obtained at low fermentation pH and compared to neutral pH results.

Acid	Low pH fermentation optimization				Neutral pH fermentation optimization			
	Final titer (g/L)	Final pH	Strain	Reference	Final titer (g/L)	Final pH	Strain	Reference
Acetic	200	~2	*Acetobacter*	(Arnold et al., 2002)	Not optimized			
Citric	240	2.1	*Aspergillus niger*	(Papagianni et al., 1999)	Not optimized			
Fumaric	20	3.5	*Rhizopus oryzae*	(Engel et al., 2011)	126	~6	*R. oryzae*	(Ling and Ng, 1989)
Gluconic	140	2.1	*A. niger*	(Sankpal et al., 1999)	504	6.5	*Aureobasidium pullulans*	(Anastassiadis et al., 1999)
Itaconic	90	2	*Aspergillus terreus*	(Kuenz et al., 2012)	113	~7	*Pseudozyma tsukubaensis*	(Bodinus et al., 2011)
L-Lactic	135	3.0	Yeast strain	(Miller et al., 2011)	210	6.2	*Lactobacillus lactis*	(Bai et al., 2003)
Succinic	96	3	*Saccharomyces cerevisiae*	Verwaal[a]	146	~7	*Corynebacterium glutamicum*	(Okino et al., 2005)

[a] Presentation at BBPOA (Symposium on Bio-based Production of Organic Acids) Frankfurt, 10-11 May 2012

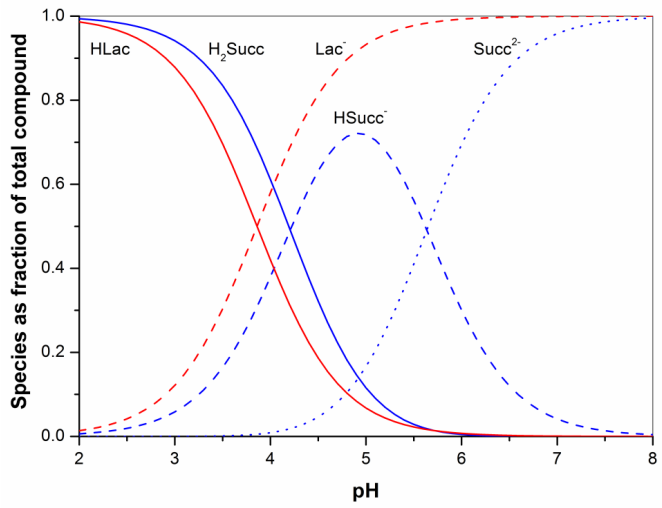

Figure 2. Undissociated fraction (solid lines), monodissociated fractions (dashed lines) and double dissociated fraction (dotted line) of the total of lactic acid species and of the total of succinic species.

Despite the low pH values in the left hand side of Table 6, part of the carboxylic acids will still be dissociated. The calculated proportion dissociated and undissociated acid as function of pH for some carboxylic acids is given in Figure 2. Clearly, to achieve a proportion of 99% undissociated acid requires a pH that is two units below the lower pK_a value as given in Table 2. Thus, for lactic acid this pH is somewhat lower than for succinic acid, as shown in the figure, but according to Table **Table 2**2 very low pH values need to be achieved for pyruvic, fumaric, and citric acid. The calculations for Figure 2 assume dilute aqueous solutions, but the conditions mentioned in Table 6 generally involve ionic strengths exceeding 0.2 mol/L. At such high ionic strengths, favorable electrostatic interactions between dissolved ions favor the acid dissociation reactions and lead to apparent pK_a values significantly lower than those in Table **Table 2**2, so the actual extent of dissociation might be somewhat lower than in Figure 2 (Roa Engel et al., 2013).

2.3.2 Fermentation at neutral pH by conversion of carboxylic acid into carboxylate

In this context, "neutral pH" is roughly the range 6-8, which is above the (higher) pK_a of the carboxylic acid.

If, directly after its formation at neutral pH, the –COOH group of the carboxylic acid can be converted, the carboxylic acid formation reaction might not lead to acidification of the fermentation medium. Reactions like esterification or amidation will require catalysts and nonaqueous condition to proceed to a significant extent. In fermentation medium, no easy conversion of the -COOH group is available besides conversion into carboxylate (-COO^-) salt, which merely requires base addition. Nonetheless, while at neutral pH the stress imposed over the cell by acidic conditions is avoided, the carboxylate and its counterion can also exert an inhibitory effect.

Examples of neutralization bases that might be used are given in Table 4. Of course the bases' price is important, but also the waste disposal needs to be taken into account, and in later sections some options for recycling will be given.

Carbonate bases will release CO_2 upon carboxylate formation. This will be favorable for several carboxylic acid fermentations, such as those for citric, succinic, malic, fumaric and

itaconic acid, because the metabolic pathways of these fermentations involve carboxylation of pyruvate or phosphoenolpyruvate, thus consuming CO_2. Increased concentrations of dissolved CO_2 may increase the yield of product on carbohydrate feedstock, and increase the productivity (Rogers et al., 2006).

NH_3 (and potentially also other nitrogen-containing bases such as trimethylamine) can be used as nitrogen source by the production organism. In that case cell growth, which may interfere too much with carboxylic acid formation, may be controlled using limitation of other elements required for growth, for example phosphorous (Riscaldati et al., 2000).

Although ammonia and trimethylamine will be toxic, the prevalent species at neutral pH will be ammonium and trimethylammonium ion. When using the latter, lactic acid fermentation by *Lactobacillus* sp. MKT878 proceeded better than when using calcium or ammonium counterion (Hetenyi et al., 2011).

The tolerance of the microorganism to the cation of the base is important, because this cation will reach high concentrations. However, osmolarity was only marginally important in succinate fermentations using *E. coli* (Andersson et al., 2009). Flocculation of *A. succinogenes* was reported when using sodium carbonate but not when using magnesium carbonate (Liu et al., 2008). The solubility behavior of the carboxylate due to the cation will also matter. For succinate production using *E. coli*, $Ca(OH)_2$ was more favorable as neutralizing agent than NaOH or KOH, probably because of calcium succinate precipitation (Lu et al., 2009). A comparable situation was found when comparing $CaCO_3$ and $NaHCO_3$ for fumaric acid production using *R. oryzae* (Zhou et al., 2002). It was assumed that the higher productivity when using $CaCO_3$ would be offset by the complications caused by calcium fumarate precipitation in the fermentor.

In summary, the neutralization of carboxylic acids during fermentation will alleviate inhibition of acid-sensitive microorganisms. The neutralizing base choice is determined by its price, the potential of the base to be C- or N-source to the microbial cells, the solubility of the resulting carboxylate, and the ease to remove the cation in the subsequent process stages. Nevertheless, the desired high carboxylate titers might also have adverse effects in the fermentation performance.

2.3.3 Control of fermentation pH by ISPR

ISPR (in-situ product removal or recovery) is generally used to allow the prolonged production of an inhibiting or toxic product (Woodley et al., 2008). In this sense, removal of carboxylic acids during their fermentative production can be beneficial because both the dissolved carboxylic acid and the associated H^+ concentration may be inhibiting.

The removal of the carboxylic acid can be used to control the fermentation pH, as removed carboxylic acid does not decrease the pH in the fermentor. Several strategies can be used and all deal with the transfer of the acid to another phase (solid, immiscible liquid, or vapor). As can be anticipated, such a process might imply a primary recovery of the acid product, leading to a potentially simplified downstream processing. Due to that, specific ISPR options will be discussed in further sections. The default and simpler *ex-situ* removal options will be treated first.

2.4 Primary recovery

After removing cells from fermentation broth, the aqueous carboxylic acid or carboxylate solution will contain numerous impurities such as sugars and salts remaining from the fermentation feed, fermentation by-products such as proteins and undesired carboxylic acids, and debris derived from the cell lysis and/or decay. In many recovery strategies, the bulk of the impurities can be removed by selectively transferring the product to another phase. This can be an extractant phase, adsorbent phase, a precipitate phase, or an aqueous phase behind a membrane. Such capture or primary recovery steps are collectively treated in this section because usually one of them will be selected.

Adsorption and extraction dominate the literature on primary recovery of carboxylic acids. Numerous options have been suggested, due to the wide variety of interactions between carboxylic acids and amine-based adsorbents and extractants, as discussed before (see Table 5). The high prices of the adsorbents and extractants as compared to the recovered carboxylic acids, and the high costs of treating them as waste, are incentives to regenerate the adsorbents and extractants virtually completely for use in a next cycle. Adsorbents have features very different from extractants, such as equipment to be used, price, process losses, fouling behavior, and safety aspects.

Adsorption is usually done using packed beds and extraction using different types of stirred extraction columns. For extraction, contacting of the phases may also be done via a membrane, being membrane-based solvent extraction, supported liquid membranes and pertraction the most typical modes. No details will be given of the actual equipment type used, because that has no major influence on chemicals consumption and materials integration possibilities within the overall process. However, the next sections describe for each adsorption or extraction option also the regeneration options, focusing on the associated chemicals consumption and implications for the overall process.

2.4.1 Adsorption

The power of adsorption stems from the possibility of designing the surface chemistry of the sorbent to selectively recover target molecules (Kalyanpur, 2002). One of the main advantages of adsorption operations over extraction is the ease of the auxiliary phase removal. Solid adsorbents confined in columns are effortlessly handled in comparison to liquid-liquid systems in which phase separation might require either large equipment or energy demanding operations. This aspect also has consequences in the material's consumption as a difficult phase separation causes solvent losses. A disadvantage, though, is that adsorbents are prone to fouling which may limit the operational lifetime of the material.

The adsorbents relevant to the recovery of carboxylic acids and carboxylates can be classified according to their electronic properties in ionic and nonionic materials and more thoroughly by its functionality and support morphology, as seen in Table 7. Ionic materials, also known as ion exchangers, capture carboxylic species according to the interactions described in Section 2.2.3. Thus, weak anion exchangers become charged over a limited pH range and otherwise are not able to exchange anions whereas strong anion exchangers

exchange anions over a broad pH range. It is stressed here that weak anion exchangers do not actually perform anion exchange during the capture process, instead, an overall exchange is complete when the carboxylic acid is desorbed. A more detailed description of this process will be given in the coming section.

Key factors such as diffusivity, ion selectivity, exchange kinetics and osmotic stress resistance depend on the morphology of the bead. Microporous (or gel-type polymer) and macroporous (or macroreticular polymer) ion exchangers prevail (Alexandratos, 2009). Other supporting materials such as silica and zeolite are available, but their application is restricted due to mechanical stability, particle size and availability.

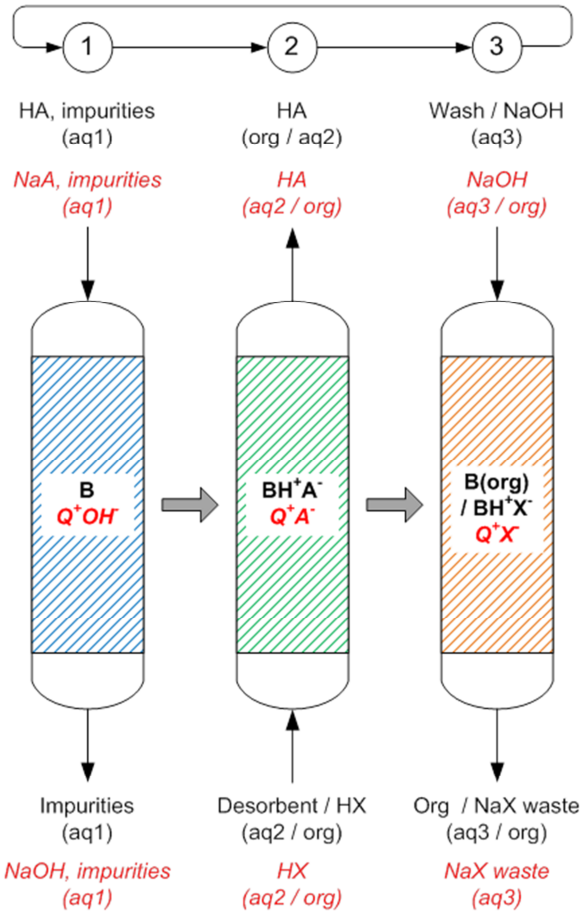

Figure 3. Operation scheme of a sorption, anion exchange-based, recovery of carboxylic acids and carboxylates. Text in black and red corresponds to a weak and strong anion exchange operation, respectively. Numbers on top refer to the cyclic steps of the operation: 1. Sorption, 2. Desorption, 3. Washing / regeneration (if required).

To be industrially applicable, Davison et al. (2004) have estimated that for industrial applicability a minimum capacity for carboxylic acid of 0.05 g/g is required, and this should remain during prolonged processing. Figure 3 depicts an operation cycle for a recovery of carboxylic acids or carboxylates based on anion exchange in a packed column, being the most representative sorption technique relevant to our discussion. In the way described, the process produces directly a purified acid rather than a salt, as could be the case depending on the desorbent. During stage 1 the solute interacts with the functional group on the resin and impurities will flow through the column. In stage 2, the acid is desorbed as the characteristics of the resin (counterion, embedded solvent) are changed. Stage 3 allows the reuse of the resin

in a new cycle by different means in a process known as regeneration. Intermediate washing steps and resin re-packing might be needed and were not considered in the scheme. The discontinuous nature of this operation requires careful scheduling of each stage. Semi-continuous simulated moving beds have been already applied to the purification of lactic (Lee et al., 2004) and citric acids (Wu et al., 2009). In the coming sections, the characteristics of each step in terms of interactions, chemical requirements and waste generation will be analyzed.

Adsorption of carboxylic acids using ion exchange materials

Consider an aqueous solution of monocarboxylic acid *HA* (phase indicated by "*aq1*") such as obtained from fermentation at low pH or at neutral pH with subsequent acidification by inorganic acid. Most impurities will not be adsorbed by anion exchange resins. Consequently, significant purification can be achieved.

Using weak anion exchangers

These materials, functionalized with a weak base group *B* such as a pyridine, imidazole or primary, secondary or tertiary amine, will adsorb the target carboxylic acid. If desorption is performed with a base such as NaOH, the resin is recovered for reuse in a next capture cycle:

Adsorption $$HA\,(\text{aq1}) + B\,(\text{resin}) \rightleftarrows BH^{+}A^{-}\,(\text{resin}) + (\text{aq1}) \quad (1)$$

Desorption: $$BH^{+}A^{-}\,(\text{resin}) + NaOH\,(\text{aq2}) \rightleftarrows B\,(\text{resin}) + NaA\,(\text{aq2}) + H_2O \quad (2)$$

Overall: $$HA\,(\text{aq1}) + NaOH\,(\text{aq2}) \rightarrow (\text{aq1}) + NaA\,(\text{aq2}) + H_2O \quad (3)$$

Equilibrium studies on adsorption of carboxylic acids have shown that reaction 1 is typically favorable as high resin capacities and affinities have been found. Poly(4-vinylpyridine) adsorbents were effective to capture carboxylic acids at low concentrations (~0.5%) with a capacity (on dry basis) for lactic, malic and citric acids of 0.20, 0.54 and 0.83 g/g, respectively (Kawabata et al., 1981). Similarly, Davison et al. (2004) tested more than 25 sorbents for uptake of succinic acid from aqueous solution and selected a weak base pyridine polymer which provided adequate capacity and stability through sorption and desorption cycles.

In general, tertiary amine adsorbents have shown slightly lower adsorption capacities (Dethe et al., 2006, Gao et al., 2010, Li et al., 2009). Most of the experimental equilibrium data are presented as adsorption isotherms, e.g. Langmuir type, and deviations from this model are an indication of other mechanisms taking place. Reaction 1 depicts a very simplified acid-base interaction mechanism in which a 1:1 stoichiometry is followed, but a multiple stoichiometric complexation is possible depending on the acid structure and valence, leading to resin overloading. This effect was found for succinic and formic acids sorbed on Dowex® MWA-1 where multiple acid sorption occurred per site by ion pairing and hydrogen bonding (Husson and King, 1999).

Table 7. Selected adsorbents for primary recovery of carboxylic acids and carboxylates

Type	Name	Functional group	Pore type	Matrix	Application reference
Weak anion exchanger	Duolite A7	Polyamine (Secondary amine in majority)	N/A	Phenol-formaldehyde-polyamine	(Tung and King, 1994)
	Amino SBA-15	Primary, secondary and tertiary amines	Mesoporous	SBA-15 Silica	(Jun et al., 2007a)
	Amberlite® IRA-67	Tertiary amine	Gel	Polyacrylic	(Gluszcz et al., 2004) (Gao et al., 2010)
	Amberlite® IRA-35	Tertiary amine	Macroporous	Polyacrylic	(Tung and King, 1994) (Cao et al., 1996) (Husson and King, 1999) (Davison et al., 2004)
	Dowex® MWA-1	Tertiary amine (90%) and quaternary amine	Macroporous	Polystyrene-DVB	(Garcia and King, 1989) (Tung and King, 1994) (Husson and King, 1999)
	Indion® 860	Tertiary amine	Macroporous	Polystyrene-DVB	(Dave et al., 1997) (Dethe et al., 2006)
	Reillex® 425	Pyridine	Macroporous	Poly(4-vinylpyridine)-DVB	(Tung and King, 1994) (Evangelista et al., 1994) (Cao et al., 1996) (Husson and King, 1999) (Davison et al., 2004)
	VI-15	Imidazole	Gel	Acrylamide	(Evangelista et al., 1994)
	NERCB 09	Weak base	N/A	Polystyrene	(Li et al., 2009)

Table 7 (continuation). Selected adsorbents for primary recovery of carboxylic acids and carboxylates

Strong anion exchanger	Dowex® Marathon A	Quaternary amine, Type I	Gel	Polystyrene-DVB	(Leite et al., 2008)
	Amberlite® IRA-400	Quaternary amine, Type I	Gel	Polystyrene-DVB	(Sosa et al., 2001) (Cao et al., 2002) (Fu et al., 2009)
	Amberlite® IRA-420	Quaternary amine, Type I	Gel	Polystyrene-DVB	(Monteagudo and Aldavero, 1999)
	Dowex® Marathon MSA	Quaternary amine, Type I	Macroporous	Polystyrene-DVB	(López-Garzón et al., 2012)
	Amberlite® IRA-900	Quaternary amine, Type I	Macroporous	Polystyrene-DVB	(Cao et al., 1996)
	Indion® 810	Quaternary amine, Type I	Macroporous	Polystyrene-DVB	(Dethe et al., 2006)
	Amberlite® IRA-410	Quaternary amine, Type II	Gel	Polystyrene-DVB	(López-Garzón et al., 2012)
	NERCB 04	N/A	N/A	Epoxy	(Li et al., 2010a)
Nonionic	Silicalite®	Not functionalized	N/A	High surface silica	(Davison et al., 2004)
	CBV 28014	Not functionalized	Microporous	High silica ZSM-5	(Efe et al., 2010b)
	CT 3000 SG	Not functionalized	N/A	α-Al_2O_3	(Gulicovski et al., 2008)
	Hematite	Not functionalized	N/A	α-Fe_2O_3	(Hwang and Lenhart, 2008)
	XFS-40422	Not functionalized	N/A	Polymer	(Davison et al., 2004)
	Hytrel 8206	Not functionalized	N/A	PBT-Polyether	(Hepburn and Daugulis, 2012)

Reaction 2 depicts the deprotonation behavior leading to desorption of the acid. As pH will influence directly the protonation of the resins, loading capacity is expected to have a strong dependence on pH. The increase in pH decreases the availability of protons and therefore the possibility of ion pairing between the protonated amine group and the carboxylate. In general, commercial weak anion exchangers will sustain most of their adsorption capacity up to the pK_a of the acid and then undergo a sharp decrease up to neutral pH. The progressive decay in capacity with pH depends on the pK_a of the acid and the basicity of the resin (Husson and King, 1999, Li et al., 2009, Tung and King, 1994). The more concentrated the NaOH solution, the more concentrated the obtained carboxylate solution. However, the carboxylic acid has been converted in carboxylate salt (NaA) and additional steps will be required for re-conversion as confirmed in a publication on lactic acid recovery using tertiary amine resin (González et al., 2006).

If desorption is done using salts such as NaX instead of bases, a regeneration step with NaOH is required to get the resin back to its original state:

Adsorption: $HA\,(\mathrm{aq1}) + B\,(\mathrm{resin}) \rightleftarrows BH^+A^-\,(\mathrm{resin}) + (\mathrm{aq1})$ (1)

Desorption: $BH^+A^-\,(\mathrm{resin}) + NaX\,(\mathrm{aq2}) \rightleftarrows NaA\,(\mathrm{aq2}) + BH^+X^-\,(\mathrm{resin})$ (4)

Regeneration: $BH^+X^-(\mathrm{resin}) + NaOH\,(\mathrm{aq3}) \rightleftarrows B\,(\mathrm{resin}) + NaX\,(\mathrm{aq3}) + H_2O$ (5)

Overall: $HA\,(\mathrm{aq1}) + NaX\,(\mathrm{aq2}) + NaOH\,(\mathrm{aq3}) \rightarrow (\mathrm{aq1}) + NaA\,(\mathrm{aq2}) + NaX\,(\mathrm{aq3}) + H_2O$ (6)

The need for a regeneration step will depend on the basicity of A^- and X^-. If, for instance, sodium carbonate is used in the desorption step it will regenerate the resin with the associated carbon dioxide and water production. Neutral salts are not likely to desorb carboxylate anions as reaction 4 will not be favorable. Therefore, this strategy has no clear advantages but may be used in fundamental studies of the ion-exchange behavior.

It is more convenient to elute carboxylic acid instead of carboxylate. To achieve this, a mineral acid HX can be used as eluent:

Adsorption: $HA\,(\mathrm{aq1}) + B\,(\mathrm{resin}) \rightleftarrows BH^+A^-\,(\mathrm{resin}) + (\mathrm{aq1})$ (1)

Desorption: $BH^+A^-\,(\mathrm{resin}) + HX\,(\mathrm{aq2}) \rightleftarrows HA\,(\mathrm{aq2}) + BH^+X^-\,(\mathrm{resin})$ (7)

Regeneration: $BH^+X^-(\mathrm{resin}) + NaOH\,(\mathrm{aq3}) \rightleftarrows B\,(\mathrm{resin}) + NaX\,(\mathrm{aq3}) + H_2O$ (8)

Overall: $HA\,(\mathrm{aq1}) + HX\,(\mathrm{aq2}) + NaOH\,(\mathrm{aq3}) \rightarrow (\mathrm{aq1}) + HA\,(\mathrm{aq2}) + NaX\,(\mathrm{aq3}) + H_2O$ (9)

The selection of the acid desorbent is critical for the process as it should be able to displace the carboxylic acid bound to the resin. Although most common mineral acids have lower pK_a values than the organic acids relevant to this review (see Table 3) and therefore are expected to establish a stronger interaction with the basic group, they might have equilibrium characteristics unfavorable when compared with the target acid. HCl was assessed as possible

eluent for lactic acid sorbed on tertiary amine resins, but it showed weaker adsorption than lactic acid, which will not facilitate the indicated desorption (Dave et al., 1997).

Another way to achieve elution of sorbed carboxylic acids is using an organic solvent such as methanol (MeOH) as eluent in which the acid will have better partition. After elution, the resin pores will contain methanol instead of water. A thermal regeneration step might be applied to liberate the alcohol as vapor which can be condensed for reuse:

Adsorption: $HA\,(\text{aq1}) + B\,(\text{resin}) \rightleftarrows BH^+A^-\,(\text{resin}) + (\text{aq1})$ (1)

Desorption: $BH^+A^-\,(\text{resin}) + MeOH\,(\text{org}) \rightleftarrows B\,(\text{resin containing MeOH}) + HA\,(\text{org})$ (10)

Regeneration: $B\,(\text{resin containing MEOH}) \xrightarrow{\Delta} B\,(\text{resin}) + MeOH\,(\text{org})$ (11)

Overall: $HA\,(\text{aq1}) + MeOH\,(\text{org}) \rightarrow HA\,(\text{org}) + (\text{aq1})$ (12)

If the regeneration is done by eluting with water, or if the methanol is left on the resin for the next adsorption cycle, it will end up in an aqueous steam from which it can potentially be recovered by distillation. In a later process step HA might be crystallized or distilled from its solution in methanol, allowing recycling of this portion of methanol. As example, lactic acid was sorbed using tertiary amine weak base resins and successfully desorbed with methanol (Dethe et al., 2006). However, complete desorption required 40-60 bed volumes of methanol, leading to heavy dilution. Other acids such as acetic, butyric, lactic and adipic adsorbed in a poly(4-vinylpyridine) resin required about 3 bed volumes of methanol for complete elution. Malic and citric acid needed up to 13 bed volumes, indicating that polyvalent acids are sorbed stronger (Kawabata et al., 1981).

So far all the reviewed options have dealt with the desorption step at expenses of consumption of chemicals and waste salt production. A very innovative alternative was proposed by Husson and King (1998) in which the acid was desorbed using trimethylamine (TMA) in an organic solvent, thereby forming a new acid-base complex prone to thermal decomposition. Ideally, after the thermal treatment the acid remains in solution and the vaporized TMA is reabsorbed in the corresponding solvent to be reused:

Adsorption: $HA\,(\text{aq1}) + B\,(\text{resin}) \rightleftarrows BH^+A^-\,(\text{resin}) + (\text{aq1})$ (1)

Desorption: $BH^+A^-\,(\text{resin}) + TMA\,(\text{org}) \rightleftarrows B\,(\text{resin}) + TMAH^+A^-\,(\text{org})$ (13)

Cracking: $TMAH^+A^-\,(\text{org}) \xrightarrow{\Delta} HA\,(\text{org}) + TMA\,(\text{org})$ (14)

Overall: $HA\,(\text{aq1}) \rightarrow HA\,(\text{org})$ (15)

Reaction 13 occurs due to the difference in basicity between the resin functional group and TMA, being effective in the cases where the former is a weaker base. Although an aqueous solution of TMA can be used in the desorption step, such aqueous environment leads to incomplete thermal cracking of the salt and therefore an organic solvent is preferred (Poole and King, 1991). Due to this, intermediate steps of resin washing to remove water (prior to

desorption) and solvent (to use the resin in a new adsorption cycle) are required. Moreover, the TMA vaporized during thermal cracking should be absorbed in the organic solvent for a new desorption step. The final product of this recovery sequence is the acid dissolved in an organic solvent, which should be separated as required.

The resin Dowex MWA-1 loaded with succinic and lactic acid was desorbed with TMA dissolved in methyl ethyl ketone (MEK) (Husson and King, 1998). The ketone was selected as a solvent as it stabilizes the undissociated acid more than the acid-TMA complex, favoring the thermal decomposition reaction. Using a large molar excess of TMA, all the tertiary sites loaded with acid were desorbed. Interestingly, the TMA complex of succinic acid appeared to be very insoluble in MEK and precipitated whereas the lactic acid complex remained in solution. Solid trimethylammonium succinate and the dissolved trimethylammonium lactate were quantitatively cracked, at temperatures of 150 and 80 °C, respectively. A patent based on this technology claims that the overall process provides an efficient alternative for the recovery of carboxylic acids from aqueous streams, neither consuming large amounts of chemicals nor generating waste by-products (King and Poole, 1995), making this alternative attractive for industrial purposes. Nonetheless, to the knowledge of the authors such a process has not been applied to any relevant carboxylate at large scale. The main reason for that might be the strong and unpleasant odor of TMA, suggesting that the virtually complete removal required for most applications could be difficult to achieve. Moreover, energy requirements and mass and heat transfer during the cracking step may also complicate the scenario.

Finally, temperature is another parameter influencing the sorption equilibrium that can be used for promoting desorption. Anion exchange processes are exothermic (Irving et al., 1977) and therefore sorbent capacities will decrease with temperature. This dependency was studied for the adsorption of citric and lactic acids on Amberlite® IRA-67 where the equilibrium loading capacity of citric acid was reduced by 35% for a temperature rise from 20 to 60 °C. The reduction was much lower (6%) for lactic acid over the same temperature interval (Gao et al., 2010, Gluszcz et al., 2004). Such drop in capacity indicates that a desorption scheme based on temperature swing, e.g. using hot water, will require high temperatures and flows to be effective. Although promising for succinic acid, this has seemed to be cumbersome (Davison et al., 2004).

Using strong anion exchangers

Materials functionalized, predominantly, with quaternary ammonium compounds constitute strong anion exchangers. As a result of its degree of substitution, the quaternary ammonium cation is permanently charged and carries a negative counterion to maintain its electroneutrality. Thus, when adsorbing carboxylates by ion pairing, a strong anion exchanger will always release its counterion in stoichiometric equivalents. Starting with a quaternary ammonium hydroxide Q^+OH^-, the exchange reaction will resemble the aforementioned scheme composed by reactions 1-3:

Adsorption: $HA\,(\text{aq1}) + Q^+OH^-\,(\text{resin}) \rightleftarrows Q^+A^-\,(\text{resin}) + (\text{aq1}) + H_2O$ (16)

Desorption: $Q^+A^-\,(\text{resin}) + NaOH\,(\text{aq2}) \rightleftarrows Q^+OH^-\,(\text{resin}) + NaA\,(\text{aq2})$ (17)

Overall: $HA\,(\text{aq1}) + NaOH\,(\text{aq2}) \rightarrow H_2O + NaA\,(\text{aq2})$ (18)

Given the basicity of the strong anion exchangers, the interaction between Q^+ and the carboxylate is much stronger as compared with other amine groups. Therefore reaction 17 will need a more concentrated base to proceed, offering no clear advantages over weak anion exchangers. Lactic acid was adsorbed using a strong base resin in the hydroxide form and its equilibrium isotherm was compared with other weak base resins (Evangelista et al., 1994). A lower capacity and higher affinity for lactic acid were obtained which implies higher resin and desorbent requirements. This approach was applied to diluted solutions of fumaric acid for which packed bed column studies demonstrated a favorable adsorption (Fu et al., 2009).

Instead of regenerating the resin through desorption with NaOH, a salt desorption step could be used. The applicability of this step will be governed by the selectivity of the resin for the different anions. A more detailed description will be given in the next section.

Carboxylic acids can also be sorbed by using a resin in a different form than hydroxide. Consider, for instance, a resin with a quaternary ammonium salt Q^+X^- which will adsorb the acid by hydrogen bonding:

Adsorption: $HA\,(\text{aq1}) + Q^+X^-\,(\text{resin}) \rightleftarrows Q^+X^- : HA\,(\text{resin}) + (\text{aq1})$ (19)

Desorption: $Q^+X^- : HA\,(\text{resin}) + NaOH\,(\text{aq2}) \rightleftarrows Q^+X^-\,(\text{resin}) + NaA\,(\text{aq2}) + H_2O$ (20)

Overall: $HA\,(\text{aq1}) + NaOH\,(\text{aq2}) \rightarrow (\text{aq1}) + NaA\,(\text{aq2}) + H_2O$ (21)

The thermodynamic equilibrium position of reaction 19 will depend on the characteristics of X^-, and for certain inorganic anions anion exchange will proceed to some extent. In comparison with quaternary ammonium hydroxides, less affinity is expected improving the performance of the desorption step. As an example, lactic acid was adsorbed from pH 2.0 solutions by a quaternary anion exchange resin that was in the sulfate form (Cao et al., 2002). The non-langmuirian behavior observed and the negligible influence of salts in the desorption step suggested that lactic acid was captured by hydrogen bonding, hydrophobic interaction or a combination of mechanisms. The authors explored several desorption options such as sulfuric acid, ammonia, methanol and pure water, obtaining moderate acid recoveries between 70 and 80%.

Adsorption of carboxylic acids using nonionic materials

For uncharged carboxylic acids, adsorption without ion-exchange may occur. Then, hydrophobic interactions may prevail. The adsorption of dicarboxylic acids on mineral materials such as alumina (Gulicovski et al., 2008) and hematite (Hwang and Lenhart, 2008)

was initially explored. Although effective at a pH higher than their pK_a values, sorption capacities were too poor to be considered as an effective recovery method.

To fully avoid the consumption of acids and bases, high-silica sorbents have been explored, which are hydrophobic and have almost no ion exchange capacity (Efe et al., 2010b). Desorption with pressurized water at 150 °C was advocated (Efe et al., 2010a). This led to minimization of chemicals use, because the regeneration of the adsorbent would merely be cooling. Instead, desorption with water-soluble organic solvents could have been done at ambient conditions, but this would have necessitated a high-temperature regeneration step to remove the strongly adsorbing organic solvent molecules.

Recently, a PBT-Polyether copolymer was evaluated as a succinic acid adsorbent from carbon dioxide acidified crude fermentation broth (Hepburn and Daugulis, 2012). The adsorption capacities were low, but the sorbent showed good stability and was not affected by the presence of cells or related biocompounds. Desorption alternatives were not discussed, though.

For lactic acid, which is more polar than succinic acid, adsorption on silicalite was relatively poor and the recovery upon desorption was only 75% (Aljundi et al., 2005). No reason was given for this.

In summary, non-ionic materials for recovery of undissociated acids are still in an early stage of development. Relatively low capacities and affinities for short chain carboxylic acids are typical for these materials given the polarity of the target molecules. Moreover, the stability of most of the studied mineral materials is an issue that impedes further practical applications. In order to become an alternative to weak basic adsorbents, developments in both aspects are needed.

Adsorption of carboxylates using anion exchange materials

The starting point here is an aqueous solution of a carboxylate obtained after fermentation, for instance, the sodium salt NaA of a carboxylic acid. A usual way to recover the carboxylate is by capturing the organic anion on a strong anion exchanger Q^+X^-. Initially, we consider the case of desorbing with a salt NaX, so that the resin is simultaneously regenerated:

Adsorption: $$NaA\,(\text{aq1}) + Q^+X^-\,(\text{resin}) \rightleftarrows NaX\,(\text{aq1}) + Q^+A^-\,(\text{resin}) \quad (22)$$

Desorption: $$Q^+A^-\,(\text{resin}) + NaX\,(\text{aq2}) \rightleftarrows Q^+X^-\,(\text{resin}) + NaA\,(\text{aq2}) \quad (23)$$

Overall: $$NaA\,(\text{aq1}) + NaX\,(\text{aq2}) \rightleftarrows NaX\,(\text{aq1}) + NaA\,(\text{aq2}) \quad (24)$$

The purity and concentration of the recovered carboxylate will depend on the concentration and nature of the resin counterion X^- and specifically on its selectivity, which is the thermodynamic description of the preference the resin has toward an ion given a reference ionic state. Thus, the selectivity is defined as the concentration-based equilibrium constant of the ion exchange reaction between the sorbent in a determined ionic state and the carboxylate.

Table **6** indicates the usual selectivity sequence for common inorganic anions found in fermentation media, bio-based carboxylates and potential resin counterions.

Table 6. Anions in order of decreasing selectivity for strong anion exchangers.[a]

Anion	Charge
Citrate	3-
Hydrogen citrate	2-
Sulfate	2-
Hydrogen phosphate	2-
Hydrogen sulfate	1-
Nitrate	1-
Fumarate ≈ Itaconate ≈ Ketoglutarate	2-
Succinate ≈ Malate	2-
Chloride	1-
Dihydrogen citrate	1-
Hydrogen fumarate / itaconate / ketoglutarate	1-
Hydrogen succinate / malate	1-
Hydrogen carbonate ≈ Dihydrogen phosphate	1- 2-
Formate	1-
Acetate ≈ Lactate	1-
Propionate ≈ Butyrate	1-
Carbonate	2-
Hydroxide	1-

[a] The sequence was composed taking hydroxide anion as reference. Polymer structures considered were styrene-divinylbenzene. Other support materials might affect the sequence. For most of the anions, the reported selectivities were measured for type 1 quaternary ammonium groups. For type 2, the anions will show a lower selectivity but the sequence is likely to remain unaffected. Exceptions may apply though. References: (Dow, 2013, Helfferich, 1962, Kikuchi et al., 1994, Sosa et al., 2001, Takahashi et al., 2003).

For simultaneous desorption and regeneration, the selectivity toward X^- will influence the equilibrium position of reactions 22 and 23. A good resin counterion for sorption is such with a low selectivity coefficient, preferentially lower than for the target carboxylate, leading to higher usable column capacities. However very concentrated NaX solutions will be needed to achieve a good desorption. In addition, from the carboxylate concentration aspect, thermodynamics dictate that NaA can be more concentrated in the obtained aqueous carboxylate solution "aq2" than feed carboxylate solution "aq1" if NaX in "aq2" is more concentrated than NaA in "aq1". Moreover, the obtained NaX (aq1) solution cannot easily be reused as NaX (aq2) because it will contain the removed impurities and will have a lower concentration than required in reaction 23. Summarizing, a good counterion for adsorption is not a good desorbent-regenerant and vice versa. Uncoupling of the former processes, using specific desorbents and regenerants might be more adequate.

From a purity standpoint, the obtained aqueous carboxylate solution will be purer than feed the feed because non-adsorbing impurities will remain in aq1. Yet, a strong anion exchanger will adsorb other anions present in the feed solution, according to the resin

selectivity. It is then important to consider the selectivity sequence in the formulation of the fermentation medium and strain optimization efforts (elimination of by-products). Sulfate salts might be kept at concentrations just sufficient to provide the sulfur requirements for the microorganism, and if possible, chloride salts can be used instead. Acetate production should be avoided in butyrate-producing microorganisms as they will compete strongly, whereas this will not be a big issue for succinate producing microorganisms.

Examples of the aforementioned scheme make use of anions with low selectivity coefficient, therefore prioritizing the sorption step. A bed of quaternary anion exchange resin in the carbonate form was used in the primary recovery of clarified sodium lactate fermentation broth (Vaccari et al., 1993). Desorption using ammonium carbonate regenerated the resin and yielded an ammonium lactate solution with good purity, but probably substantially more dilute than the feed. Inorganic anions such as phosphate gradually accumulated on the resin and were removed by NaOH after 15 cycles. The ammonium lactate was acidified using a cation exchanger. Integration possibilities were discussed as the liberated sodium carbonate could be used for pH control during fermentation. Later, such integration was carried out aiming to overcome inhibitory effects of lactate on lactic acid bacteria and controlling the fermentation pH (Monteagudo and Aldavero, 1999). Higher yields of lactic acid on sucrose were obtained, but a net consumption of hydrochloric acid was involved to regenerate the cation exchange columns.

The hydroxide form of an anion exchange resin has also been used. Tung and King (1994) showed good adsorption of lactate and succinate on a quaternary ammonium resin in the OH^- form. Later, this form of the resin was used for pH control in the fermentative production of succinate (Li et al., 2010a). Sodium hydroxide (0.7 mol/L) was used to desorb the succinate allowing up to 30 sorption-desorption cycles with model medium. The feasibility of the system was demonstrated in a batch fermentation coupled to a packed bed of resin. Although the productivity of the fermentation system was improved, no information was given regarding base consumption and final succinate concentrations.

There are also anion exchange schemes possible where different anions are used during adsorption and desorption, as a way to improve the performance of each step. For example, hydroxide during adsorption and chloride during desorption. Then, a regeneration step is required to get the resin in its original state for the next cycle:

Adsorption: $$NaA\,(\text{aq1}) + Q^+OH^-\,(\text{resin}) \rightleftarrows Q^+A^-\,(\text{resin}) + NaOH\,(\text{aq1}) \quad (25)$$

Desorption: $$Q^+A^-\,(\text{resin}) + NaCl\,(\text{aq2}) \rightleftarrows Q^+Cl^-\,(\text{resin}) + NaA\,(\text{aq2}) \quad (26)$$

Regeneration: $$Q^+Cl^-\,(\text{resin}) + NaOH\,(\text{aq3}) \rightleftarrows Q^+OH^-\,(\text{resin}) + NaCl\,(\text{aq3}) \quad (27)$$

Overall: $$NaA\,(\text{aq1}) + NaCl\,(\text{aq2}) + NaOH\,(\text{aq3}) \rightleftarrows NaOH\,(\text{aq1}) + NaA\,(\text{aq2}) + NaCl\,(\text{aq3}) \quad (28)$$

This strategy may lead to an overall lower chemicals consumption, depending on the selectivity coefficient of the carboxylate anion. However, the use of concentrated feed solutions of both NaOH and NaCl, which will become impure and diluted upon elution, might not be attractive for industrial application. Therefore the most important use of this

strategy is probably in fundamental studies of ion-exchange, where these disadvantages are often not important.

The aforementioned desorption reactions can also be performed with desorbents containing other cations than NaCl, for example KCl, which would give KA as carboxylate and thus lead to cation exchange (despite using an anion exchange resin), in addition to primary recovery. This cation exchange may be useful in subsequent downstream processing. Similarly, the regeneration might be done with regenerants containing other cations than NaOH, for example NH_4OH, yielding NH_4Cl in the effluent. Such an option should be considered if recycling of effluent is not pursued anyhow.

More interesting is the option to use a mineral acid for desorption, for instance HCl. Then the desired carboxylic acid is obtained upon desorption:

Adsorption: $$NaA\,(\text{aq1}) + Q^+Cl^-\,(\text{resin}) \rightleftarrows Q^+A^-\,(\text{resin}) + NaCl\,(\text{aq1}) \quad (29)$$

Desorption: $$Q^+A^-\,(\text{resin}) + HCl\,(\text{aq2}) \rightleftarrows Q^+Cl^-\,(\text{resin}) + HA\,(\text{aq2}) \quad (30)$$

Overall: $$NaA\,(\text{aq1}) + HCl\,(\text{aq2}) \rightleftarrows NaCl\,(\text{aq1}) + HA\,(\text{aq2}) \quad (31)$$

In this primary purification the counterion is simultaneously removed and also ends up in the same solution as the removed impurities. Besides, if the sorption equilibrium is good enough, a separate regeneration step of the resin might not be required.

This option has been used for the recovery of lactate at pH 5 using quaternary ammonium resin in the sulfate form (Cao et al., 2002). The sorption isotherms showed a high competition between sulfate and lactate anions. Elution with 2 mol/L sulfuric acid gave 97% lactic acid recovery. The concentration had decreased from 88 g/L lactate to 21 g/L lactic acid, though.

In a similar example, lactate was recovered from crude fermentation broth without cell removal in an expanded bed column with a strong anion exchanger in the hydroxide form, which was subsequently eluted using 4 mol/L hydrochloric acid (Sosa et al., 2001). The authors stated the need to use the resin in the OH^- as strong competition between chloride and lactate was observed. A maximum recovery of 87% was obtained.

Although a feasible option according to Table 5, examples of sorption of carboxylates using sorbents functionalized with primary or secondary amines or their salts (including tertiary ammonium salts) were not found in the literature. In the case of the popular tertiary amine resins, is not possible to determine whether their salts could indeed capture carboxylates by ion exchange as most of the authors use the free base form in their studies. For these resins in free base form, no interaction is expected with the carboxylate anion, but most of the equilibrium studies in which the effect of pH was analyzed demonstrated a residual capacity at pH higher than the pK_a of the acid (Evangelista et al., 1994, Husson and King, 1999, Li et al., 2009). The equilibrium data provided by Evangelista *et al.* for lactate adsorption on Dowex® MWA-1 showed basic pH values at equilibrium, suggesting that the resin could contain traces of quaternary ammonium groups which sorbed lactate anions by ion exchange and then released hydroxide anions.

2.4.2 Extraction

Extraction has been the most studied technology for the primary recovery of carboxylic acids. Depending on the mechanism, carboxylic acids can be extracted by solvation with aliphatic and aromatic hydrocarbons, carbon-bonded oxygen-bearing extractants, phosphorous-bonded oxygen bearing extractants and by several interactions with amine-based compounds (Eyal and Canari, 1995, Kertes and King, 1986). The term "reactive extraction" has been coined in the field to categorize extraction operations in which either an association complex or a chemical compound is formed between the solute and extractant as a result of intermolecular or chemical interactions, respectively. Although these interactions can be represented with a reaction equation, reinforcing the usage of the term "reactive", such denomination is misleading since it should refer only to interactions conducing to chemical reactions, e.g. ion exchange. The reader should then be aware of the often vague use of this term in the literature. Although initial reports about the application of extraction as a recovery technique for carboxylic acids date back to the late 1960s, its industrial implementation pose practical difficulties largely related with the stripping of the acid from the extraction solvent. For just a few acids industrial application was achieved as discussed in section 2.9.

The nature of the extraction solvent has evolved from single component organic extractants to a tailor-made multicomponent solvent in which all the important characteristics involved in the extraction process are optimized, such as capacity, phase separation, stability and biological compatibility. To achieve this, modern extraction solvents for carboxylic acids are generally composed of an extractant, a modifier and a diluent. The extractant is the active component primary responsible for the transfer of the carboxylic acid or carboxylate to the solvent phase. In this section, amine-based extractants, ammonium, phosphonium and imidazolium salts as extractants would be treated. It should be taken into account, however, that if the solvent is composed of only one chemical species, both denominations, i.e. extractant and solvent can be used indistinctly.

Some extractants containing hydrophilic functional groups were designed with hydrophilic substituents, for instance long alkyl chains, to reduce their solubility in water. Moreover, the characteristics of the alkyl substituents (structure, length and functionality) influence the interaction chemistry and to a large extent the phase behavior of the extractants. The presence of these substituents affects transport properties and therefore, if used pure, undesired mass transfer characteristics would be present. Hence, a diluent is used to improve properties like viscosity and interfacial tension which will impact the mass transfer and phase separation. Typical diluents are alkanes, alcohols and halogenated hydrocarbons.

Most of the extractants reviewed in this section form complexes with the target acid. Once the complex is formed it needs to be solvated in the extraction solvent. If the diluent does not have the required solvation power (see Table 10), the complex will form a separate third phase leading to separation problems. In some cases a modifier, which mainly improves the solvation of the complex is used. Modifiers in general are less economical than diluents and do not provide enough good transport properties to be solely used together with the extractant. Long chain alcohols are the most frequently used modifiers. They also influence the basicity of amines and improve phase separation (Bízek et al., 1993, Marinova et al., 2005). Table 9 shows a selection of extractants used in primary recovery along with their solvents

and modifiers (if required). The classification of the extractants in amine-based, ionic and neutral has implications in the type of mechanism available for extraction. Such classification will be used throughout this section.

Once the composition of the extraction solvent has been defined, the extraction operation should be carried out in an appropriate manner. Figure 4 depicts the primary recovery process of carboxylic acids using extraction. Three main processes, extraction, back-extraction and regeneration are performed. Each step might span over several stages and may not be performed in a mixer and settler configuration as shown schematically in the figure. Many other equipment configurations such as extraction columns or centrifugal contactors can be used, but their description is outside the scope of this review.

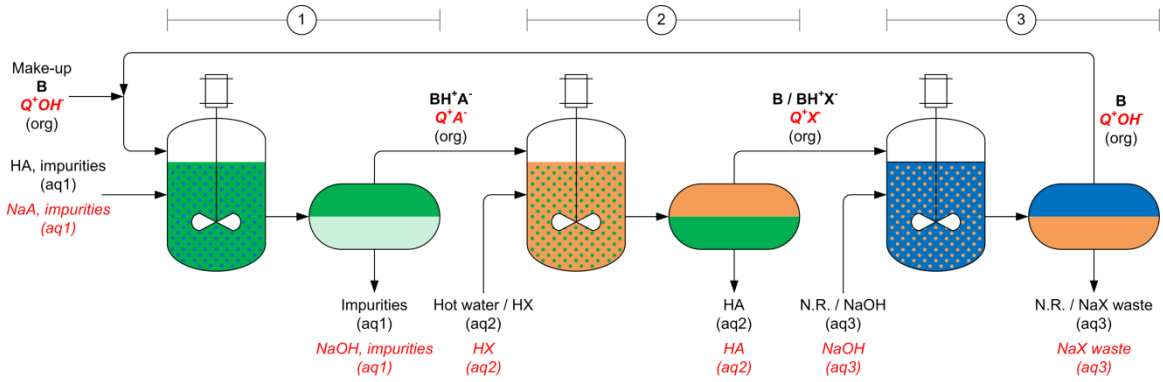

Figure 4. Scheme for amine-based recovery of extraction of carboxylic acid and carboxylate. Text in black and red corresponds to amine-based and ionic solvents respectively. Numbers on top refer to a step in the primary recovery operation: 1. Extraction, 2. Back-extraction, 3. Regeneration.

In the first step, a clarified carboxylic acid or carboxylate stream from a fermentation process is extracted leaving fermentation impurities in the aqueous stream. The loaded organic phase is then processed to back-extract the carboxylate to an aqueous phase using different means such as temperature swing or acid displacement. After phase separation, the aqueous stream is the purified carboxylic acid product and the solvent, depending on the applied back-extraction method might need regeneration in step 3 before used in a new cycle.

In the following sections, the extraction of carboxylic acid and carboxylates using amine-based, ionic and neutral/solvating extractants will be discussed. Since the interactions between the extractants and solutes are close to those treated in the adsorption section, the reader will be referred to the reactions described in such section.

Extraction of carboxylic acids using amine-based extractants

Carboxylic acids can be extracted using primary, secondary or tertiary amines resembling closely the reactions described for sorption on weak anion exchangers. Due to their functionality, they interact mainly via ion pairing and hydrogen bonding creating a complex which stoichiometry depends on the number of carboxylic groups and the characteristics of the extraction solvent. The degree of ion-pair formation depends on the acid pK_a and the basicity of the amine, being important only if $pK_{a,amine} > pK_{a,acid}$ (Eyal and Canari, 1995).

Table 9. Selected extractants for primary recovery of carboxylic acids and carboxylates

System	Extractant name	Functional group	Structure characteristics	Solvent (modifier)	Reference
Amine-based	N1923	Primary amine	Methyloctadecyl amine	1-Octanol	(Wang et al., 2009)
				Butyl acetate	
				Hexane	
	Primene® JM-T	Primary amine	Branched alkyl chains C16-C22	Kerosene	(Eyal and Canari, 1995)
	Amberlite® LA-2	Secondary amine	Asymmetric alkyl chains C12-C15	Diethyl carbonate	(Uslu et al., 2009)
				Methyl isobutyl ketone	
				1-Hexanol	
				Kerosene (1-Octanol)	(Reisinger and King, 1995)
	Tris(2-ethylhexyl)amine	Tertiary amine	2-Ethylhexyl alkyl chains	Kerosene	(Eyal and Canari, 1995)
	Trihexylamine	Tertiary amine	Hexyl alkyl chains	1-Octanol	(Kurzrock and Weuster-Botz, 2011)
	Tri-n-octylamine (Alamine® 300)	Tertiary amine	Straight octyl chains	Kerosene	(Eyal and Canari, 1995)
				Dodecane (1-Decanol)	(Marinova et al., 2005)
					(Yankov et al., 2004)
				n-Paraffins (Isodecanol)	(Poposka et al., 1997)
				Heptane(1-Octanol, tripropylamine)	(Hong and Hong, 2000)
				Methyl isobutyl ketone	(Hong et al., 2001b)
				Methyl isobutyl ketone (1-Octanol)	(Schunk et al., 2004)
					(Procházka et al., 2005)
				1-Octanol	(Jun et al., 2005)
					(Jun et al., 2007b)
	Triisooctylamine	Tertiary amine	Isooctyl alkyl chains	Chloroform	(Malmary et al., 1998)
				Heptane	
				1-Octanol	
	Alamine® 336	Tertiary amine	Straight alkyl chains C8-C10	2-Octanol	(Yang et al., 1991)
				Kerosene	(Eyal and Canari, 1995)
				1-Octanol	(Reisinger and King, 1995)
				Decanol	(Wasewar et al., 2002)

Table 9 (continuation). Selected extractants for primary recovery of carboxylic acids and carboxylates

	Aliquat® 336	Quaternary ammonium salt	Linear alkyl chains C8-C10 and methyl substituent	2-Octanol, Kerosene 1-Octanol Shellsol® A Dodecane (1-Decanol) 1-Hexanol Hexane	(Yang et al., 1991) (Reisinger and King, 1995) (Jaquet et al., 1999) (Syzova et al., 2004) (Keshav et al., 2009) (Coelhoso et al., 1996) (Kyuchoukov et al., 2004) (Kyuchoukov et al., 2005) (Marinova et al., 2005) (Uslu and İsmail Kırbaşlar, 2009) (Wasewar et al., 2011a)
Ionic liquids	[Bmim][PF6]	Imidazolium salt	Butyl and methyl substituents	None, tributylphosphate	(Matsumoto et al., 2004)
	[Bmim][BF4]	Imidazolium salt	Butyl and methyl substituents	None	(Lin et al., 2007)
	Trioctylamine-bis(2-ethylhexyl)phosphoric acid	Tertiary amine - organophosphate salt	Linear octyl chains mostly	Xylene	(Juang and Huang, 1994)
	Aliquat 336 - bis(2-ethylhexyl)phosphoric acid	Quaternary ammonium - organophosphate salt	Linear alkyl chains C8-C10-C16 phosphate	Isopar K	(Syzova et al., 2004)
	Cyphos IL104	Phosphonium - alkylphosphinate salt	Linear alkyl chains C10-C16 phosphinate	Dodecane	(Martak and Schlosser, 2007)
	Tributylphosphate	Phosphate ester	Linear butyl chains	Dodecane Hexane Sunflower oil	(Malmary et al., 1994) (Bouraqadi et al., 2007) (Kyuchoukov et al., 2008) (Labbaci et al., 2009) (Matsumoto et al., 2001) (Wasewar et al., 2011b)
Neutral/ solvating	Tri-n-octylphosphine oxide	Organophosphorous oxide	Linear octyl chains	1-Octanol Hexane	(Reisinger and King, 1995) (Keshav et al., 2008)

In general, the complexation stoichiometry can described in a similar way as in reaction 1, but reactions 32-34 also take into account the complexation in the organic phase of multiple acids molecules per tertiary amine extractant molecule:

$$HA\,(\text{aq1}) + R_3N\,(\text{org}) \rightleftarrows R_3NH^+A^-\,(\text{org}) + (\text{aq1}) \quad (32)$$

$$2HA\,(\text{aq1}) + R_3N\,(\text{org}) \rightleftarrows R_3N(H^+A^-)_2\,(\text{org}) + (\text{aq1}) \quad (33)$$

$$nHA\,(\text{aq1}) + R_3N\,(\text{org}) \rightleftarrows R_3N(H^+A^-)_n\,(\text{org}) + (\text{aq1}) \quad (34)$$

Subsequent steps such as back-extraction and regeneration of the solvent are analogous to the respective desorption and regeneration in section 2.4.1 by appropriately adjusting the stoichiometry. Such complicated extraction stoichiometry leads to complex extraction models in which several equilibrium constants are involved.

A consequence of the simultaneous occurrence of several extraction mechanisms is the stoichiometric overloading of the amine, producing a complex difficult to solvate in the organic phase generating, as a side effects, third phase formation and a great extent of water co-extraction (Bízek et al., 1993). Thus, solvation of the formed complex is important as it will reduce the occurrence of these problems. The characteristics of the diluent and/or modifiers will strongly influence the solvation and therefore its selection is critical. Table 7 provides useful selection guidelines for modifiers and diluents based on their solvation power, with those mostly used located in the extremes of the table, respectively.

Table 7. Diluents used in extraction systems, ordered according to decreasing solvation power according to extractant loading.[a]

Solvent class	Diluent
Alcohols	2-Ethyl-1-hexanol, 1-octanol, 1-decanol
Halogenated proton donor	Chloroform, dichloromethane, 1,2-dichloroethane
Ketone and ester	Methyl isobutyl ketone, diisobutyl ketone, butyl acetate
Halogenated aromatic	Dichlorobenzene, chlorobenzene
Alkyl aromatic	Toluene, xylene
Aliphatic hydrocarbon	Hexane, octane, dodecane

[a]Extractant loading is defined as the ratio of total acid concentration to total amine concentration, both in the organic phase. References: (Tamada et al., 1990, Tamada and King, 1990, Wang et al., 2009).

Often, the main complication when comparing different extraction systems is that assumptions behind the adoption of a particular stoichiometry are not clearly stated impeding a better understanding of the phenomena. As expected, the scenario is even more complex when dicarboxylic and tricarboxylic acids are involved.

Using primary and secondary amines

The use of primary and secondary amines has been restricted given their water solubility and their tendency to form amides upon heating. The former issue is particularly problematic for primary amines, however the latest technology of extractants have circumvented the

problem by attaching a secondary carbon to the amine group to which a pair of long aliphatic chains are attached, diminishing losses in the aqueous phase. Nonetheless, too long alkyl chains in the amine reduce its molar concentration in the system, reducing available complexation pairs, increasing the alkyl nature of the system and hence decreasing acid extraction (Hong et al., 2001a).

The equilibrium of propionic acid by N1923, a primary amine with 19-23 carbon atoms, was studied in 1-octanol, butyl acetate and hexane as diluents (Wang et al., 2009). Overloading of the extractant was observed, evidencing acid-acid interactions in the organic phase leading to a maximum of three acid molecules extracted per amine group. Among the diluents, octanol led to the highest values of the extraction constants promoting 1:1 interactions. In accordance to such extraction mechanism, the partition coefficient varied strongly with the acid concentration, ranging from 18 at acid concentrations of 0.05 mol/L to about 7 at 0.18 mol/L.

The secondary amine Amberlite LA-2 (*N*-lauryl-*N*-trialkylmethyl amine with 24-28 carbon atoms) was used in the extraction of lactic acid and compared with the tertiary amine Alamine 336 (tri-octyl/dodecyl amine) dissolved in 1-octanol and chloroform, respectively (Miller et al., 1996). The secondary amine presented slightly higher lactic acid distribution ratios at low (0.14 mol/L) and high (0.30 mol/L) lactic acid concentrations. In the case of succinic acid, extraction using the secondary amines diisooctylamine and dihexylamine in a mixture of 1-hexanol and 1-octanol gave an extraction yield of 84% (Kurzrock et al., 2011). Subsequent back-extraction with almost two equivalents trimethylamine in an aqueous solution gave a yield of 95%. The extractant was successfully recycled three times.

Using tertiary amines

In the case of tertiary amine extractants, there is a vast amount of literature focused on extraction equilibrium, effect of pH and solvent composition on partition; however very limited attention has been given to the back-extraction of the recovered acid. We will focus on the most relevant cases and in the available back-extraction alternatives.

Triisoctylamine in two different solvents, chloroform and a mixture of heptane and 1-hexanol, was tested as a solvent for the extraction of citric, lactic and malic acids (Malmary et al., 1998). The partition coefficients, measured for an organic to aqueous mass ratio of 2, showed a maximum at about 25% of amine in the heptane – hexanol mixture and were 41.5, 25.9 and 59 for citric, lactic and malic acids respectively. The authors suggested the recovery of the acid from the solvent by back-extraction with hydrochloric acid, which will require further regeneration as indicted in reaction 8.

The extraction of citric acid from unfiltered fermentation broth has been performed at pilot scale (Wennersten, 1983). Using Alamine 336 in kerosene as extractant, 97% citric acid was extracted, but the extract was more dilute than the feed. Back extraction with water was only marginally tested at 63 ^{o}C, though.

The extraction of succinic acid using a mixture of tripropyl and trioctylamine dissolved in heptane with octanol as a modifier was studied at different acid and amine concentrations (Hong and Hong, 2000). In several extraction studies for di and tricarboxylic acids, the

formation of a third phase has been reported (Bízek et al., 1993). By the addition of a short chain amine such problem was avoided even at high acid concentration, enhancing also the extraction power of the solvent. For a mixture of 8:2 of tripropyl and trioctylamine, a partition coefficient close to 3 was determined at an acid concentration of 0.12 mol/L. A temperature-swing alternative was discussed by the authors to strip off the amine from the solvent, no data was presented however.

Yankov et al. (2004) studied in great detail the extraction of lactic acid using trioctylamine. The effect of several diluents (alkanes) and modifiers (alcohols) was studied in order to optimize the extraction solvent. Although hexanol and octanol led to higher distribution coefficients, they were discarded due to the possible toxicity to lactic acid bacteria and decanol was selected. Among the alkanes, no considerable effects were seen on the partition coefficient and dodecane was chosen as the diluent. A solvent composed of 30% trioctylamine, 20% decanol and 50% dodecane give the best compromise in terms of phase separation and provided a partition coefficient of 2, which was considered as adequate. Trials with synthetic fermentation broth at pH=5.0 showed a dramatic reduction of the distribution coefficient as ion pairing interaction was reduced. Treating the extraction solvent with hydrochloric acid transformed the amine from free base to hydrochloride form and therefore switched the extraction mechanism to ion exchange, recovering partially its ability to extract lactate at expenses of mineral acid consumption.

In other cases, tertiary amines are used to remove impurities. For instance, in the production of succinate at neutral pH instead of acidifying the broth to such an extent that the carboxylic acid can be extracted, Song et al. (2007) acidified the broth to a pH above the pK_{a1} of succinate but below the pK_a of contaminating carboxylic acids. This allowed selective extraction of such contaminants, including acetic, lactic, formic and pyruvic acids.

Although, as reviewed above, tertiary amine extractants are very effective in capturing undissociated carboxylic acids, alternative methods for regeneration of carboxylic acid-tertiary amine extracts have proven to be difficult. In the early 1990s, Poole and King (1991) proposed an interesting approach in which the acid was back-extracted using trimethylamine (TMA) in aqueous solution generating a thermally crackable trimethylammonium salt. This approach served as a base for similar developments in adsorption-based recovery, as discussed in section 2.1.

In their initial experiments, aqueous solutions of lactic, succinic and fumaric acid were extracted with Alamine 336 dissolved in methyl isobutyl ketone and further back-extracted completely when using aqueous TMA in equivalent concentration. The aqueous solutions of trimethylammonium lactate, succinate and fumarate where heated under N_2 atmosphere. TMA was released after almost complete water evaporation. For lactate, a viscous mass was obtained due to intermolecular esterification, hampering TMA removal. Succinic and fumaric acid were largely obtained as crystals, but ~20% TMA was not removed without extra purification steps. Keshav and Wasewar (2010) applied the same method for the back-extraction of propionic acid from loaded organic phases comprising trioctylamine in 1-decanol and methyl isobutyl ketone. The acid was effectively recovered when TMA was used in slightly higher stoichiometric amounts.

As mentioned earlier for adsorption, the complete thermal decomposition of the complex and complete TMA removal is critical. Using reduced pressure, >99% removal from lactate

has been achieved (Wasewar et al., 2004a). Kurzrock et al. (2011) evaporated at 160 °C and 4 mbar trimethylamine from an aqueous solution that originated from back-extracting fermentative succinate. A yield was obtained of >99% of 99.5% pure succinic acid crystals. Impurities were trimethylamine and the extraction solvents.

Other approaches for acid stripping usually involve an increase in temperature to influence to reverse the extraction equilibrium. For propionic acid production, a process has been simulated using extraction by trioctylamine in ethyl acetate (Posada and Cardona, 2012). Ethyl acetate lost to the aqueous phase had to be recovered. Back-extraction of propionic acid to water was proposed using a combined effect of evaporating the ethyl acetate and increasing the temperature.

Cargill-Dow developed a process in which sodium lactate is produced by fermentation and the broth is concentrated and extracted with a tertiary amine solvent mixture under CO_2 pressure, thus using CO_2 to acidify. This yields a sodium bicarbonate precipitate and an amine lactic acid extract. The latter is back-extracted with hot water at 140 °C and 7 atm to produce a lactic acid solution and a regenerated amine solvent mixture that is recycled (Datta and Henry, 2006). Also the sodium bicarbonate may be reused, as indicated in section 7.4.

Extraction of carboxylic acids using ionic extractants

Using quaternary ammonium and imidazolium salts

One of the first reports on the extraction of carboxylic acids by water insoluble ammonium compounds was published by Yang et al. (1991) who investigated the use of Aliquat 336 (*N*-methyl-*N*,*N*-dioctyloctan-1-ammonium chloride) dissolved in kerosene or 2-octanol for the recovery of acetic, lactic, propionic and butyric acid from aqueous solutions. When studying the effect of pH on the distribution coefficient of the mentioned acids it was verified that Aliquat was able to extract both the undissociated and dissociated forms of the acid. Even though a quaternary ammonium group is able to perform ion exchange at pH > pK_a of the acid, at low pH such mechanism is not thermodynamically feasible as carboxylate anions are much stronger bases than chloride (Aliquat counterion) impeding hydrochloric acid formation as ion exchange product. Therefore, at low pH conditions extraction of uncharged carboxylic acids by ammonium groups occurs by other mechanisms such as hydrogen bonding (Eyal and Canari, 1995). At low pH, the distribution coefficients for butyric and propionic acid were 10 and 3.8 respectively, much higher than for acetic and lactic acid for which values lower than 1 were obtained. The described interactions between the quaternary ammonium group and the acid coincide closely to those using strong anion exchange resins in reactions 19-21.

Comprehensive studies have been performed on the extraction performance of lactic acid using a carbonate form of Aliquat 336 (Kyuchoukov et al., 2004) and on extraction mechanisms by the chloride form of the extractant (Kyuchoukov et al., 2005). The extraction degree achieved using the carbonate counterion was up to 60% higher than the chloride version at pH values lower than the pK_a of lactic acid. A difference of 20% was maintained at higher pH. Such difference can be explained by the lower selectivity coefficient of the carbonate anion compared to chloride (see Table 8), favoring anion exchange reactions at a wider range of acidity.

The influence of several diluents on the distribution coefficient of itaconic acid extracted by Aliquat 336 was recently reported (Wasewar et al., 2011a). Kerosene, toluene and hexane provided little solvation for the formed complex if compared to ethyl acetate for which a maximum distribution coefficient of 2.65 was found. Even though not discussed by the authors, the relatively high solubility of the ester in water will imply great diluent loses in the aqueous phase which would need to be recovered. The authors suggested a back-extraction method based on the use of volatile bases such as trimethylamine, however based on related published results for sorption the basicity of TMA is not high enough to achieve stripping (Husson and King, 1998). Back-extraction of acids from quaternary ammonium extractants will require the use of acids or bases as discussed in section 2.4.1.

In an exploratory work, imidazolium-based ionic liquids were examined as potential extractants for several acids including propionic, lactic, pyruvic and butyric acid (Matsumoto et al., 2004). Overall the distribution coefficients were very low, being below unity for all except butyric acid. In addition, the solubility of well-known amine extractants was also limited impeding their use as extraction diluents. The authors also evaluated the toxicity of the ionic liquids finding a good biocompatibility, not affecting the growth of lactic acid bacteria.

Using binary extractants

Also known as ABC (acid-base coupled extractants), comprise a coupling between a hydrophobic amine, ammonium or phosphonium compound and a hydrophobic organic acid or anion. Although initially described early in the mid-nineteen's they have received increased attention just recently. Until now, the described extractants are composed mostly of trioctylamine and di(2-ethylhexyl)phosphoric acid (Juang and Huang, 1994) and Cyphos IL104 or Aliquat 336 with di(2-ethylhexyl)phosphate (Syzova et al., 2004).

Their potential as extractants for carboxylic acids has been proven but the motivation behind further developments is to extract carboxylate salts as whole by providing interaction sites to the metal cation. So far, different interactions have proven to be occurring in these systems, from proton transfer (ion coupling) and solvation (Syzova et al., 2004) to hydrogen bonding (Martak and Schlosser, 2007).

Lactic acid was extracted using trihexyl(tetradecyl)phosphonium bis 2,4,4-trimethylpentylphosphinate (Cyphos IL-104). Using this ionic liquid, hydrogen bonding and ion exchanged were the dominating mechanisms (Martak and Schlosser, 2007). The distribution coefficient was above 40, but only at low lactic acid concentrations. After a similar extraction of lactic, malic, and succinic acid, the acids could not be stripped off from the ionic liquid by distillation (Oliveira et al., 2012). Back extraction with an aqueous solution containing a two-fold excess of sodium hydroxide was required, partially recovering the acids and yielding sodium carboxylate solutions.

In a recent report, several acids including propionic and butyric acids were extracted using trioctylmethylammonium di(2-ethylhexyl)phosphate (Kholkin et al., 2013). Despite the high partition coefficients presented, the acid back-extraction remains as the main issue although the authors claim that simple stripping with water might be possible. Experimental demonstration of such straightforward method was not provided.

In conclusion, there is no clear motivation or advantage for the use of ionic liquids as extractants for undissociated acids. In some cases, distribution coefficients are somewhat higher than those obtained with amine-based extractants, but acid back-extraction demonstrated to be very inefficient, requiring strong acids or bases.

Extraction of carboxylic acids using neutral/solvating extractants

Regular extractants consist of hydrocarbons or oxygenated hydrocarbons without charged groups. Partition coefficients between such nonionic extractants and water have been compiled by Kertes and King (1986) for a range of fermentative carboxylic acids. Generally, these partition coefficients were poor, with the best values (up to 4) for relatively nonpolar carboxylic acids (propionic and fumaric) in relatively polar extractants (cyclohexanone, 1-butanol and isobutanol). Usually, however, the partition coefficient was below 1, leading to dilution of carboxylic acid upon extraction. In cases with more favorable partition coefficients, relatively much extractant will be lost to the aqueous phase, and this will have to be recovered, for example by extraction with hexane and distillation of this extract.

The advantage of the more volatile nonionic extractants is that back-extraction of the carboxylic acid can be avoided by evaporating the extractant. For propionic acid, this is not critical because it can be evaporated from nonvolatile extractants (Xu et al., 2011), but for nonvolatile carboxylic acids primary recovery using volatile nonionic solvent might still be considered in case of favorable partitioning.

Organophosphorous liquids such as tributylphosphate and trioctylphosphine oxide (TOPO) often show better partition coefficients than oxygenated hydrocarbons, and have been studied for extraction of some carboxylic acids (Kertes and King, 1986, Xu et al., 2011). The distribution coefficient of butyric acid in an extraction solvent comprising tributylphosphate in decanol decreased with the concentration of both acid in the aqueous phase and phosphate in the solvent. For the conditions tested the partition coefficients were always above one, even at high acid concentration (Bouraqadi et al., 2007). TOPO dissolved in hexane was also explored as a solvating extractant for propionic acid (Keshav et al., 2008). As the extractant is toxic for most bacteria, its concentrations in the extraction solvent were limited to 0.1 mol/L for which a distribution coefficient close to one was obtained. There are no clear benefits from using TOPO as extractant as it is uncertain if a temperature swing will promote acid stripping.

For citric acid recovery, clarified broth was concentrated to contain only 20% water, and pouring this in acetone (Shishikura et al., 1992). This does not lead to a liquid-liquid extraction, but to precipitation of polar impurities, mainly sugars. Upon dissolving CO_2 up to a pressure of ~25 bar the solution became less polar and additional impurities precipitated. After settling of the precipitate, citric acid was purified from the acetone solution as described in a later section.

Extraction of carboxylates using ionic extractants

Extraction using ionic liquids finds is importance in the recovery of carboxylates as these liquids usually are capable of anion exchange. Extraction using anion exchange extractants

can be represented by a reaction equation, just like has been done for adsorption by strong anion exchange adsorbents in section2.4.1. As a consequence, these extractants are applied to carboxylate streams produced at neutral pH fermentations, capturing the carboxylate anion and producing a mineral salt in the aqueous phase.

Salts of primary, secondary and tertiary amines can be ionic liquids or be dissolved in organic liquids. Eyal and Canari (1995) suggested these salts could undergo ion exchange and therefore their use as anion exchangers is feasible. However, since no references on this have been found it will not be treated here. The focus of this brief section is on quaternary ammonium and phosphonium salts in the newly designed binary extractants.

Aliquat 336 in the chloride form dissolved in Shellsol A was used to capture lactate anions from sodium lactate aqueous solutions at a pH 6.3 (Coelhoso et al., 1996). Modeling of the ion exchange process through the experimental determination of the reaction equilibrium constant allowed the prediction of the distribution coefficients for different lactate concentrations. The partition was adequate (above 1) only at very low lactate concentrations (0.05 mol/L). The stripping of the lactate was performed using sodium chloride as shown in reaction 23. The stripping was efficient only using concentrated salt solutions of 1-2 mol/L producing dilute sodium lactate as product. Similar studies for lactate using phosphonium salts have been mentioned in section 2.4.2.

2.4.3 Precipitation

Precipitation of carboxylates for primary recovery

Precipitation of carboxylates themselves is included here under primary recovery and the removal of counterions of carboxylates is dealt with in a later section. A soluble carboxylic acid HA or soluble carboxylate such as NaA is converted into an insoluble carboxylate by performing a double replacement reaction. The main example is the primary recovery of citric acid (H_3A), which is precipitated as its calcium salt with calcium hydroxide:

$$H_3A\ (\text{aq}) + 1.5Ca(OH)_2\ (\text{s}) \rightarrow CaA\ (\text{s}) + 3H_2O\ (\text{l}) \qquad (35)$$

Calcium carbonate can be used as well (Heding and Gupta, 1975). The precipitated citrate can be filtered off, thus separating it from most impurities and water. However, not *all* citric acid is recovered, due to the aqueous solubility of calcium citrate. The solubility value of 0.96 g/L given in Table 8 corresponds to 0.57 g/L citric acid, which is less than 0.5% of the amount produced by fermentation and acceptable as loss. However, solubility product calculations should be used to determine the exact solubility of citrate as function of pH and added amount of calcium ion.

Table 8 shows higher solubilities for some other carboxylates of calcium, magnesium, and sodium. Only calcium L-malate and calcium succinate are reasonably insoluble. The calcium succinate solubility can be decreased by increasing the temperature. Some calcium succinate precipitated during a fermentation where the pH was controlled at 6 by using calcium hydroxide (Datta, 1992). After filtration, heating of the filtrate to 80 ^{o}C led to additional precipitate. Cells and proteins were not retained by the filters. Magnesium succinate is not

mentioned in the Table, but seems to have a low solubility (Van Krieken and Van Breugel, 2010).

Gao et al. (2009) developed a fermentation that produced 220 g/L calcium lactate, partly suspended in the broth. This was dissolved at 80 °C for clarification of the broth, and subsequent cooling would lead to ~70% calcium lactate crystallization. Recovering the remaining 30% would need much more effort and probably a thermal evaporation step.

Ammonium and potassium salts will have solubilities as high as sodium salts. Polyvalent cations other than calcium or magnesium may lead to low carboxylate solubilities, but these cations are expensive or toxic. Antisolvents such as alcohols might be added to decrease carboxylate solubilities, but the recovery of such antisolvents will complicate the process too much. Switching to a temperature where the solubility is low will be more feasible.

Thus, carboxylates can be precipitated with high yield from filtered fermentation broth in rare cases where they are very insoluble. After recovery of the salts, cation removal should be performed, as described in section 5.

Table 8. Aqueous solubilities of some carboxylate salts. Note that solubility products determine solubilities in multicomponent mixtures.

Carboxylate	Solubility (g/L)	T (°C)	Reference
Ca acetate	320	24.7	(Saury et al., 1993)
Ca citrate	0.86	20	(Zabozlaev et al., 2007)
Ca citrate.4H_2O	0.96	23	(Weast, 1979)
Ca fumarate.3H_2O	21.1	30	(Weast, 1979)
Ca D-gluconate	33	15	(Weast, 1979)
Ca L-lactate	61	25	(Cao et al., 2001)
Ca L-malate	12.2	37.5	(Weast, 1979)
Ca succinate.3H_2O	19.3	10	(Weast, 1979)
Ca succinate	12.3	20	(Zabozlaev et al., 2007)
Ca succinate.3H_2O	8.9	80	(Weast, 1979)
Mg acetate.5H_2O	1200	15	(Weast, 1979)
Mg L-lactate	62.9	20.5	(Apelblat et al., 2005)
Na acetate.3H_2O	762	0	(Weast, 1979)
Na citrate.2H_2O	720	25	(Weast, 1979)
Na fumarate	220	25	(Zhou, 1999)
Na succinate.6H_2O	214	0	(Weast, 1979)

Precipitation of carboxylic acids for primary recovery

According to Table 2 and Table 8, fumaric acid has a low aqueous solubility whereas sodium fumarate has a high aqueous solubility. This allows primary recovery of fumarate as solid fumaric acid (HA) according to the following scheme:

$$NaA\ (\mathrm{aq}) + HX\ \ (\mathrm{aq}) \rightarrow HA\ (\mathrm{s}) + NaX\ \ (\mathrm{aq}) \qquad (36)$$

Impurities and counterions are simultaneously retained in the aqueous solution, which is favorable. There is no need for a separate carboxylate conversion to carboxylic acid such as described in section 2.5.

The aqueous solubility of furan-2,5-dicarboxylic acid is even lower than that of fumaric acid, and a precipitate of this compound has been recovered in a similar way from fermentation broth (Ruijssenaars et al., 2012).

Although not a solid, a comparable principle has been applied to butyric acid. Already in 1878, addition of sulfuric acid to aqueous sodium butyrate, obtained via fermentation, led to an oily layer of butyric acid on top of the aqueous phase (Benninga, 1990). This indicates that the presence of salts decreases the solubility of otherwise completely miscible butyric acid. Recently, Wu et al. (2010) showed that in such a system, with $CaCl_2$ as salt, the butyric acid phase will contain less acetic acid than the aqueous phase, thus leading to some purification.

2.4.4 Nanofiltration and Reverse Osmosis

Nanofiltration (NF) and reverse osmosis (RO) are pressure-driven membrane techniques. NF membranes allow passage of water and of somewhat larger molecules. Ions are retained more than molecules of the same size, which indicates a potential for concentrating as well as purifying carboxylic acid or carboxylate solutions. RO membranes have smaller pores, mainly allowing water permeation.

NF has been tested, for example, for removal of contaminating multivalent anions and cations from ammonium lactate solution. Despite cascading, permeation of all lactate was prevented because of accumulated sulfate (Kim et al., 2012). Such limitations have led to relatively few publications on NF in the area of recovery of carboxylic acids.

NF has also been tested, together with reverse osmosis (RO) for dewatering of filtered lactic acid fermentation broth. The separation properties of tested NF membranes were better (Timmer et al., 1994). Protein fouling occurred, and it was recommended to remove proteins by ultrafiltration before NF, but this will lead to additional costs.

Ultrafiltered butyric acid fermentation broth has been subjected to NF and RO (Cho et al., 2012). At low pH, both membrane types allowed separation of butyric acid and water from larger molecules and ions. NF gave a good recovery but a low purity whereas the reverse was the case for RO. Neither method led to a concentrated permeate.

Overall, NF and RO membranes do not seem to be the best option for primary recovery.

2.4.5 Conventional electrodialysis

Figure 5 shows the principle of conventional electrodialysis (CED). A feed solution of a carboxylate salt is introduced between cation and anion exchange membranes. Driven by an electrostatic potential, cations and anions diffuse in opposite directions, but they can pass only cation or anion exchange membrane, respectively. This leads to a more concentrated solution and a more dilute solution of carboxylate salt.

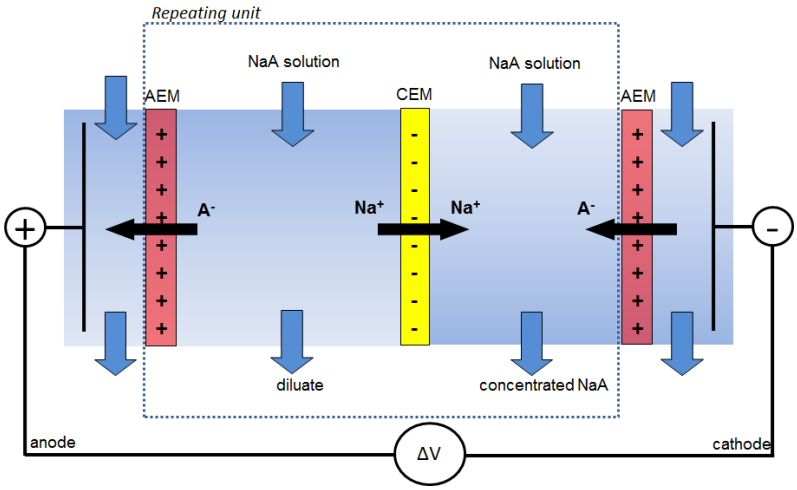

Figure 5. Principle of CED in a two compartment configuration, using as example the concentration of a sodium carboxylate (NaA) solution. CEM: cation exchange membrane, AEM: anion exchange membrane.

Obviously, CED can be used as a concentration step only before counterion removal. To some extent, CED can also simultaneously increase the purity of the target acid salt, since residual sugars or other impurities that do not rapidly pass the membranes can be removed.

CED has been used, for example, to concentrate sodium lactate solutions. In a batch desalting electrodialysis unit, aqueous solutions of 100 g/L of lactate at pH 5.5 were slightly concentrated up to 120 g/L (Gyo Lee et al., 1998). However, depending on the membrane, a maximum concentration of about 140-300 g/L can be achieved (Bailly et al., 2001).

Glassner and Datta, (1992) have described the use of an electrodialysis step as a primary recovery operation for a succinate salt. The whole fermentation broth containing succinate, acetate, cells and other impurities was circulated through a conventional electrodialysis stack in order to concentrate succinate and remove cells together with other nonionic materials. An initial run concentrated succinate from 22 to 55 g/L and acetate from 6 to 13 g/L. 10% of the proteins and amino acids were also transported to the concentrated stream. Clarification of the fermentation medium and an increase of the electrical current density by almost 50% led to a succinate concentration factor of 4 and a total succinate removal from the feed stream of 80%. As expected, acetate was concentrated accordingly.

During continuous fermentation, succinate and the side-product acetate were removed from cell free solution by CED in a pilot plant (Meynial-Salles et al., 2008). This concentrated the succinate from 20 to 80 g/L. The diluted exit stream with unconsumed nutrients was recycled to the fermentor, and this prevented that inhibiting succinate concentrations were achieved.

Citric acid has also been concentrated using CED. Ling et al. (2002) performed concentration experiments of citric acid in which a feed stream of 21 g/L was concentrated by a factor of two. A pH value of the feed stream was not specified and therefore is difficult to assess the dissociation degree of the carboxylate.

Although the applicability of this technique has been demonstrated for several carboxylates, there are still hurdles for its widespread use. Improving antifouling characteristics and increase selectivity for co-ions are the main application-related optimization goals (Huang et al., 2007). Moreover, the cost of the membranes needs to be

reduced in order to broaden its application. An economic feasibility study indicated that CED is much more attractive for monovalent than for multivalent sodium carboxylates, due to the larger electric currents required for multivalent carboxylates and lower membrane fluxes for larger anions (Moresi and Sappino, 2000).

2.5 Removal of counterions of carboxylates

In many cases carboxylate salts are formed whereas carboxylic acids are desired. When converting carboxylate into carboxylic acid, H^+ has to replace the counterion of the carboxylate (e.g. Na^+). Depending on the required procedure, the removal will lead to a (sodium) salt or base as co-product. This co-product can be removed from the carboxylic acid if both products end up in different phases. An example has been given in section 2.4.3 (conversion of sodium fumarate into solid fumaric acid and dissolved sodium salt).

When the counterion of the original carboxylate salt, such as formed during fermentation, is not easily removed, prior ion-exchange or precipitation may be used to obtain another carboxylate, which is much better or much worse water-soluble. For example, poorly water soluble magnesium succinate can be converted into much better soluble sodium succinate using sodium hydroxide.

$$MgSucc\ (\mathrm{s}) + 2NaOH\ (\mathrm{aq}) \rightarrow Mg(OH)_2\ (\mathrm{s}) + Na_2Succ\ (\mathrm{aq}) \qquad (37)$$

Magnesium hydroxide will precipitate, and can be filtered off and recycled to the fermentation for pH control (Van Krieken and Van Breugel, 2010). The sodium succinate still needs to be converted into succinic acid by one of the methods described below.

2.5.1 Removal of counterions by precipitation

Suppose that a salt *MA* of a carboxylic acid, either solid or in aqueous solution, has been obtained after fermentation or primary recovery. This carboxylate can be converted back into the desired carboxylic acid using an inorganic acid HX with a lower pK_a than the carboxylic acid while an insoluble salt is produced. The following general reaction proceeds:

$$MA\ (\mathrm{s, aq}) + HX\ (\mathrm{aq}) \rightarrow HA\ (\mathrm{aq}) + MX\ (\mathrm{s}) \qquad (38)$$

It is evident that the stoichiometry should be adjusted depending on the valences of *M*, *A* and *X*. Several combinations of metal cations and inorganic acids can be used leading to different salts. Table 12 presents the solubilities of the expected salts formed from the most common metals associated with carboxylates upon reaction with sulfuric, phosphoric and carbonic acid. Hydrochloric acid has been left out of this analysis due to the high solubility of chloride salts. Salts of sodium, potassium and ammonium are too soluble, narrowing down the options to calcium and magnesium salts. Bringing back the discussion held in section 2.2.1, sulfuric acid might be the most cost-effective acid for counterion precipitation and therefore will be treated in more detail. An overview of precipitation process based on carbon dioxide will also be given.

Starting with a calcium carboxylate, a reaction with sulfuric acid yields $CaSO_4$ as the side product (in particular $CaSO_4$ dihydrate, known as gypsum). This has a low aqueous solubility and can be filtered off. Such combination has proven to be very convenient and therefore applied to most of the carboxylates treated in this review. The main examples are the conversion of calcium citrate to citric acid (Heding and Gupta, 1975, Soccol et al., 2006) and calcium lactate to lactic acid (Min et al., 2011). In the latter case, when a clarified *Lactobacillus* fermentation broth was treated, the recovery of lactic acid was about 92% with a purity of 71%. Similarly, calcium succinate can be converted to succinic acid (Luque et al., 2009). The aqueous carboxylate solution has to be processed as described in section 2.6 and the precipitated gypsum as in section 2.8.

Table 9. Aqueous solubilities (at 25 °C, on basis of anhydrous content) of sulfate, phosphate and carbonate salts of relevant metals that may be formed upon converting carboxylate into carboxylic acid using the respective inorganic acid.

Salt cation	**Solubility[a] (g/kg)**		
	Sulfate	Phosphate	Carbonate
Na	281	145	307
K	120	1060	1110
Ca	2.05[e]	0.0012[c]	0.0066[c]
Mg	357[f]	0.0009[c,d]	1.8[c]
NH_4	764	250	1000[b]

[a](Haynes et al., 2012). [b]15 °C. [c] 20 °C. [d] Pentahydrate. [e] Hemihydrate and dihydrate (at 20 °C) show the same solubility. [f] Monohydrate and heptahydrate show the same solubility.

Precipitation of ammonium sulfate has been done after removing most water from ammonium lactate, dissolving it in alcohol, and adding sulfuric acid (Cockrem and Johnson, 1993). Ammonium sulfate has advantages to gypsum with respect to filterability and economic value, and the obtained lactic acid in alcohol is ready for esterification. Similar approaches have been performed for other carboxylates. For example, sodium sulfate has been removed from sodium succinate to obtain succinic acid in ethanol (Orjuela et al., 2011).

Calcium carbonate salts are also very insoluble and could easily precipitate out from a calcium carboxylate solution. Despite this fact, acidification using carbon dioxide will require very high pressures to achieve the required pH (see section 2.2.1). A way to aid this process is by removing the formed carboxylic acid from the aqueous phase. Miller et al., (1996) removed calcium counterion from lactate under carbon dioxide pressure while extracting lactic acid using a secondary amine extractant (Amberlite LA-2) in octanol. Maximum calcium carbonate formation was observed at pressures of 7.2 bar and low lactate concentrations (0.07 mol/L). However the capacity of the extractant was low, which limits further application.

Removal of counterions using ion exchange adsorbents or extractants

In some of the primary recovery schemes given in section 2.4, carboxylates were converted into carboxylic acids using an inorganic acid and an anion exchange resin or anion exchange

extractant. Such primary recovery and acidification can also be done separately. Then, the acidification can be performed not only using anion exchange groups but also with cation exchange groups. Only the latter will be explained here.

A strong acid cation exchange group will be indicated by R^-H^+. An example is sulfonic acid type cation exchange resin. When contacted with a carboxylate solution such as NaA, this will yield a carboxylic acid solution and a cation exchange resin such as R^-Na^+. The latter can be regenerated with a second aqueous solution (aq2) of an inorganic acid such as HX:

Adsorption: $$NaA\ (\text{aq1}) + R^-H^+\ (\text{resin}) \rightleftarrows R^-Na^+\ (\text{resin}) + HA\ (\text{aq1}) \quad (39)$$

Desorption: $$R^-Na^+\ (\text{resin}) + HX\ (\text{aq2}) \rightleftarrows R^-H^+\ (\text{resin}) + NaX\ (\text{aq2}) \quad (40)$$

Overall: $$NaA\ (\text{aq1}) + HX\ (\text{aq2}) \rightarrow HA\ (\text{aq1}) + NaX\ (\text{aq2}) \quad (41)$$

If the aqueous solutions of the overall reaction would directly be mixed, the obtained carboxylic acid and inorganic salt would not be in separate aqueous solutions. Although the carboxylic acid could be recovered in later steps with different methods, e.g. desalting, extraction or chromatography, the performance of those processes would also be affected by the presence of competing anions. Thus, using a cation exchange resin competition between the organic acid and inorganic acids or anions in further purification steps is prevented (Cao et al., 2002). On top of that, in some cases ion-exchange adsorption in a column process may have advantages such as higher outflow concentration or partial removal of contaminants.

Clarified lactate fermentation broth has been converted into lactic acid solution using strong acid cation exchange resin (González et al., 2006). Lewatit S2568H in the hydrogen form removed sodium, potassium, magnesium and calcium from a *Lactobacillus* broth. The treatment acidified the broth to pH 1.5 which then was fed to an anion exchanger for lactic acid purification. Regeneration of the resin required 2.5 bed volumes of 1 mol/L HCl, resulting in a saline effluent that needs proper treatment.

In a related example, ammonium cation was removed from a model lactate broth using the strong exchanger Duolite C-464 (Evangelista and Nikolov, 1996). The column effluent reached a pH of 2.1 and fractions were collected until pH 3. The acidified broth was also purified further using weak anion exchangers. Regeneration using sulfuric acid was performed. It was noticed that the cation exchanger removed colored compounds from the broth, improving the final purity of the produced lactic acid.

This technique has also been applied to dicarboxylates. Kushiku et al. (2006) have developed a purification process based on cation removal by ion exchange. A bacterial fermentation broth containing diammonium succinate, other carboxylates and amino acids was clarified, passed through an H-type strong cation exchange resin, then evaporated creating a succinic acid slurry which was cooled to crystallize succinic acid. As a comparison, the fermentation broth was processed in the same way but the acidification step was replaced by sulfuric acid addition. The obtained succinic acid purities for each case were 99.8 and 96.4% respectively. The authors noticed that during the cation exchange step amino acids were removed, which led to a high purity product.

Analogous to the aforementioned case, acid groups might also be present in a liquid extractant. Examples are alkyl phosphoric acids like di-(2-ethylhexyl)phosphoric acid (HDEHP), derivatives of sulfonic acids like dinonylnaphthylsulfonic acid (Khopkar, 2007) and Versatic acid 10, a brand name for an α,α-dibranched decanoic acid mixture. Albeit the availability of these materials, no references of their use in the counterion removal of fermentative carboxylates have been found.

Evaporation of volatile bases acting as counterions

Bases such as ammonia, methylamine, dimethylamine and trimethylamine are gaseous, having boiling points of -33, -6, 7 and 3 °C, respectively (Weast, 1979). These bases can be used during fermentation to control the pH (see Section 2.3.2) and lead to obtaining the respective carboxylate salt. By heating such carboxylates, the base may be liberated, thus obtaining the carboxylic acid. For example, for the ammonium salt of the desired carboxylic acid HA:

$$NH_4^+A^- \xrightarrow[\Delta]{} NH_{3(g)} + HA \tag{42}$$

This would allow recycling of ammonia as base for pH control in the fermentation or for desorption or back-extraction during primary recovery. However, a consecutive amidation between the ammonia and carboxylic acid occurs. This is due to the high temperature required for the ammonia evaporation, increasing the amidation rate. Moreover, concomitant water evaporation concentrates the system and also facilitates such side reaction. The amidation occurs also with methylamine and dimethylamine but cannot occur with trimethylamine (TMA), also employed in alternative desorption (Section2.4.1) and back-extraction (Section 2.4.2) schemes. It should be reminded that thermal decomposition of trimethylammonium salts in aqueous solutions has proven to be difficult for very soluble acids and a water removal step might need to be carried out first. In an application example, TMA was successfully used as neutralizing agent in the production of lactic acid leading to higher productivities, proving that the produced trimethylammonium lactate was not inhibitory to *Lactobacillus sp.* (Hetenyi et al., 2011). After thermal decomposition of the salt, lactic acid could be further purified as discussed in previous sections.

Bipolar membrane electrodialysis

Upon electrolysis, water may be split into H^+ and OH^-. This is applied in bipolar membrane electrodialysis (BPED). Cation and anion exchange membranes, in combination with an electric potential, can be used to transport cations and anions in opposite directions, such that H^+ and OH^- combine with a carboxylate anion and its counterion, respectively. In the example of Figure 6 a sodium carboxylate solution is converted into a NaOH solution and a separate carboxylic acid solution.

$$NaA\,(\mathrm{aq}) + H_2O \xrightarrow{\Delta V} NaOH\,(\mathrm{aq}) + HA\,(\mathrm{aq}) \tag{43}$$

Instead, a two-compartment configuration with a central AEM is also possible, as well as a three-compartment configuration with separate central AEM and CEM membranes (Huang

et al., 2007). The configuration shown in Figure 6 has the advantage that only the cation (which often is smaller and diffuses faster than the anion) has to pass a membrane. The alternatives have the advantage that some neutral impurities can be kept from the product stream. The three-compartment configuration is the most complicated one but suffers least from leakage of OH^- or H^+ through the membranes resulting from steep pH gradients (Wang et al., 2011).

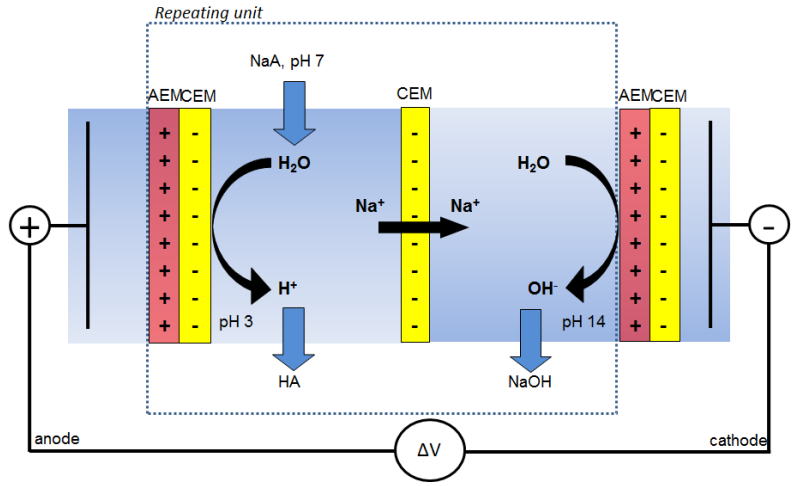

Figure 6. Two-compartment bipolar membrane electrodialysis using cation exchange membranes (CEM) and anion exchange membranes (AEM). A bipolar membrane is depicted as a stack of CEM and AEM. NaA is the carboxylate salt and HA the carboxylic acid.

The produced NaOH solution can be used to control the pH in the fermentation, thus recycling the Na^+ cations. If the carboxylate feed is pure enough, the carboxylic acid only needs to be recovered from the aqueous solution, which will be treated in later sections.

Thus, BPED splits the carboxylate salt into carboxylic acid and inorganic base, as desired. Nevertheless, there are some less attractive features (Bailly, 2002, Huang et al., 2007):

- At the cathode and anode, H_2 and O_2 are formed, respectively.
- H^+ formed at the left hand side of Figure 6 can pass the cation exchange membrane in the center and recombine with OH^- at the cathode, leading to a futile cycle.
- Typical feed concentration should exceed 1 mol/L of carboxylate groups to prevent too large membrane and operating costs
- Multivalent cations need to be removed beforehand to ppm level because they form products with a low aqueous solubility, such as $Ca(OH)_2$, which precipitate and foul the membranes.

BPED has been used, for example, for acidification of a fermentation solution containing sodium acetate, so that liquid-liquid extraction of acetic acid could be performed (Katikaneni and Cheryan, 2002). Concentrates of 200 g/L lactic acid and 4.5 mol/L NaOH have been obtained from 40 g/L sodium lactate, leaving less than 1 g/L in the feed (Börgardts et al., 1998).

BPED was shown to be economically favorable for recovering lactic acid (Bailly, 2002, Börgardts et al., 1998, Datta et al., 1995) or gluconic acid (Wang et al., 2011) from fermentation broth. The costs of the bipolar membranes are high, and to reduce this membrane area, prior concentration using CED has been proposed as shown in Figure 7(Bailly, 2002).

Such a process strategy has been applied to the recovery of sodium gluconate and citrate and compared with a simple three-compartment bipolar electrodialysis (Novalic et al., 2000). A significant reduction in the required bipolar and anion exchange membrane area was found for both cases, but at the expense of higher cation exchange membrane requirements as a result of the double transport of Na^+ in the combined process. A reduction of the specific energy consumption was noticed, being more important in the case of sodium citrate. An optimization of the process should be carried out taking into account the energy and different membrane costs.

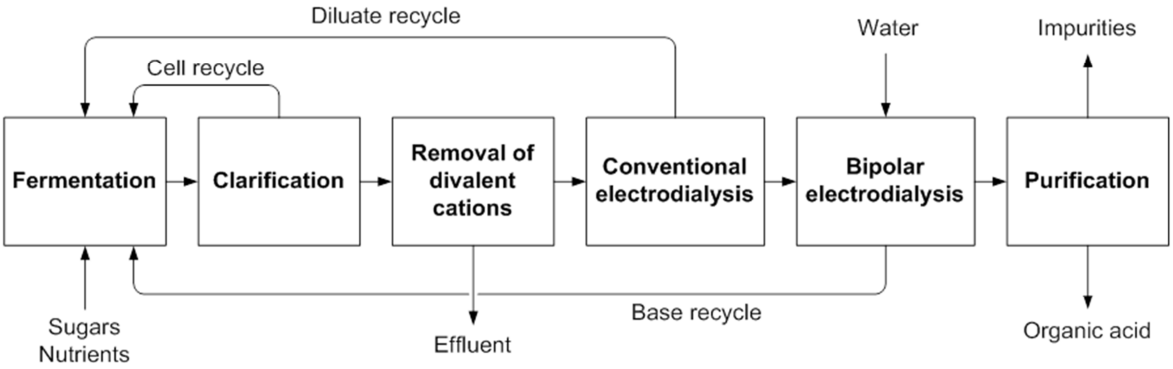

Figure 7. Configuration proposed for CED and BPED. Adapted from Bailly (2002)

2.6 Water removal and carboxylic acid purification

A large portion of water may be removed during primary recovery, but often a separate water removal step has to be introduced. Several methods are available. These may be used at different positions in the process, to concentrate the carboxylic acid, its salt, or an inorganic salt. The removal of water is often integrated with purification by distillation or crystallization.

2.6.1 Reverse osmosis

If reverse osmosis (RO) membranes allow passage of only water, a concentration effect is obtained that may be exploited for crystallization (Cuellar et al., 2009). This RO may be energetically more favorable than heat-driven evaporation. However, the proportion of permeated carboxylic acid should be negligible, because that will be lost product.

Fundamental studies on RO of lactic acid, ammonium and sodium lactate have been carried out to understand the effect of their physicochemical properties on the main process operating variables (Liew et al., 1995). Such salts were selected based on the type of neutralizing agent used in fermentation. It was found that both salts were better rejected than the undissociated acid (83, 91 and 99% respectively) but at the expense of the permeate flux. The authors found that, besides its convenience in fermentation, ammonium lactate provided the best balance between flux, concentration factor and total solute loss. Nevertheless, at high feed concentrations (5.8%) a concentration of only 5% was achieved accompanied with a

solute loss close to 10%. Similar cases and results have been reviewed elsewhere (Pal et al., 2009).

Instead of using pressure-driven reverse osmosis, forward osmosis of butyric acid solutions has been published, for example by Cho et al.(2012), using a concentrated salt solution at the permeate side for driving water permeation. This salt solution will become more dilute. For reuse, it will have to be concentrated by another process step. So, forward osmosis does not lead to net water removal.

2.6.2 Evaporation and distillation

In evaporation, water and maybe volatile impurities are removed from nonvolatile carboxylic acid, whereas in distillation also the carboxylic acid is volatile and is separated from other volatile components (such as water) in a countercurrent evaporation-condensation method.

Evaporation is the default process for removing water. Evaporation costs are high for dilute aqueous solutions due to the energy required to evaporate water. Therefore it is important to obtain concentrated solutions by fermentation, and to concentrate rather than dilute during primary recovery and counterion removal. A first estimate of energy costs can be made assuming that water needs to be heated up from 30 to 100 ^{o}C and needs to evaporate, requiring 2.55 MJ/kg. Reasonable assumptions are that 50% of this heat can be saved by heat integration, that steam can provide 2.26 MJ/kg (Vane, 2008), and that the steam price is 0.012 $/kg (SuperProDesigner, version 8.5). This leads to evaporation energy costs of 0.0068 $/kg of water. Evaporating all water from a 50, 100 or 200 g/L carboxylic acid solution would then require 0.13, 0.06, or 0.03 $/kg carboxylic acid, respectively. Such numbers allow comparison of potential evaporation costs for fermentations such as indicated in Table 6, whereas these fermentations can also be compared with respect to inorganic acid and base costs using data previously given. However, substrate and equipment cost differences, a.o., should also be estimated.

As indicated before, water evaporation may be integrated with distillation for volatile carboxylic acids. According to the boiling points given in Table 2, distillation is possible with acetic, propionic and butyric acid. A prerequisite is that these acids are undissociated. Since the distillation of these compounds in petrochemical processes is well-known, not much experimental work is done with bio-based carboxylic acids. Vapor-liquid data and models are incorporated in modern process design software. Using such models, simulations indicate for example that propionic acid can be readily purified by a final distillation (Posada and Cardona, 2012). Acetic acid has a boiling point close to that of water, and several extractive distillation schemes have been evaluated to facilitate the purification (Garcia and Caballero, 2011).

Direct distillation of lactic acid from a crude acidified fermentation broth at normal temperature and pressure for recovery of the lactic acid has not been acceptable because lactic acid forms high boiling internal esters as dimers and polymers during the distillation, resulting in poor yields of lactic acid (Cockrem and Johnson, 1993). Therefore, crude lactic acid is usually esterified with alcohols such as methanol, and such volatile esters are removed

by distillation. This can lead to high purification. The esters can be used for further processing or can be hydrolyzed to lactic acid (Datta and Henry, 2006).

Pervaporation may be used to enhance the selectivity of removing water from carboxylic acid solutions via the vapor phase. Thus, pervaporation has been done for concentrating lactic acid using inorganic NF membranes (Duke et al., 2008). However, the fluxes were assumed to be far too low for commercial application (Pal et al., 2009).

In case of carboxylic acids that need crystallization, part of the water will be evaporated. Volatiles contaminating carboxylic acids, such as acetic acid, can be removed during evaporation (Huh et al., 2006).

2.6.3 Acid polishing / Chromatography

Before crystallizing a carboxylic acid, its solution may still contain impurities that were not removed during previous processing steps and, depending on the application, may need to be removed. It might be evident at this point that most of the primary recovery methods are able to recover the target acid from many types of but they may lack the required selectivity to separate it completely from other organic acids. Albeit most of the metabolic engineering efforts in strain development are focused on minimizing by-product formation (and therefore improving the carbon yield of the product), many carboxylic acids are produced in closely related metabolic pathways and therefore their simultaneous production is difficult to avoid. In relation to this, such acids might be structurally similar, e.g. fumarate as by-product in succinate fermentation, complicating even further the purification.

In such cases, a polishing step may be included to remove troublesome acid impurities often present in relative low concentrations. Adsorptive chromatographic methods are among the best suited for this purpose and have been applied to several fermentative carboxylic acids.Due to the expected production scale for these molecules, continuous counter-currentprocesses such as simulated moving bed (SMB) operations can be implemented. Figure 8 depicts an eight columns, four zones SMB in which the four ports, feed, desorbent, extract and raffinate, switch periodically by a column bed and in the direction of the mobile phase to achieve complete separation of the target acid and the critical impurity.

Like in other sorption-based separation techniques, the selection of the solid phase and the desorbent are crucial for the performance of the separation. These will impact the number of columns needed, desorbent consumption, achievable purity, recovery yield, waste production, final concentration of the target acid and economics. In a compelling application example, fermentative lactic acid was separated from acetic acid, the major acidic impurity in the fermentation broth (Lee et al., 2004). A poly(4-vinylpyridine) resin (PVP) was used based on its selectivity and capacity toward lactic acid. Complete separation of the acids was possible and thus very high purities (99.9%) and recovery yield (93%) for lactic acid were achieved. Nonetheless, the use of deionized water as desorbent led to a dilution of the purified acid by a factor of 7, increasing final evaporation costs.

Nam et al. (2011) studied the adsorption equilibrium of succinic and lactic acid on a hydrophobic polystyrene resin as a first step in the design of a SMB process for separating the two acids. The dependence of the adsorption equilibrium on temperature revealed that the

affinity toward succinic acid is greater than for lactic acid, allowing complete separation of both acids. As in the previous example, a drastic 10-fold dilution of the purified succinic acid relative to the feed solution is expected. A similar equilibrium study showed that primary and secondary amine functionalized SBA-15 silica can also be used for removal of pyruvic acid from concentrated succinic acid solutions (Jun et al., 2007a).

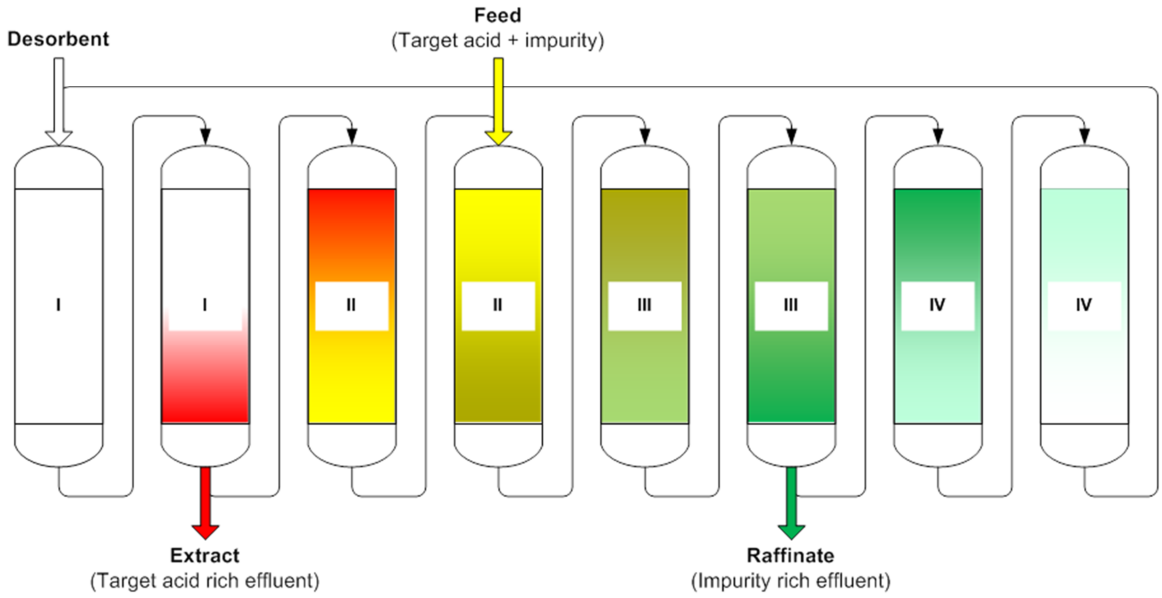

Figure 8. Four zone simulated moving bed (SMB) chromatography for the purification of carboxylic acids.

In an analogous setting, however focused on a primary recovery rather than polishing step, the separation of fermentative citric acid from unconverted glucose was carried out in a SMB using a PVP resin and deionized water as an eluent (Wu et al., 2009). After optimization of the process conditions, 99.8% purity and 97.2% recovery yield were attained at high feed concentrations (640 g/L of citric acid) with an extract concentration reduced by 50% compared to its concentration in the feed.

Besides other carboxylic acid impurities, critical impurities may comprise cations and inorganic anions. Chromatographic operations using selected cation and anion exchangers can be used to remove such contaminants. A two-step process including cation removal by a strong cation exchanger and anion decontamination by a weak anion exchanger in the free base form was designed to purify a concentrated succinic acid effluent after precipitation and acidification (Datta, 1992). Residual calcium was completely removed after cation exchange and sulfate was captured by the weak exchanger. Residual proteins were also removed in both columns. As a result of this polishing step, the purity of succinic acid was increased from 89.6% to 94.2%.

2.6.4 Crystallization

Solubility data are crucial for determining the potential of crystallization. Aqueous solubilities at room temperature are given in Table 2. For determining the influence of

temperature and co-solutes, detailed data or models are required, such as given for succinic, glutaric, and malic acid by Clegg and Seinfeld (2006a, b). Some carboxylic acids do not crystallize easily from water, because they are too soluble. Water removal (usually by evaporation) and cooling will lead to supersaturation, so that crystallization can occur. The extent of water removal has to be stopped at the concentration where the solution also becomes supersaturated by one or more of the contaminating nonvolatile solutes. The amount of carboxylic acid crystallized at that point determines the yield of the crystallization.

Ideally, the obtained crystals would be pure, but some impurities might be built in the crystal structure if they fit well. Impurities that attach at crystal surfaces can we washed off at the expense of some loss by dissolution.

Amongst fermentative carboxylic acids, citric acid crystallization has been studied best because of its commercial relevance. Citric acid crystallizes at temperatures above 36.6 °C as anhydrate, and at lower temperatures as a monohydrate (Nyvlt and Vaclavu, 1972). The highest crystallization yields can be obtained at the lower temperature. Therefore, the monohydrate has been the focus of fundamental research. For example, growth rate dispersion of citric acid monohydrate occurring in continuous crystallizers was explained using a mathematical model (Berglund and Larson, 1984). A modest influence of some fermentation impurities (KH_2PO_4, $MgSO_4$, $FeSO_4$) on primary nucleation was found (Bravi and Mazzarotta, 1998).

For itaconic acid, not much fundamental data have been published, but commercial crystallization has been described (Okabe et al., 2009). An itaconic acid solution, obtained by clarifying fermentation broth, is concentrated to 350 g/L for crystallization at 15 °C. The mother liquor is subjected to a second, similar crystallization. Recrystallization is performed after active carbon treatment. The mother liquor of the 2nd crystallization still contains substantial amounts of itaconic acid, but the presence of impurities such as glucose impede further crystallization (Zhang et al., 2009).

Succinic acid crystallization is currently of key interest. For batch crystallization at 4 °C, Huh et al. (2006) concentrated a succinic acid solution, obtained by using HCl to acidify calcium succinate from a fermentation. The crystal purity was 98% but the yield of the crystallization step was only 68%. Others have not been able to achieve better results using comparable methods (Li et al., 2010b, Luque et al., 2009). Prior removal of some contaminating organic acids by extraction increased the crystal purity to 99.8% and the crystallization yield to 73% (Huh et al., 2006). The moderate yields may be due to dissolved calcium chloride that was present at stoichiometric level in the succinic acid solution.

Crystallization or recrystallization from organic solvent may be more effective than from water. Obviously, solubility data in organic solvents are required, but their availability may be limited. It is important to notice that the solubility in water-immiscible organic solvents may increase by a factor of ~5 if the solvent is water-saturated (Starr and King, 1992). In fumaric acid and adipic acid crystallization from organic extracts, the energy required to evaporate this water will be large as compared to the total evaporation energy.

As described in the section on primary recovery, Shishikura et al. (1992) obtained citric acid in acetone solution containing CO_2. More CO_2 was dissolved in this solution up to ~50 bar pressure, leading to citric acid crystallization, because CO_2 decreased the polarity of the

solution. The purity and recovery of crystals were 99.8 and 96.4%, respectively. Oxalic acid, which was a key impurity, remained dissolved in the supernatant. After drying the crystals, food grade citric acid was obtained (Shishikura et al., 1994).

2.7 Destination of inorganic salts formed in the process

Most of the aforementioned process options for carboxylic acid fermentation and recovery lead to stoichiometric amounts of inorganic salts. This is not a problem if these salts have a useful destination, but in any case they influence the economics of the recovery process. Again, there are many different options, and these will be treated here to facilitate evaluation of alternative carboxylic acid processes.

2.7.1 Disposal of salt as waste

If no useful destination can be found for the co-produced salt, it will become waste. The costs of this will vary according to local legislation and facilities. Close to sea, some dissolved inorganic waste salts may be purged for free. In the Netherlands, tariffs are used of 0.085 €/kg sulfate or chloride in wastewater streams (Anonymous, 2012). For Na_2SO_4 this is equivalent to ~0.16 $/kg. Since the amount of waste salt produced is about 1 kg per kg carboxylic acid, such a number might easily be an unaffordable contribution to the production cost of the carboxylic acid (which is typically 0.5-5 $/kg). Alternative destinations of the salt should be considered.

Use of salt as co-product

For all waste salts one should consider selling them. Approximate salt prices are given in Table 10. These revenues may be obtained if the salt such as produced during the fermentation process can be processed to meet the specifications required by the market. Such processing may easily be too costly if it involves evaporation of water from a dilute aqueous salt solution or removal of impurities.

Gypsum ($CaSO_4.2H_2O$), which is directly obtained as wet solid during classical citric acid and lactic acid production processes, will not need much processing for specific applications. Disposal of gypsum is a potential problem (Milsom and Meers, 1985a), but various internet sources indicate the use of such gypsum as a kind of fertilizer in agriculture, but not for wallboard, which is the main market of gypsum. The gypsum for wallboard is usually mined.

As fertilizer, ammonium sulfate is more important and valuable than gypsum. It accounts for about 4% of the world nitrogen fertilizer market (IHS, 2013). Usually, ammonium sulfate is obtained as co-product in chemical processes or directly from ammonia and sulfuric acid (Zapp et al., 2000). In a fermentation process, ammonium sulfate is obtained *indirectly* from ammonia and sulfuric acid. Crystallization and further processing of this ammonium sulfate will be required. With the prices given in Table 10, it will not be profitable to produce ammonium sulfate from downstream processing of carboxylate fermentation processes, but if

the costs can be kept low, co-producing ammonium sulfate may overall be less costly than co-producing another inorganic salt.

Similar evaluations may be performed for other salts mentioned in Table 10. Potassium sulfate has also an attractive price and its processing might be economical, if compared to ammonium sulfate, due to its lower solubility (Table 12). However its market is limited as a specialty fertilizer which may hinder its commercialization. Thus, the choice of the base used for neutralization in the fermentation process requires a good knowledge of the market of the waste salt obtained.

Table 10. Market prices of waste salts and the constituting acids and bases

Base	Approximate price ($/kg)	Costs of acid + base [d] ($/kg)
Na_2SO_4	0.10 [a]	0.17
K_2SO_4	0.70 [a]	0.51
$CaSO_4.2H_2O$	0.09 [b]	0.08
$MgSO_4$	0.15 [c]	0.18
$(NH_4)_2SO_4$	0.15 [c]	0.14

[a] (Orjuela et al., 2013) based on ICIS 2012 Chemical price report.

[b] (Founie, 2007).

[c] The source (Anonymous, 2006) gives price ranges and various qualities, so the actual value may deviate considerably.

[d] Calculated from the stoichiometric amounts of inorganic acid and base required to make the salt, and the acid and base prices given in previous Tables.

Bipolar membrane electrodialysis of inorganic salt

As discussed before, BMED can split the target carboxylate salt into carboxylic acid and a hydroxide. Here we discuss an alternative. An inorganic salt such as produced as side product when forming carboxylic acid can be subjected to BMED, to regain this salt's constituent inorganic acid and inorganic base. For example, aqueous Na_2SO_4 can be split into aqueous NaOH and aqueous H_2SO_4. The aqueous NaOH can be reused for pH control during fermentation and the aqueous H_2SO_4 can be reused for recovering the carboxylic acid by acidifying the fermentation broth.

This BMED may be profitable if the processing costs per kg of salt are lower than the costs of buying fresh inorganic acid and base, according to prices such as given in Table 3 and Table 4, plus the costs of disposing inorganic salt. Note that costs of disposing inorganic salt are negative when some revenue is obtained for it, but even then BMED may be profitable.

Thermal cracking of inorganic salt

Inorganic salts composed of volatile acids or bases such as CO_2 and NH_3 can be decomposed by evaporation of these volatile components upon thermal treatment. In this way, the acid and base can be reused in the process. Obviously, the energy required is high to

invert the exothermic reaction of acid with base to salt, especially if the acids and bases are strong. At the high temperatures used and the resulting high or low pH values, many organic components will degrade, so their presence has to be minimized.

For a number of inorganic salts, thermal cracking has been suggested as a process option:

Eyal et al. (1986) mentioned that ammonium sulfate is decomposed at 200 °C to ammonium hydrogen sulfate:

$$\left(NH_4\right)_2 SO_4\ (s) \xrightarrow{\Delta} NH_3\ (g) + NH_4HSO_4\ (s) \tag{44}$$

No details were given. A patent on succinate production indicates that the remaining mass almost quantitatively agreed with the expected mass of NH_4HSO_4, after heating at 300 °C (Berglund et al., 1999). Apparently liberation of a second ammonia molecule does not occur because sulfuric acid is a too strong acid.

Baniel et al. (1996) indicated that heat treatment or other techniques may be used to convert solid sodium bicarbonate, such as produced during lactic acid recovery, into more useful CO_2 and sodium carbonate:

$$2NaHCO_3\ (s) \xrightarrow{\Delta} CO_2\ (g) + Na_2CO_3\ (s) + H_2O\ (g) \tag{45}$$

Similarly, it has been indicated that ammonia and CO_2 can be recovered from ammonium carbonate or bicarbonate that was produced in a lactate process option (Sterzel et al., 1995):

$$\left(NH_4\right)_2 CO_3\ (s) \xrightarrow{\Delta} CO_2\ (g) + 2NH_3\ (g) + H_2O\ (g) \tag{46}$$

$$\left(NH_4\right)HCO_3\ (s) \xrightarrow{\Delta} CO_2\ (g) + NH_3\ (g) + H_2O\ (g) \tag{47}$$

In recent patent applications related to carboxylate production, magnesium chloride obtained after acidification of magnesium carboxylate with hydrochloric acid was thermally decomposed at ~350 °C producing magnesium oxide and the respective inorganic acid in a typical pyrohydrolysis reaction (Cerdà Baró et al., 2013, De Haan et al., 2013):

$$MgCl_2\ (s) + H_2O\ (g) \xrightarrow{\Delta} 2HCl\ (g) + MgO\ (s) \tag{48}$$

The produced magnesium oxide can be further transformed into magnesium hydroxide upon contact with water, providing the base required during bacterial fermentation. Furthermore, the produced hydrochloric acid can be absorbed in water to be reused in the acidification step or used in its gas form to directly acidify magnesium carboxylate in an integrated absorption operation.

Another inorganic ammonium salt that may be obtained is that of zeolite Y. This zeolite consists of silica and alumina groups, which cations compensating the negative charge of the alumina groups. Thus, it can be considered as cation exchange material that can resist heating. If it is in the ammonium form, ammonia can be removed from it by heating:

$$NH_4Y\ (s) \xrightarrow{\Delta} NH_3\ (g) + HY\ (s) \quad (49)$$

Zhou (1999) passed ammonium fumarate solution through an acidic Y-zeolite column that retained the ammonium and liberated the fumaric acid. At 300-400 °C, ammonia was liberated, thus regenerating the zeolite in the H^+ form.

The above described processes lead to alternatives in which waste streams are avoided at the expense of energy consumption. The thermal decomposition of the solids can be carried out in specialized furnaces or roasters (Cerdà Baró et al., 2013).

2.8 Combining process steps

In-situ product removal

Removing carboxylic acid or carboxylate during fermentation can prolong the time before the fermentation stops due to inhibiting product concentrations. Moreover, removing the undissociated carboxylic acid can reduce the consumption of pH-controlling base and the associated inorganic salt production.

During extractive fermentation, the broth is internally or externally contacted with an extractant. This has been studied by many groups and for many different carboxylic acids (Yang et al., 2007). As mentioned before, the fermentation will decrease the pH, but carboxylic acids can be extracted much better than the corresponding carboxylates. Therefore the fermentation pH can be controlled by balancing fermentation and extraction instead of using base addition.

For a successful implementation of in-situ extractive fermentation, the extractant should be not toxic to the cells and should even be efficiently extracting at relatively low carboxylic acid concentration. Moreover, the phase separation characteristics of the extraction system (density difference, viscosity and interfacial tension) should not impact adversely the bioconversion itself. Such prerequisites can be partially overcome with external contacting or pertraction (membrane aided extraction). A popular extractant is trioctyl amine in oleyl alcohol. Regeneration of such extractants, however, is often done via back-extraction with stoichiometric amounts of strong inorganic bases. This offsets the avoidance of such bases during the fermentation. In another perspective, the amine extractant is a base that neutralizes the fermentation pH. Consequently, the formed ammonium salt of the carboxylic acid is relatively stable, and not easily decomposed into its constituent amine and acid.

Similarly, adsorptive fermentation can be done. Figure 9 shows a possible operation strategy in which an adsorption column is integrated with the fermentor (step 2) selectively removing the target carboxylic acid or carboxylate, after desorption (step 4) the column is ready for a new cycle. Similar strategies have been used elsewhere, for example, Cao et al. (1996) used a rotary biofilm contactor as fermentor for *Rhizopus oryzae*, in combination with an adsorption column. The produced fumaric acid was removed from the broth by the resin in a recycle loop, reducing product inhibition and thus increasing the production rate and sustaining cell viability. A strong quaternary ammonium resin in the hydroxide form and a polyvinyl pyridine (PVP) weak anion exchange resin were selected as adsorbents because this

yielded the highest loading capacity for fumaric acid (0.22 and 0.31 g g^{-1} dry weight, respectively). Desorption of the fumarate and regeneration of the resin to its hydroxide form led to stoichiometric inorganic acid and base consumption and associated salt production, however.

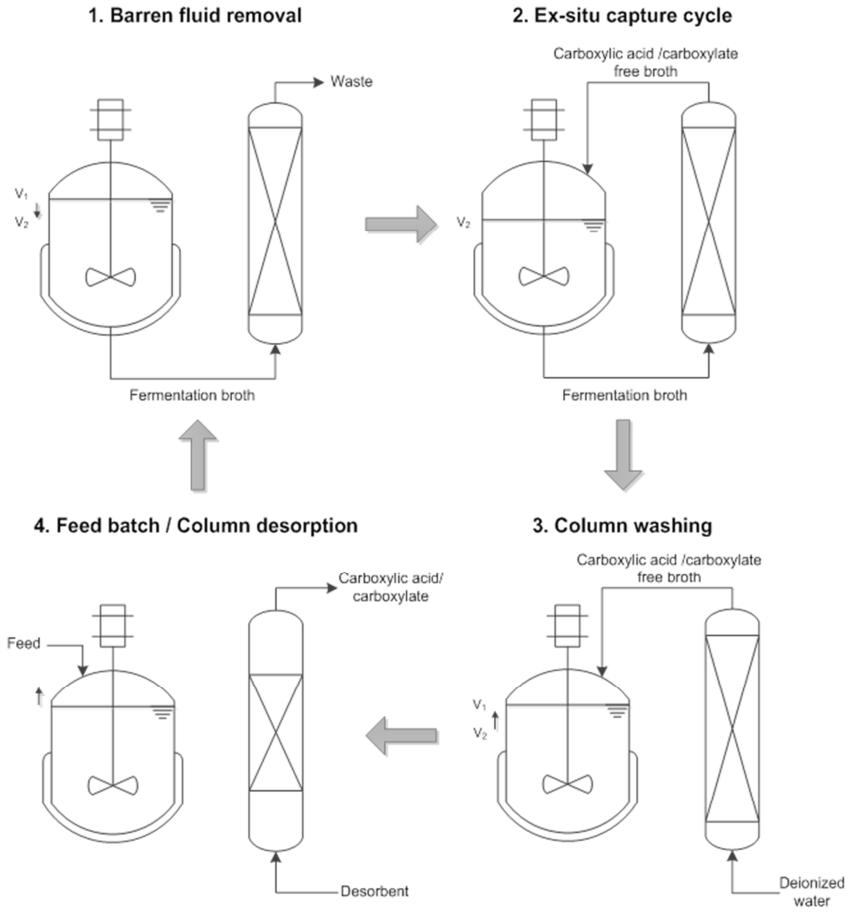

Figure 9. Operation strategy for the integration of a fed-batch fermentation and adsorptive carboxylic acid / carboxylate removal. During steps 1-3 the column operates in expanded bed mode and desorption is performed in fixed bed mode. Integration is also possible using an intermediate cell removal step instead.

During a fermentation that produced sodium fumarate at pH 5, Zhou (1999) cycled the broth over a column of Amberlite IRA-900 resin (strong base) in the OH^- form. The adsorption of fumarate released OH^- to the fermentation medium, which controlled the fermentation pH. After eluting the loaded column with 1 mol/L ammonium hydroxide solution, ammonium fumarate solution was obtained.

Lactic acid was removed in-situ in a dual fluidized bed of immobilized cells and weak anion exchange resin Amberlite IRA-67 (Patel et al., 2008). Desorption from the resin with NaOH was integrated in the system and led to a sodium lactate solution.

In a fed-batch fermentation, Li et al. (2011) cycled magnesium succinate-containing broth through an expanded bed of anion exchange resin in hydroxide form. Succinate was bound

and hydroxide was liberated, so that it could control pH of the fermentation. The resin neither adsorbed cells nor decreased cell growth and succinic acid production, thus prolonging the fermentation. As expected, elution of the succinate from the resin and regeneration of the resin led to stoichiometric salt production.

Alternatively, different membrane processes have been integrated with the similar goals. During nanofiltration of fermentation broth, lactic acid permeated better than sodium lactate, whereas lactose and other feed components such as magnesium ions were mostly retained (Jeantet et al., 1996). Also cells were retained without much membrane fouling, thus facilitating ISPR for fermentations at relatively low pH. Permeation fluxes were low, however.

Integration of lactic acid fermentation and a pH controlled electrodialysis module was applied to increase the overall productivity of the process (Hongo et al., 1986). The current applied to the electrodialysis module was controlled to maintain the fermentation pH at adequate levels for the microorganism. During the whole duration of a batch, the pH was controlled to 5.5 by removing the lactate anion in the concentrate side. Although fouling of the membrane was noticed, the productivity of the integrated system was comparable to the conventional fermentation system in which a base is used as neutralizing agent.

Gluconic acid removal by electrodialysis during enzymatic glucose oxidation to gluconic acid has been achieved (Arora et al., 2007). Without any base addition, the pH was controlled at ~4.5. Similarly, lactic acid producing cells were immobilized within the ED unit. The system will need further development to reduce fouling of membranes and to increase product concentrations.

Insoluble carboxylic acids can be removed in-situ by properly adjusting process conditions. Fumaric acid has been removed from fermentations at pH 3.5 by cycling filtered broth along a cooling crystallizer at 0-5 ^{o}C (Roa Engel, 2010). No primary recovery, counterion removal or concentration needed to be performed in this option, but only a modest portion crystallized per cycle. Alternative in-situ crystallization for a range of organic acids has been discussed by Urbanus et al. (2012)

In all these systems, the carboxylic acid rather than the carboxylate needs to be removed to control the pH and minimize product inhibition. However, the lower the acid tolerance of the used strain, the higher the feasible fermentation pH, and the lower the ratio of carboxylic acid to carboxylate. The best ISPR methods should selectively remove carboxylic acid as compared to carboxylate, and should not convert the removed carboxylic acid into carboxylate.

Overall, proving that in-situ recovery is beneficial is not easy. For example, various justifications are given for in-situ adsorption:

1. The fermentation may proceed faster and longer by removing inhibiting product
2. Base addition for controlling fermentation pH is prevented
3. Selective adsorption leads to primary purification and/or to a more concentrated product stream

Ad 1. The improvement of fermentation has to be weighed against the decrease of the performance of adsorption, which probably cannot be done anymore at the most favorable conditions. This will require comparison of the base case and the in-situ case in process models.

Ad 2. This makes sense if regenerating the adsorbent does not consume base, but such regeneration may not be available yet.

Ad 3. When the target was merely primary recovery or concentration, there is no clear advantage in using in-situ instead of ex-situ adsorption.

Integration with esterification or other reactions

Carboxylic acids can sometimes directly be used in consumer products, for example citric acid is used as food ingredient. However, more often carboxylic acids need to be converted into derivatives. If the follow-up conversion can bypass the isolation and purification of the carboxylic acid, favorable process integration may be achieved. Some examples will be given here.

Polymers are an important destination of carboxylic acids (see Table 1). Many of these polymers can industrially be prepared in a more convenient way from methyl or ethyl esters of the carboxylic acids. Such esters may be purified by distillation, which is simpler than the purification by crystallization of carboxylic acids. For lactic acid and succinic acid, this has led to methods for esterification without intermediate purification of the carboxylic acid. In section 2.5 it was already indicated that solutions of lactic acid in methanol can be obtained for direct esterification. Similarly, succinic acid can directly be esterified (Orjuela et al., 2011). The excess of sulfuric acid used for precipitating sodium sulfate from an ethanolic sodium succinate mixture can be used as esterification catalyst.

When ammonium lactate was extracted by tributyl phosphate under vacuum at high temperature, to evaporate ammonia and water, and the extract was esterified with ethanol in the presence of acid ion-exchange catalyst, 78% conversion was obtained, and 95% selectivity to ethyl lactate (Kasinathan et al., 2010). The evaporated ammonia might be reused for controlling the fermentation pH.

Barve et al. (2012) published another way to obtain esters directly from carboxylates while recovering inorganic base. They dissolved anhydrous sodium acetate, sodium benzoate, sodium salicylate, and calcium lactate in excess methanol under 40-60 bar CO_2 pressure and obtained at 170 ^{o}C up to 81% methyl ester. Alkali carbonate precipitated and might be reused for pH control during the fermentation. This is a system of equilibrium reactions where the liberated water is partly used for formation of H_2CO_3 from CO_2.

Biphasic esterification of aqueous succinic acid solutions with long chain alcohols could be applied as "reactive extraction" of the carboxylic acid from fermentation broth (Delhomme et al., 2012). Using 1-octanol as an extractant and reagent, succinic acid is removed to the organic phase as dioctyl succinate. Among several homogeneous and heterogeneous catalysts, dodecylbenzenesulfonic acid and Novozym 435 were selected as the most active for the esterification reaction in the biphasic system. Although the obtained conversions are promising, the obtained esters might not have an attractive market. If purified succinic acid is required, the formed ester can be hydrolyzed and the alcohol reused as extractant, but such an option is costly as it involves distillation steps of high boiling point compounds.

Another interesting option for avoiding waste salt from neutral pH fermentations is to couple complexation and acidification with tributylamine and carbon dioxide, respectively

(Eggeman and Verser, 2005). A proof of concept was carried out using calcium acetate as starting carboxylate. Upon acidification with CO_2 and removal of calcium carbonate, aqueous acetic acid was contacted with tributylamine forming a water soluble complex that can be extracted using a long chain alcohol. Reactive regeneration by biphasic esterification is done producing an organic phase rich in the ester and the amine. After distillation, the amine and excess of alcohol can be recycled within the process. It was determined that the performance of the complex extraction and esterification is affected by the length of the alcohol chain, and the best compromise was found when 1-hexanol was used. Although the process is promising and should minimize waste salt production, the produced long chain esters might not be convenient, as discussed previously.

A new integrated process for ester formation bypasses the need for acidification of the carboxylate salts found in esterification reactions. In this direct downstream catalysis approach, adsorptive recovery and upgrading to esters via *O*-alkylation are integrated to minimize waste salt production (López-Garzón et al., 2012). An aqueous carboxylate is captured using a strong anion exchange resin, which is also the catalyst for the conversion of the sorbed carboxylate to its respective ester. The reaction also regenerates the resin to the proper ionic form for reuse. A proof of principle was performed using aqueous sodium succinate and chloroethane as alkylating agent. Good ester yields and favorable atom economy were obtained. The use of dimethyl carbonate as alkylating agent allowed further integration with fermentative production (López-Garzón et al., 2014). A carbonate base, which can be used as neutralizing agent, is coproduced with the dimethyl ester in this new reaction scheme. In the case of succinate, the carbonate base also provides the inorganic carbon required for the best producing bacteria. Almost quantitative ester yields and adequate kinetics were found. In spite of its benefits and potentially neutral waste production, the resin may not be stable enough to withstand the cyclic changes between aqueous and organic solutions, furthermore as the reaction temperature is close to the upper operational limit of the resin, irreversible defunctionalization might occur. Improvements in such aspects would lead to a more attractive process alternative.

In summary, the integration of bio-based production processes and chemical transformations through recovery operations can pave a new processing paradigm in which sustainability goals could be better reached. New auxiliary materials/catalysts together with green chemical transformations in aqueous or biphasic media are required to exploit the possibilities of downstream transformations of fermentative carboxylic acids.

2.9 Industrial recovery processes

Open sources provide only limited data about industrial processes, but the main process steps are known for large-scale production of carboxylic acids.

2.9.1 Citric acid

Citric acid is produced using low pH fermentation. A flowsheet of the traditional industrial recovery process for citric acid has been given by Heinzle et al. (2007). After

filtering off the cell mass, calcium hydroxide is added to precipitate calcium citrate and thus remove water and impurities. The precipitate is filtered off, and acidified using sulfuric acid yielding soluble citric acid and gypsum as by-product. Citric acid is crystallized from the solution. So, despite the low pH fermentation, stoichiometric amounts of gypsum are produced for recovering pure citric acid.

To avoid neutralization with lime and production of gypsum, Novasep offers simulated bed adsorption (SMB) technology, analogous to such described in section 2.6.3 (Novasep, 2013). Prior to SMB processing, ultrafiltration and evaporation of broth are required to increase feed concentration.

Citric acid extraction using a tertiary amine extractant has also been applied on large scale (Kertes and King, 1986). Moresi and Sappino (2002) mention Pfizer Inc. in Europe and Haarman and Reimer Corp. in the USA. Other companies now own these businesses.

After concentrating citric acid to >80% of the saturation value, extraction was done with a recycled amine extractant - citric acid solution to produce a more concentrated amine extractant - citric acid solution and aqueous citric acid raffinate, which was mostly recycled to the evaporation step. The concentrated amine extractant - citric acid solution was back-extracted with hot water. A patent describes 90% recovery (Baniel and Gonen, 1991). Pilot plant studies indicate the technical feasibility of such option (Wennersten, 1983).

2.9.2 Gluconic acid

Milsom and Meers (1985b) showed a possible layout of a plant suitable for gluconic acid production. Clarified and decolorized broth (previously freed from cells of the organism and containing minimal residual glucose) is concentrated by evaporation. To obtain a 50% gluconic acid solution, the remaining liquor may be passed through a cation exchanger in the hydrogen form to remove sodium ions. This final ion exchange step can be performed continuously according to Novasep (Novasep, 2013)

2.9.3 Lactic acid

The traditional fermentation and recovery process of lactic acid such as performed by Corbion Purac, e.g., is well known (Gao et al., 2010, Miller et al., 2011, Pal et al., 2009, Rogers et al., 2006, Wasewar, 2005). The bacterial fermentation is neutralized by lime and performed up to the solubility of calcium lactate. The cell mass is filtered off, and after adding sulfuric acid to the filtrate, gypsum is filtered off. The lactic acid is concentrated by evaporation and purified by methods involving short path distillation. In Thailand, the gypsum from Corbion Purac's 100,000 t/a lactic acid plant is sold to a local company and replaces natural gypsum (Groot and Borén, 2010).

ThyssenKrupp Uhde builds lactic acid plants that use ammonia-neutralized fermentation, acidification by sulfuric acid, removal of cell mass by filtration, simulated moving bed adsorption, polishing, and evaporation. The co-produced ammonium sulfate is used as fertilizer.

NatureWorks has used the traditional calcium lactate process, but introduced in 2008 a yeast-based fermentation for their 180,000 t/a lactic acid production in Blair, Nebraska, USA. This uses significantly lower pH, thereby also significantly reducing the use of calcium hydroxide and sulfuric acid, and in turn reducing the gypsum production significantly (Vink et al., 2010).

In the past, commercial purification of concentrated lactic acid has been done in the USA and UK using countercurrent extraction with diisopropyl ether (Benninga, 1990).

2.9.4 Itaconic acid

For itaconic acid, a scheme consisting of decoloration, clarification, evaporation and crystallization steps has been given (Milsom and Meers, 1985b). Okabe et al. (2009) present a scheme consisting of evaporation and crystallization steps (both twice) and a recrystallization after active carbon treatment. Primary recovery is skipped, at the expense of the crystallization yield and purity. They suggest that solvent extraction, ion exchange, and decoloration are required for high purity itaconic acid.

2.9.5 2-Keto-L-gulonic acid

Information about recovery of 2-keto-L-gulonic acid is scarce. According to Yu et al. (2000), the fermentation pH is controlled using sodium carbonate, and ion exchange is used to obtain 2-keto-L-gulonic acid. This is esterified with methanol, using sulfuric acid as catalyst, for further conversion into vitamin C. Although not reported, the ion exchange of 2-keto-L-gulonate may be anion exchange so that subsequent desorption as 2-keto-L-gulonic acid by using sulfuric acid in methanol might yield the desired esterification solution.

2.9.6 Succinic acid

Succinic acid is an upcoming fermentation product and a range of recovery processes is pursued by several companies. The strong competition makes it unlikely that all announced processes can become profitable.

Novasep (2013) portfolio includes purification alternatives for succinic acid. For BioAmber they have designed a 3000 t/a pilot plant in Pomacle, France. The recombinant *E. coli* fermentation broth contains ammonium or sodium succinate, which is clarified using inorganic crossflow membranes. Electrodialysis removes sodium or ammonium hydroxide. After polishing by ion exchange and nanofiltration to remove colors, this leads to 99.5% purity and 96% recovery. In a recent press release (BioAmber, 2013), BioAmber has announced a switch to low pH yeast technology from Cargill which will directly impact their processing structure, therefore it is uncertain whether Novasep technology would still be applied in BioAmber's production plants worldwide. BioAmber is currently constructing a 17,000 t/a biosuccinic acid plant in Sarnia, Canada, which is expected to start in 2013.

Depending on the location, an alternative scheme can be applied to process succinate salts from bacterial fermentations, using cation exchange in simulated moving bed mode, with regeneration by H_2SO_4; and production of sodium or ammonium sulfate. After nanofiltration this leads to 99% purity and 90% recovery.

ThyssenKrupp Uhde uses their lactic acid recovery technology also to build a 13,500 t/a succinic acid plant for Myriant in Lake Providence, Louisiana, USA (R. Kleinhammer, personal communication, 2012). An *E. coli* fermentation is used with neutralization by ammonium carbonate. Solids are removed by centrifugation and ultrafiltration to avoid that cell mass gets mixed with filter aid. The polishing involves active carbon treatment and nanofiltration to remove colors, sulfate and 50% of remaining sugars.

A joint venture of BASF and Corbion Purac has piloted succinic fermentation at neutral pH using *Basfia succiniproducens*, followed by using the recovery technology Purac is using for lactic acid. A 25,000 t/a plant is planned to be built near Barcelona, Spain.

DSM and Roquette Freres have developed a recombinant *Saccharomyces cerevisiae* strain to produce succinic acid by low pH fermentation, thus minimizing inorganic acid and base consumption. Their recovery scheme resembles the aforementioned itaconic acid recovery scheme, but involves recrystallization after decoloration and ion-exchange (R. Verwaal, lecture at BBPOA, Frankfurt, May 2012). Commercial production of 10,000 t/a in Cassano, Italy, has started. This is a favorable option according to a life cycle assessment study (Cok et al., 2014), which includes some process details.

2.9.7 Acetic acid

The main industrial production process of acetic acid is based on petrochemistry and not relevant for this review. Fermentative acetic acid is used for vinegar and requires no recovery process other than cell mass removal after the low pH oxidation of aqueous ethanol.

2.10 Conclusions

Numerous methods have been suggested to recover fermentative carboxylic acids. Because of the very different physical properties of different carboxylic acids and the different fermentations, there is not a single method that is useful for all. Usually, several methods have to be combined to removing major impurities (in a primary recovery step), water, and minor impurities (in a purification step). Moreover, in many cases a carboxylate salt needs to be converted into a carboxylic acid. A destination needs to be found for the stoichiometrically co-produced inorganic salt. There may be no market for it, and it may be unacceptable as waste.

In a few cases such salts have been split into acid and base, using thermal methods or bipolar membrane electrodialysis, so that potentially no waste salt is produced. These salt splitting methods are relatively difficult. A more rigorous solution is preventing the salt formation problem by performing the fermentation at low pH. This has been achieved for several carboxylic acids, but may require in-situ carboxylic acid removal. In-situ extraction

using tertiary amines is the most popular in-situ removal method, but regeneration of the amines can easily lead again to undesired stoichiometric inorganic salt production.

Systematic comparison and evaluation of published recovery methods is difficult. All recovery processes consist of a series of steps, with several alternatives per step. Most publications deal with one or two individual steps and are not explicit about the function of the studied steps within the overall process. Therefore, the consequences on the overall process may not be overseen. Comparison of several alternatives of a complete process is required but time-consuming.

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3

Strong anion exchange recovery of aqueous dicarboxylates: Extraction and sorption equilibrium comparison

Ion-exchange can be used for primary recovery of aqueous dicarboxylic acids at neutral pH, where there is almost complete acid dissociation. The equilibria of anion exchange of aqueous dicarboxylate anions (fumarate, itaconate, malate and succinate) with anions (Cl^- and OH^-) bound to quaternary ammonium compounds (Aliquat extractant and Dowex sorbent) were quantified. All equilibria could be described by the same model. The four dicarboxylates behaved similarly, and were exchanged by two Cl^- or OH^- anions. Sorption gave a much better exchange than extraction and OH^- gave a much better exchange than Cl^-.

Keywords: Dicarboxylates, ion exchange equilibrium, extraction, sorption.

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3.1 Introduction

In recent years, interest in bio-based production of dicarboxylic acids has increased as they have shown a clear potential of becoming relevant renewable chemical building blocks. Their diacid functionality provides a versatile platform for different chemical transformations into alcohols, esters, furans, and lactone-related compounds, among others, driving the production of new specialty chemicals, solvents, polymers, detergents and many other intermediate and consumer products (Zeikus et al., 1999). Biotechnological production of dicarboxylic acids has been investigated extensively and has reached technological maturity for some molecules, in particular for succinic acid leading to different industrial ventures worldwide (Straathof, 2014). The economic and sustainability hurdles associated with the feasibility of large scale production of dicarboxylic acids are closely related to the biological host chosen, as it determines the fundamental structure of the production process. During fermentation, production of carboxylic acids will decrease the pH of the medium, creating a challenging acidic environment for most natural producer microorganisms. Neutrophilic microorganisms will require the removal of the produced diacid either by neutralization reaction or other physical or chemical methods. Alternatively, an acid-tolerant microorganism can be used provided it has enough acid stress resistance. As expected, there is a trade-off in selecting the production microorganism, higher titers and productivities have been observed at neutral pH conditions for most relevant dicarboxylic acids (López-Garzón and Straathof, 2014).

From a recovery and purification standpoint, neutral pH fermentations will require a more extensive processing and thus might compromise the overall economics of the process, even with a favorable fermentation. At neutral pH, dicarboxylates are present as bivalent salts associated with metal cations originating from the base used during fermentation. During downstream processing, the dicarboxylate should be recovered from the bulk liquid, converted back to its undissociated form, concentrated, and purified to the required grade. Several sequences of operations are possible to achieve this (López-Garzón and Straathof, 2014), but the choice of the primary recovery method is often critical as it has a great influence on subsequent operations. Different technologies for primary recovery of carboxylates are available. They are mostly based on the transfer of the carboxylate to an extractant or sorbent auxiliary phase. Anion exchangers comprise an interesting class of extractant or adsorbent auxiliary phases, potentially allowing integration of several of the above described downstream steps, possibly resulting in an efficient and sustainable bio-based dicarboxylate process.

Although the extraction and sorption of carboxylic acids using ion-exchange has been studied for over three decades, most of the research has been focused on monocarboxylic acid at the time, e.g. lactic acid, at a processing pH below the pK_a where the majority of the acid exists as the undissociated species. There is a limited amount of equilibrium data for capture of dicarboxylates that is, at a pH above their second pK_a. Moreover, a myriad of different models and parameters has been used to characterize the equilibrium phenomena of such operations, impeding thorough and accurate comparisons across the existing literature.

Limiting the scope of the present study to fully dissociated acids produced at neutral pH, favorable chemical interactions for primary recovery occur using strong anion exchangers rather than using weak anion exchange resins. Strong anion exchange materials typically contain a quaternary ammonium-based functional group, so they are capable of ion pairing leading to anion exchange reactions. As a consequence, the target carboxylate can be captured, and depending on the selected displacement and regeneration steps, further acidified and purified. The phase of the exchanger will determine the process technology to be used, liquid exchangers leading to extraction operations and their solid analogues to sorption operations.

In the case of ion exchange extraction of dicarboxylates, the anion exchanger Aliquat 336 Cl^- (Figure 1a) will be studied as extractant. Although it has been reported to be non-toxic to anaerobic bacteria (Playne and Smith, 1983), it is a very viscous liquid, limiting its applications in pure form. Thus, diluents and modifiers are commonly used diluted to improve extraction characteristics.

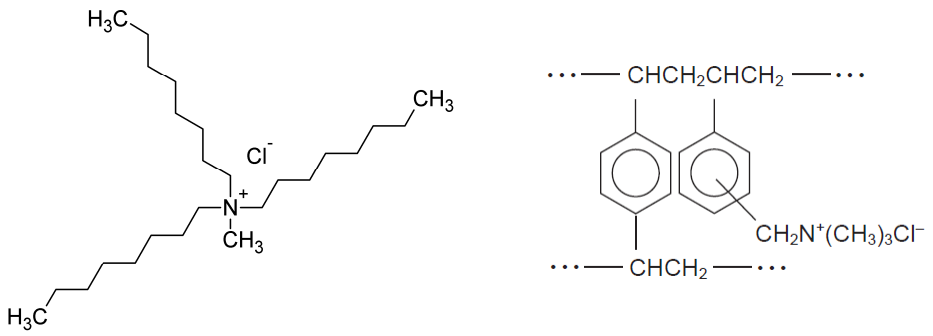


Figure 1. Strong anion exchangers used in this study. a) Aliquat 336 Cl-, although shown as *N*-methyl-*N*,*N*,*N*-trioctylammonium chloride, its commercially available as a mixture of octyl (predominant) and decyl chains. b) Dowex Marathon A, a styrene-divinylbenzene gel matrix functionalized with trimethylammonium chloride.

In the case of solid exchangers, a variety of polymeric matrixes and functional groups are commercially available, but their characteristics are often broadly lumped by the manufacturer and only a few details about their chemical composition are known. Gel type resins have great permeability and allow higher number of functional groups as a true porous structure is absent. Strong anion exchange resins are usually classified by their basicity as Type I or II, the former being the stronger base, thus able to ion pair in a broader pH range. Dowex Marathon A is a gel, type I resin with uniform size, which might provide high capacity for the sorption of dicarboxylates.

As can be concluded from the discussion presented above, the definition of an ion exchange-based primary separation process for dicarboxylates requires a comparison between extraction and sorption operation. Consequently, this study will focus on a capture equilibrium comparison between extraction and sorption for several model dicarboxylates. Fumaric, itaconic, succinic and malic acid will be used as model compounds as have been considered promising chemical building blocks from biomass (Werpy et al., 2004) and for

which bio-based processes are available at neutral pH (Straathof, -2014). The capacity and affinity of such dicarboxylates will be established and compared using the already presented Aliquat 336 and Dowex Marathon A as model ion exchange extractant and sorbent, respectively. Finally, the influence of two typical counter-ions, Cl^- and OH^-, on the mentioned equilibrium characteristics will be considered.

3.2 Materials and methods

3.2.1 Materials

Fumaric, itaconic, succinic and malic acid used were of analytical grade (Sigma-Aldrich). Aqueous solutions were prepared using deionized water from a Milli-Q water purification system (Millipore Corp.) All the acid solutions were adjusted to pH 7.00 ±0.5 in two steps, using solid sodium hydroxide (Merck) to a pH close to 6 and to neutral pH with 1 mol/L NaOH solution by means of an automatic titrator (Metrohm Titrino system). The prepared solutions were analyzed to determine the exact carboxylate concentration prior to use. Extraction experiments were performed using Aliquat 336 (Sigma-Aldrich), a commercial ion exchange extractant composed of a mixture of trialkylmethylammonium chlorides (C_8-C_{10} alkyl chains) with an average molecular weight of 404.16 and a density of 884 kg/m^3. The extractant was converted to the OH^- form as described in section 2.2. 1-Decanol 98% (Fluka), 1-octanol (Sigma) and *n*-dodecane (Sigma) were used as modifiers and diluent, respectively. Ion exchange sorption experiments were carried out using the strong anion exchange resin Dowex Marathon A (Sigma-Aldrich), composed by a styrene-DVB matrix with a type I quaternary amine functional group. Both Cl^- and OH^- forms were obtained directly from the supplier. According to the supplier, the moisture retention capacity of the resins varies between 50-60% for the Cl^- form to 60-72% for the OH^- ion. As a result of this, shipping density is higher for Cl^- than OH^-, with values of 670 and 640 g/L, respectively. The resin was washed twice and rehydrated with deionized water prior to use.

3.2.2 Conversion of Aliquat 336 Cl^- into its OH^- form

A solution (500 mL) composed of Aliquat 336 in chloride form (30% v/v), 1-decanol (20% v/v) and *n*-dodecane was contacted with an equal volume of 1.0 mol/L NaOH in a closed flask at 500 rpm using an overhead stirrer during four hours. Preliminary tests showed this time was sufficient for attain exchange equilibrium. After settling, the spent aqueous phase was drained and replaced by fresh hydroxide solution for a new contacting. By the end of the last contacting, and settling overnight in a separation funnel, the yellowish organic phase was centrifuged at 2500 rpm for 15 min, and washed with deionized water until the pH of the aqueous solution was below 7.5.

3.2.3 Extraction equilibrium isotherms

Batch extraction equilibrium experiments were carried out in 60 mL glass scintillation vials. The vials were magnetically stirred at 600 rpm with the aid of a multiple agitation plate and thermostated at 25 °C. Equal volumes (15 mL) of aqueous and organic phases were equilibrated during four hours. After equilibrium, the biphasic system was centrifuged at 2500 rpm and the aqueous phase withdrawn using a glass Pasteur pipette. The aqueous phase was then filtered using a 0.2 μm hydrophilic filter to remove any traces of entrained organic solvent and analyzed to determine carboxylate, OH^- and Cl^- concentrations as required.

Extraction equilibrium experiments without ion exchange extractant, referred as physical extraction experiments, were performed using an organic phase composed of 20% (v/v) 1-decanol dissolved in *n*-dodecane. The organic phase used in ion exchange extraction experiments was composed of Aliquat 336 (10-30% v/v, as required) in both Cl^- and OH^- form, 1-decanol (5-20%, as required) and diluted with *n*-dodecane.

3.2.4 Ion exchange sorption isotherms

Batch sorption ion exchange equilibrium experiments were performed in 30 mL scintillation vials placed horizontally in a thermostated shaker (New Brunswick Scientific) at 120 rpm and 25 °C for 4 h. In most of the experiments 1 g of wet, dewatered resin, in the required ionic form was added to the vial along with 10 mL of aqueous carboxylate solution at the desired concentration. After equilibration, the aqueous solution was analyzed for carboxylate, OH^- and Cl^- as required.

3.2.5 Analytical procedures

The pH of the initial and equilibrium aqueous phases were determined using a pH meter (Metrohm). The initial and equilibrium concentrations of the carboxylates in the aqueous phase were determined by HPLC in a Waters system with an Aminex HPX-87H column (BioRad). Aqueous phosphoric acid (0.1% v/v) was used as a mobile phase at 0.6 mL/min. Carboxylic acid detection was by UV at 210 nm. The column and detector temperatures were 60 and 40 °C, respectively. In all the experiments, the acid concentration in the respective auxiliary phase was calculated via mass balance, assuming no volume changes. Aqueous concentration of OH^- anions in the absence of carboxylate was determined by automatic titration (Metrohm Titrino system) to a pH of 7.00 using standardized 0.1 mol/L HCl. Cl^- concentration was determined using a colorimetric assay based on the improved Fried method (QuantiChrom Cl assay kit, BioAssay Systems, CA, USA). Briefly, 5 μL prediluted and acidified (pH 1-3) sample was transferred to a well of a clear bottom 96-well plate, 200 μL working reagent was added, and the plate was incubated for 5 min at room temperature. The optical density was determined at 595 nm in a microplate reader (Tecan Infinite 200 PRO) and the chloride concentration was calculated using an appropriate calibration curve.

3.3 Results and discussion

3.3.1 Ion-exchange extraction solvent design

The use of Aliquat 336 as extractant requires the formulation of an extraction solvent mixture taking into consideration, among others, extraction capacity, biocompatibility, reusability, separability, and effective mass transfer, which are mostly dependent on amount of extractant, log P_w, mutual solubility, density and viscosity, respectively. Essentially, the extraction solvent mixture should be composed to maximize the amount of Aliquat 336 while keeping a good compromise in the remaining aspects. Typically, aliphatic hydrocarbons are used as diluents and long chain alcohols as modifiers (Marinova et al., 2005). Table 1 shows an overview of relevant physical properties for Aliquat 336, 1-decanol and *n*-dodecane. These components have been selected in the extraction of other carboxylic acids (Kyuchoukov et al., 2004). As a rule of thumb it has been established that biocompatible solvents exhibit a log P_w > 3 (Jaquet et al., 1999, Pogorevc et al., 2002, van den Berg et al., 2008, Vermuë et al., 1993). Interestingly, the values lower in the table are more favorable for extraction, suggesting to increase the proportion of those component in the mixture, but the extraction liquid mixture may lose extraction capacity as Aliquat is mostly the responsible for anion exchange at neutral pH.

Table 1. Relevant physical properties of Aliquat 336, 1-decanol and *n*-dodecane at 298 K

Component	1-Octanol-water distribution ratio	Solubility		Density	Viscosity
		Organic in water	Water in organic		
	Log P_W	g/kg	g/kg	kg/m^3	mPa·s
Aliquat 336		1.2[a]		887.5[b] (880[a])	1502[b] (1450[a])
1-Decanol	4.57[d]	0.21[c,e]	33.5[c, e]	829.7[d]	11.790[f]
n-Dodecane	6.10[g]	$8.42\cdot10^{-6}$ [h]	$6.5\cdot10^{-4}$ [h]	746.0[i]	1.344[i]

[a] Manufacturer specifications. Solubility and viscosity at 303 K.
[b](Litaiem and Dhahbi, 2012)
[c] At 303 K
[d] (Haynes et al., 2012)
[e] (Maczynski et al., 2007)
[f] (Sastry and Valand, 1996)
[g] (Coates et al., 1985)
[h] (Shaw et al., 2006)
[i] (Caudwell et al., 2004)

Since Aliquat 336 is insoluble in dodecane, decanol is required to obtain a single organic phase. The required amount of decanol was evaluated at three different levels of concentration of both alcohol and Aliquat in a combinatorial manner. These tests were made in a qualitative manner, assessing the phase separation after one hour settling (see Table 2).

Table 2. Occurrence of phase separation (+) or not (-) in the extraction solvent at different concentrations of Aliquat 336 and 1-decanol in *n*-dodecane as diluent.

Aliquat 336 Cl^-	**1-Decanol**		
(% v/v)	(% v/v)		
	5	10	20
10	+	+	+
20	-	+	+
30	-	-	+

As expected, at higher concentrations of extractant, higher concentrations of modifier are required to produce a single organic phase and consequently an adequate separation from the aqueous phase. It should be stated that decanol has a negative effect on the extraction at pH > $pK_{a,acid}$. For tartaric acid extraction, decanol concentrations higher than 30% decreased the distribution coefficient by 40% (Marinova, 2005). Thus, extraction systems with >30% decanol were not considered, and it is desirable to use just sufficient decanol to create a homogeneous organic phase. Therefore, in subsequent experiments three extraction systems were tested (diluted in dodecane): A. Aliquat 336 10%, decanol 5%; B. Aliquat 336 20%, decanol 10%, C. Aliquat 336 30%, decanol 20%.

3.3.2 Influence of Aliquat 336 Cl^- concentration on dicarboxylate extraction

Several ion-exchange extraction experiments were performed using solvents with different Aliquat concentration, ranging from 0% to 30% v/v. In each extraction at equilibrium, the distribution coefficient (D_E), the ratio of total dicarboxylate (A) molar concentrations (C) in the organic (org) phase to aqueous (aq) phase, and loading factor (Z_E), the ratio of dicarboxylate concentration in the organic phase to total Aliquat (Q^+) in the organic phase, were calculated according to the equations 1 and 2, respectively.

$$D_E = \frac{C_A^{org}}{C_A^{aq}} \tag{1}$$

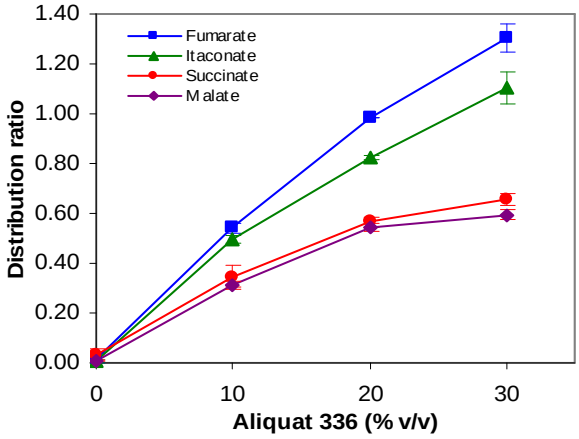
$$Z_E = \frac{C_A^{org}}{C_{Q^+_{total}}^{org}} \tag{2}$$

It was assumed that C_{Q^+} remained at its initial value.

Figure 2 shows the dependence of the distribution ratio on Aliquat 336 concentration for the four target dicarboxylates at two initial acid concentrations. It can be seen that for 0.1 mol/L initial dicarboxylate concentration the distribution ratios are about eight times higher than those obtained at 0.6 mol/L and that concentrations of 30% Aliquat 336 above 30% often do not increase the distribution ratios much further, indicating that higher extractant concentration may not lead to higher dicarboxylate recovery. Furthermore, the extraction behavior of all the dicarboxylates is similar at both concentrations. Itaconic acid showed a

poor extraction at high initial concentration (Figure 2b) probably because at these concentrations it showed a high tendency to form foam, impairing to some extent the contact between the liquid phases.

(a)



(b)

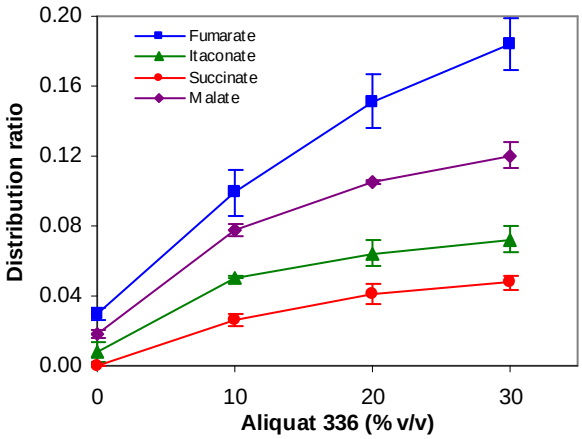


Figure 2. Influence of Aliquat 336 Cl^- concentration on distribution coefficient of various dicarboxylic acids at neutral pH. Initial acid concentration: a. 0.1 mol/L, b. 0.6 mol/L

Table 3 presents the equilibrium pH values and loading factors for the performed extractions at the above mentioned initial carboxylate concentrations. The decrease in the pH value upon extraction can be expected by replacement of dicarboxylate, which is a weak base, by Cl^- in the aqueous phase. As the loading factor is lower than 1, it suggests that no carboxylate-carboxylate interactions were present, moreover indicates that at these acidity conditions hydrogen bonding interactions are rare or absent. However, this should be confirmed by determining the anion exchange ratio at different experimental conditions.

Overall, the calculated loading factors were not improved at both high acid and Aliquat concentrations. This demonstrates the existence of several limitations other than merely extractant capacity. As described in section 3.1, having a single-phase solvent at high concentration of Aliquat requires a high decanol concentration, which negatively impacts extraction at neutral pH (Marinova, Albet, 2005). A lower concentration of decanol will negatively impact the phase separation and subsequently the proper reuse of the extractant.

Table 3. Extraction loading factor and equilibrium pH using Aliquat 336 Cl^- form for selected dicarboxylates at two initial concentrations

Dicarboxylate	Aliquat 336 Cl^- (% v/v)	C_A^{ini} = 0.1 mol/L		C_A^{ini} = 0.6 mol/L	
		pH_{eq}	Z	pH_{eq}	Z
Fumarate	0	7.02	- -	7.05	- -
	10	5.89	0.17	6.61	0.26
	20	5.67	0.12	6.42	0.19
	30	5.50	0.09	6.36	0.15
Itaconate	0	6.96	- -	7.07	- -
	10	6.88	0.15	7.17	0.08
	20	6.81	0.10	7.22	0.08
	30	6.79	0.08	7.24	0.06
Succinate	0	6.99	- -	7.06	- -
	10	6.19	0.12	7.06	0.07
	20	6.05	0.08	7.01	0.05
	30	5.97	0.06	7.02	0.05
Malate	0	6.97	- -	7.05	- -
	10	5.87	0.11	6.89	0.15
	20	5.65	0.09	6.75	0.12
	30	5.52	0.06	6.68	0.12

3.3.3 Conversion of Aliquat 336 Cl^- into OH- form

To study the influence of the exchanger counterion on the extraction equilibrium of dicarboxylates, the selected extraction solvent was transformed into the hydroxide form. The conversion occurs according to an ion exchange reaction:

$$Q^+Cl^-_{(org)} + NaOH_{(aq)} \xrightleftharpoons{K_{RE}} Q^+OH^-_{(org)} + NaCl_{(aq)} \quad (3)$$

$Q^+Cl^-_{(org)}$ is the quaternary ammonium chloride group of Aliquat 336. The conversion was performed by contacting repeatedly the extraction solvent, Aliquat 336 30%, decanol 20% in dodecane, with fresh sodium hydroxide. A change in color of the organic phase was observed through the process, from transparent to slightly yellowish after the last contact. As a possible issue, the transformed solvent tends to form spontaneous emulsions with water; but this behavior was not observed with the aqueous carboxylate solutions tested. After each contacting, the concentration of chloride ions in the aqueous phase was determined and the fractional conversion calculated. Figure 3a shows the conversion progress to the OH^- form, reaching a 46% conversion.

In the extraction system under study involving a concentrated aqueous phase, a non-ideal phase behavior is expected, so the contribution of the activity coefficients may be considerable. However, in an attempt to compare relative differences in the equilibrium of this system associated with the composition of extraction solvents using Aliquat 336 as extractant, the fractional conversion can be calculated via mass balance involving the concentration-based equilibrium coefficient. Thus, the equilibrium of the exchange reaction can be represented by the apparent concentration-based equilibrium coefficient, K_{CE}, which is a function of the concentrations of the species involved in the reaction:

$$K_{CE} = \frac{C_{QOH} C_{NaCl}}{C_{QCl} C_{NaOH}} \tag{4}$$

C_i is concentration of the species i in the respective phase. For the experimentally used phase volume ratio of 1, and assuming full dissociation of the ionic species, the mass balance of all the species can be described as:

$$C_{OH^-,eq} = C_{OH^-,0} - \varepsilon_{OH^-} \tag{5}$$

$$C_{QCl,eq} = C_{QCl,0} - \varepsilon_{OH^-} \tag{6}$$

$$C_{QOH,eq} = C_{QOH,0} + \varepsilon_{OH^-} \tag{7}$$

$$C_{Cl^-,eq} = \varepsilon_{OH^-} \tag{8}$$

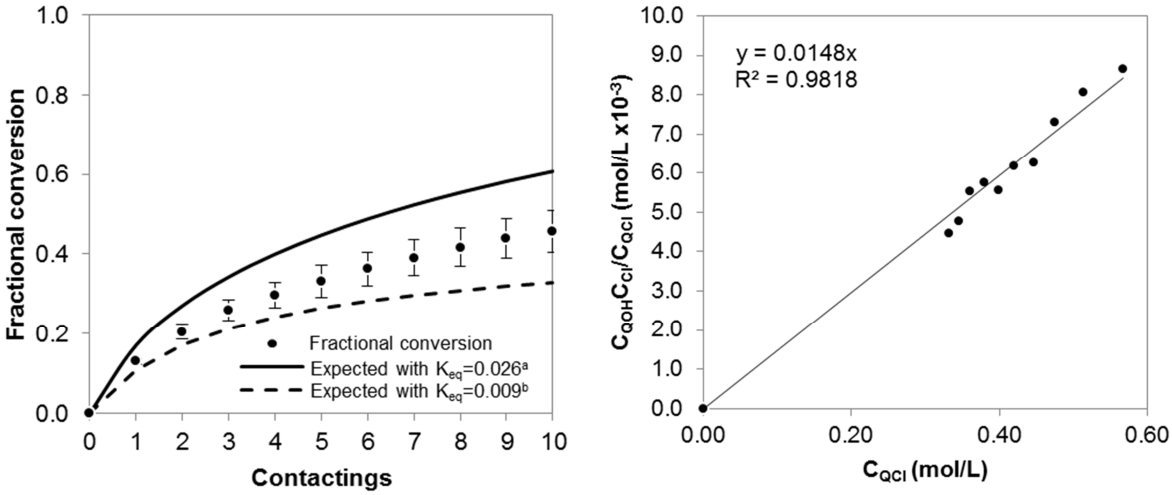
Where ε_{OH^-} is the extent of OH^- extraction in the ion exchange reaction. Substituting the mass balance expressions into the concentration-based equilibrium coefficient leads to:

$$K_{CE} = \frac{C_{QOH,0}\varepsilon_{OH^-} + \varepsilon_{OH^-}^2}{C_{QCl,0}C_{OH^-,0} - \left(C_{QCl,0} + C_{OH^-,0}\right)\varepsilon_{OH^-} + \varepsilon_{OH^-}^2} \tag{9}$$

This expression can be rearranged to a quadratic polynomial form:

$$K_{CE}C_{QCl,0}C_{OH^-,0} - \left[K_{CE}\left(C_{QCl,0} + C_{OH^-,0}\right) + C_{QOH,0}\right]\varepsilon_{OH^-} + \left(K_{CE} - 1\right)\varepsilon_{OH^-}^2 = 0 \tag{10}$$

The estimation of the concentration-based equilibrium coefficient in the tested system was done by a linear plot of Equation 4, as shown in Figure 3b.



(a) (b)

Figure 3. Conversion of Aliquat 336 Cl^- to OH^- form. a. Experimental and calculated fractional conversion based on equilibrium constant from [a]Hano et al., (1991) and [b]Galan et al., (1994) b. Concentration-based equilibrium constant

The observed conversion and concentration-based equilibrium coefficient correspond well with their respective values on the basis of calculated fractional conversions by the proposed model (Equations 5-10) and literature equilibrium coefficients. Both references, Hano et al. (1991) and Galan et al. (1994) used kerosene as diluent and the latter also 3,7-dimethyloctanol as modifier, indicating a relatively different equilibrium towards $Q^+OH^-_{(org)}$ for those solvents. As discussed by Galan et al. (1994), at high Aliquat and hydroxide concentrations required in practical applications, the system may deviates greatly from the ideal system and thus the comparison of extraction solvents based on equilibrium coefficients should be done with precaution as they are commonly calculated based on concentrations, implicitly assuming ideal behavior. However in this study the concentration-based equilibrium describes the data well, suggesting that the ratio of activity coefficients of the involved species does not change in the tested experimental conditions.

3.3.4 Ion-exchange extraction using Aliquat 336 OH^-

Extraction experiments of the target carboxylates were carried out using the modified Aliquat 336 OH^-, which still contains 54% Cl^- Figure 4 shows a comparison of the distribution ratio and loading factor for the target carboxylates depending on the Aliquat counterion. At initial dicarboxylate concentration of 0.1 mol/L, a large improvement in the extraction was obtained and virtually all the carboxylate anions were extracted, leading to very high distribution ratios. Moreover, loading factors increased significantly for the OH^- form of Aliquat, indicating better extractability and stronger acid-base interaction (Wasewar et al., 2011a).

In the case of the higher initial carboxylate concentration, the calculated loading factors for the performed extractions reached their highest values using the partially converted

extractant. Overall, these observations indicate that the partially converted extractant improve the ion exchange equilibrium as OH^- anion is exchanged more easily than Cl^- as expected by well-known ion exchange selectivity rules (Helfferich, 1962).

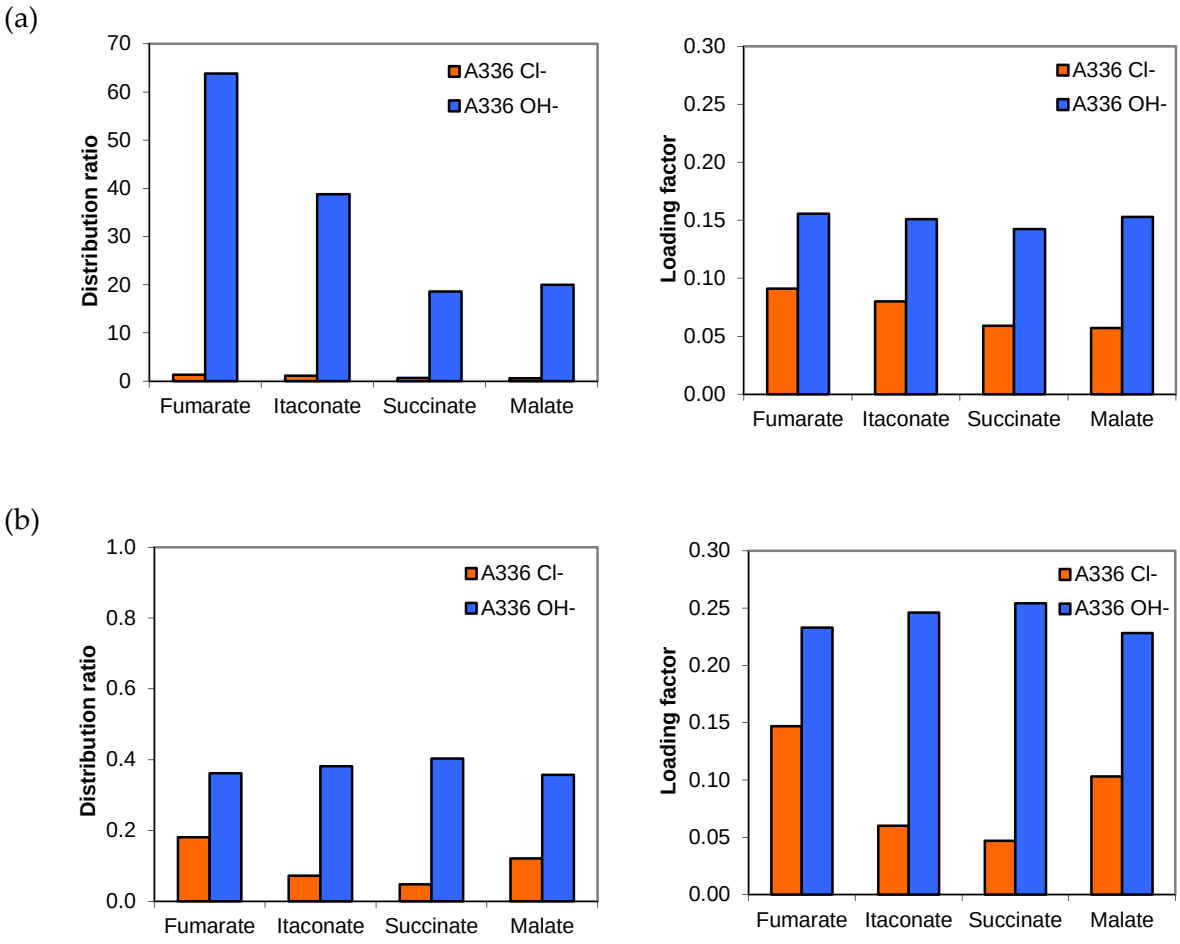


Figure 4. Extraction of selected dicarboxylates at neutral pH by Aliquat 336 in Cl^- and OH^- forms. Initial acid concentration: a. 0.1 mol/L, b. 0.6 mol/L. Composition of the organic phase: Aliquat 336 (Cl^- or OH^-) 30% (v/v), decanol 20% (v/v), dodecane.

3.3.5 Ion exchange equilibrium of extraction and sorption of dicarboxylates

Capacity considerations

Detailed experimental equilibrium data are presented in figure 5 in the form of extraction and sorption isotherms, respectively. To make the two techniques comparable, the capture or exchange capacity for species i, $q_{e,i}$, was plotted against the aqueous concentration in equilibrium, C_i. The experimental data were fitted using the known constant separation factor (C.S.F.) isotherm (Eq. 11) as described elsewhere (Arévalo et al., 2000, Green, 2008), giving information about the saturation capacity, $q_{sat,i}$, which is reached at $C_{sat,i}$, and the preference of the ion exchanger for the target anion via the separation factor K_i:

$$q_{e,i} = \frac{K_i q_{sat,i} C_i}{C_{sat,i} + (K_i - 1) C_i} \tag{11}$$

The lines correspond to the best fit that minimizes the least squares between the data and the constant separation factor isotherm. The parameters of the model, K_i, $C_{sat,i}$, and $q_{sat,i}$, were determined using a non-linear regression technique based on the generalized reduced gradient (GRG) method and are shown in Table 4.

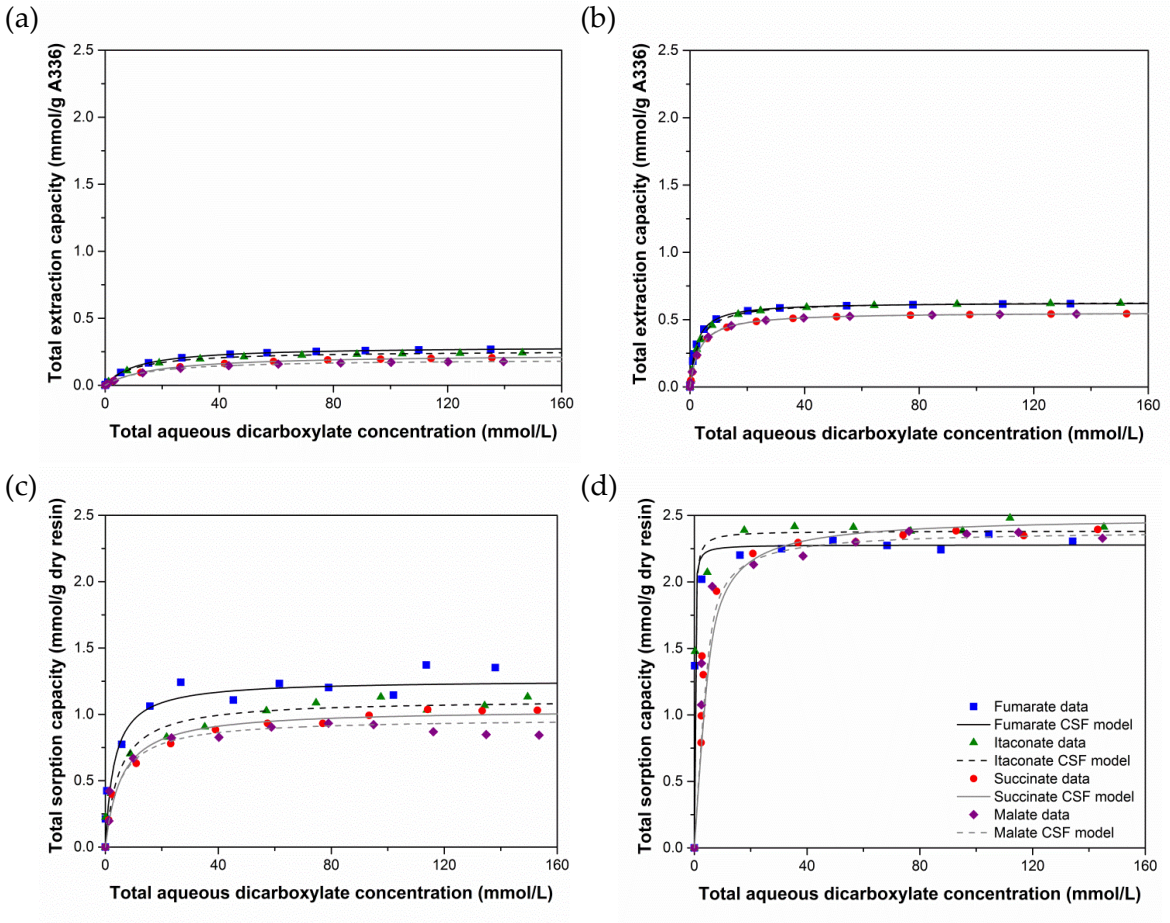


Figure 5. Ion exchange extraction and sorption isotherms, in terms of capture capacity, for selected dicarboxylates at neutral pH. a. Extraction solvent based on Aliquat 336 Cl^- 30% v/v b. Extraction solvent based on partially converted Aliquat 336 OH^- 30% v/v c. Dowex Marathon A Cl^- resin d. Dowex Marathon A OH^- resin.

All extraction and sorption isotherms show favorable, type I, shapes, indicating strong uptake preference. Overall, the total capture capacity was highest for sorption over extraction being between 3 and 4 times higher for sorption, regardless the type of counterion. If analyzed crosswise, sorption capacities found for Dowex Marathon OH^- were up to 10 times higher than those for Aliquat 336 Cl^-. As a result, and although packing density for Dowex Marathon resin is slightly lower than the bulk density of the tested liquid ion-exchange

solvent, if evaluated volumetrically a primary separation system constituted on such resin will be able to capture 10 times more mass of dicarboxylate in a single equilibrium stage than based on its analog extraction solvent.

As discussed in the previous section for extraction, the nature of the counter-ion influences strongly the exchange equilibrium. In both operations, separation factors are between 2 and 3 times higher for the A^{2-}/OH^- systems, suggesting that the OH^- counter-ion in the auxiliary phases is more easily displaced by the dicarboxylate anions compared to the standard chloride anion. The result of high capacities and separation factors encountered using the hydroxide counterion are capture processes with less equilibrium stages or less auxiliary phase requirements, in sorption operations also implies a better resin usage and lower leakage after regeneration due to a narrower mass transfer zone.

Table 4. Constant separation factor isotherm parameters for the ion exchange extraction and sorption of selected dicarboxylates, as function of exchanger counter-ion type[a].

	Capture process[b]	q_{sat}[c] (mmol/g exchanger X^-)		$K_{X^-}^{A^{2-}}$		C_{sat} (mmol/L)	
		OH^-	**Cl^-**	**OH^-**	**Cl^-**	**OH^-**	**Cl^-**
Fumarate	Extraction	0.60	0.27	22.1	14.1	48.0	148
	Sorption	2.19	1.18	26.9	15.4	2.70	45.5
Itaconate	Extraction	0.61	0.24	26.3	13.8	74.8	135
	Sorption	2.30	1.00	29.3	10.0	4.92	43.6
Succinate	Extraction	0.53	0.21	21.8	12.0	68.2	198
	Sorption	2.38	0.94	22.2	11.1	65.1	56.9
Malate	Extraction	0.53	0.18	23.1	12.3	69.5	164
	Sorption	2.29	0.87	26.1	10.2	53.7	45.7

[a] The calculated correlation coefficient was higher than 0.9 in all cases.

[b] Aliquat 336 was partially converted to the OH^- form

[c] The total saturation capacity is referred to mass of Aliquat 336 and resin in their corresponding ionic forms for extraction and sorption, respectively.

The extraction degree, defined as the percentage of dicarboxylate recovered from the initial aqueous solution, is described in equation 12 for a constant aqueous volume:

$$E(\%) = \frac{C_{A^{2-}}^{aq,init} - C_{A^{2-}}^{aq,\mathrm{eq}}}{C_{A^{2-}}^{aq,init}} \cdot 100 \qquad (12)$$

It was calculated for both capture operations using OH^- as the exchanger counterion and plotted against their aqueous equilibrium concentration (Figure 6). The extraction system (Figure 6a) maintained extraction degrees over 50% at all concentrations tested and for each of the target acids. Despite its high capacity, Dowex Marathon OH^- resin was unable to keep adequate extraction degrees at high acid concentrations under the experimental conditions

tested, dropping to 50% at aqueous equilibrium concentrations of about 55 mmol/L (Figure 6b), possibly due to a different auxiliary phase to aqueous solution ratio.

Overall, the observed capture behavior of the four target dicarboxylates was very similar. As a general trend, fumarate and itaconate showed slightly higher saturation capacities and separation factors than succinate and malate, although such behavior is less clear in OH^- based sorption due to a relatively high dispersion in the data, probably as a result of capture capacities close to the total exchange capacity of the resin.

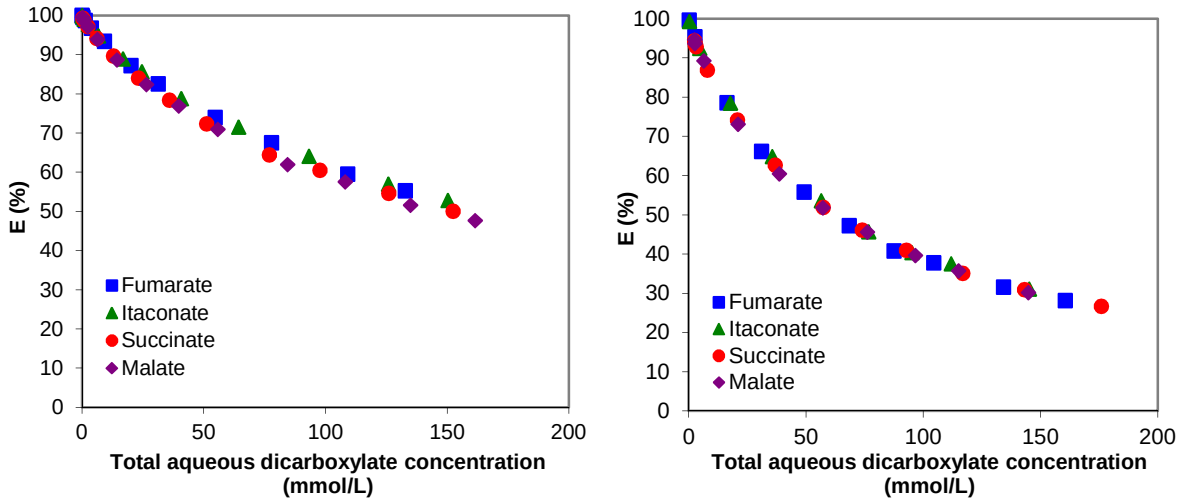


Figure 6. Comparison of the extraction degree for extraction and sorption with hydroxide counterion. a. Extraction with Aliquat 336 OH^-/Cl^- b. Sorption with Dowex Marathon OH^-.

Extraction of dicarboxylic acids using Aliquat 336 has been reported, but most of them at low pH. Malic acid was extracted by Aliquat 336 dissolved in different diesters, alcohols and ketones (Uslu and Kırbaşlar, 2009). Distribution ratios at high initial acid concentration (0.8 mol/L) were reported to be between 0.1 and 0.5, which is in the same order of magnitude as determined in this study at a similar high initial concentration (Figure 4b). In similar extraction solvents, the extraction equilibrium of itaconic acid was studied (Wasewar, Shende, 2011a). A maximum distribution ratio (D_E = 2.65) was found at high Aliquat concentration in ethyl acetate at high initial acid concentration, which is five times higher than the distribution ratio determined in the present study at neutral pH. This can be explained due to 2:1 acid to amine complexes formed at low pH. As the toxicity of this solvent is high ($\log P_w < 1$), sunflower oil was used as diluent, which a dramatic reduction in the distribution ratio to values below 0.1 at different experimental conditions (Wasewar et al., 2011b).

Sorption of succinate at pH above its second pK_a was studied using several sorbents on a wide range of basicity (Tung and King, 1994). At neutral pH, the strong base exchangers Amberlite IRA-35 and IRA-910 maintained a sorption capacity between 1.86 and 2.11 mmol/g, very close to the succinate capacity determined in this study. Exchangers with lower basicity were not able to maintain their capacity at neutral pH, which was also seen for other resins weak anion exchange resins (Sheng et al., 2016). Sorption equilibrium studies for fumarate at

pH 5.0 were carried out using Amberlite IRA-94 and IRA-400 in OH^- form (Fu et al., 2013), obtaining capacities at equilibrium concentrations (≈160 mmol/L) between 0.6 and 1.2 mmol/g in wet resin basis, which corresponds well to the respective equilibrium capacities determined with Dowex Marathon OH^- when converted to wet basis. Likewise, the equilibrium capacity for IRA-400 was also verified in fixed-bed column studies (Fu et al., 2009). Itaconic acid sorption on Purolite A-500P and PFA-300 was studied at several experimental conditions (Magalhães et al., 2016). At pH 6.3, both resins kept a sorption capacity between 0.53 and 0.76 mmol/g wet resin, which is slightly below the capacity determined for itaconate in this study.

Davison et al. (2004) established for succinic acid, that capacities above 0.05 g/g (0.42 mmol/g) sorbent should make viable a recovery process based on sorption, given a good regenerability and stability of the sorbent. Using such rule of thumb for similar dicarboxylates, it can be said that in terms of capture the resin Dowex Marathon meets the criterion, and by a larger margin when in the tested OH^- form than in the Cl^- form. However, regeneration of the ion exchange material back into the OH^- form will be more difficult than back into the Cl^- form.

Ion exchange stoichiometry

The ion exchange capture stoichiometry for dicarboxylates has not been formally described yet. Several authors including Matsumoto et al. (1999) have suggested that the extraction equilibria of carboxylic acid anions (A^{n-}) with a quaternary ammonium functional group (Q^+X^-) can be represented by:

$$nQ^+X^-_{(aux)} + M^+_nA^{n-}_{(aq)} \xleftrightarrow{K_E} Q^+_nA^{n-}_{(\mathrm{aux})} + nM^+X^-_{(aq)} \qquad (13)$$

However, no values for the stoichiometric coefficients have been reported for the selected dicarboxylates. Some researchers have suggested formation of several amine:acid complexes. Nevertheless, at neutral pH conditions merely formation of ion-pair complex may occur, that is pure ion-exchange mechanism with few to none hydrogen-bonding interactions. In order to clear this issue, the molar anion exchange ratio was determined by measuring the released of Cl^- from the auxiliary phase for all the acids in both extraction and sorption equilibria.

Figure 7 presents scatter plots for the two capture techniques evaluated, clarifying that at these neutral pH conditions only ion-pair complexes are formed. Similar results were also obtained for the OH^- resin (data not shown). In the particular case of the extractions performed using the modified ion-exchange extractant, not only hydroxide anions were exchanged.

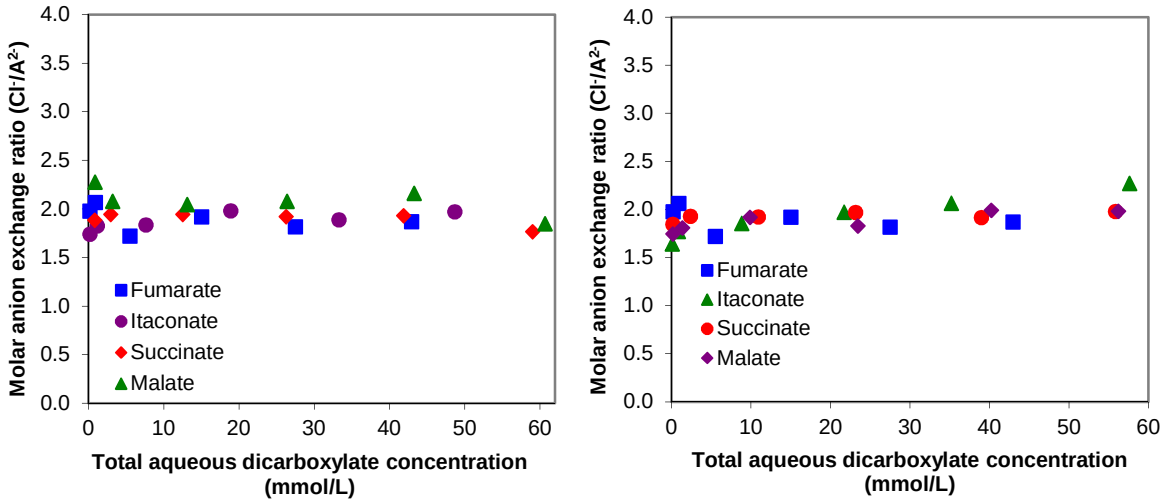


Figure 7. Molar exchange ratio for extraction and sorption with chloride counterion. a. Extraction with Aliquat 336 Cl^- 30% v/v. b. Sorption with Dowex Marathon A Cl^-.

Figure 8 shows the release of Cl^- from the unconverted Aliquat 336 species, reaching up to 11% of the exchanged counter-ions at high aqueous acid concentrations. Moreover, for all the acids the sum of Cl^- and OH^- anions was close to an anion molar exchange ratio of two. These determinations allow to conclude that the exchange stoichiometry is now known, being $n = 2$ in equation 13.

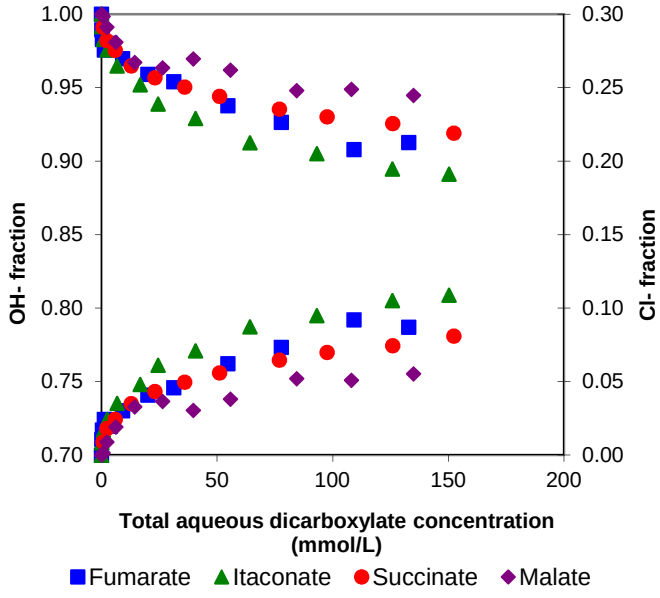


Figure 8. Exchanged counterions as fractions for extraction using partially converted Aliquat 336 OH^-/Cl^-

3.4 Conclusions

During ion-exchange extraction and sorption of the selected dicarboxylates, ion-exchange occurs to a larger extent with OH^- than with Cl^- as counterion. For the recovery systems tested, no major differences in the evaluated equilibrium parameters between fumarate, itaconate, succinate and malate were seen. Moreover, a molar anion exchange ratio of 2 was observed in all cases, resulting in a defined ion-exchange stoichiometry. Lastly, a hydroxide based sorption recovery results in the highest capture capacity and separation factors towards dicarboxylates.

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4

Direct downstream catalysis: from succinate to its diethyl ester without intermediate acidification

Seizing the potential of both chemicals and catalysts is fundamental to create a bridge between the emerging bio-based industry and the current process technology. Applying this approach to renewable succinic acid might lead to new routes for derivative production, levering its transition to industrial scale. Herein we propose a new route through direct downstream catalysis, in which a strong anion exchange resin is used not only for succinate recovery by sorption but also as a catalyst for the conversion of succinate into diethyl succinate via *O*-alkylation by gaseous chloroethane, a weak alkylating agent. A macroporous resin showed good diethyl ester yield (71%) after 1 h at 100 °C, whereas no monoester was detected. Moreover, the resin maintained its operational stability in both steps after five reuse cycles. Finally, the feasibility of a gas-solid reaction without the need of any solvent was demonstrated. Upon further development, this new process might be applied downstream of the formation of carboxylates by fermentation at neutral pH, diminishing the requirement of intermediate purification steps for the production of their respective ester derivatives.

Keywords: Succinic acid; Diethyl succinate; Adsorption; Ion Exchange; Heterogeneous catalysis; Alkylation.

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4.1 Introduction

The importance of succinic acid in the bio-based chemical platform is due to its potential to be converted into valuable derivatives, making it truly a building block for chemical synthesis (Carole et al., 2004). Such outstanding position was identified early in 2004 by the United States Department of Energy (Werpy and Petersen, 2004) and confirmed after an exhaustive revision six years later (Bozell and Petersen, 2010). Prospective transformations and uses will catapult succinic acid and derivatives market from annual values that rounded $0.4 billion in the 1990s (Zeikus et al., 1999) to exceed $1.0 billion by 2015 (Department of Agriculture, 2008). Realizing all the succinic acid potential within the biorefinery scenario will require efficient and environmentally friendly chemical upgrading technologies; advances on this field in the last decade were mostly devoted to overcome issues related with direct conversion in aqueous media (Cukalovic and Stevens, 2008, Delhomme et al., 2009) through several reduction routes. Although such advances are promising, the vast majority of those processes use pure succinic acid as starting material and therefore acid purification should be carried out first.

Succinic acid from renewable resources can be produced by fermentation using wild-type and engineered bacteria (Okino et al., 2008, Song and Lee, 2006, Wendisch et al., 2006) and recently by yeast (Raab et al., 2010, Verwaal et al., 2009, Yuzbashev et al., 2010). From well-established bacterial technology, aqueous acid purification should deal with cation removal which might involve cumbersome stages (Lin et al., 2010), is very sensitive to fermentation pH, expensive and environmentally problematic impeding industrial applications (Kurzrock and Weuster-Botz, 2010). Consequently, direct downstream transformation of succinic acid, without the need to purify it, is a way to debottleneck the industrial production of this building block (Cukalovic and Stevens, 2008).

Esters of succinic acid, in particular dimethyl and diethyl esters, are considered valuable platform chemicals and therefore excellent starting point for derivatives production (Varadarajan and Miller, 1999). However, achieving ester formation in a conventional manner implies, as expected, succinate acidification and succinic acid purification prior to an esterification reaction with an alcohol. From a fundamental perspective, to the knowledge of the authors, alternative routes have not been proposed for upgrading of succinate salts to esters. Related to this, all the research efforts have focused on improving succinic acid separation from fermentation broth at low pH and on reaction engineering aspects in the final

esterification. Here we describe a new route based on *O*-alkylation of succinate using a mild electrophilic alkylating agent. Alkylation has been used in the past for the synthesis of specialty esters and it has gained a renewed interest in the last years focused on new alkylating agents (Makowski et al., 2009, Rademann et al., 2001, Wolfenden and Yuan, 2007), applications such as methylation and carbonylation using dimethyl carbonate (Tundo and Selva, 2002), and etherification using 1-chlorobutane (Bender et al., 2010). In the proposed methodology, succinate salt is split and the organic anion sorbed by a strong anion exchange resin possessing quaternary amine functional groups (Q^+) (Figure 1, a). Subsequently the sorbed succinate reacts with gaseous chloroethane yielding the desired diethyl ester and quaternary amine in the chloride form (Figure 1, b).

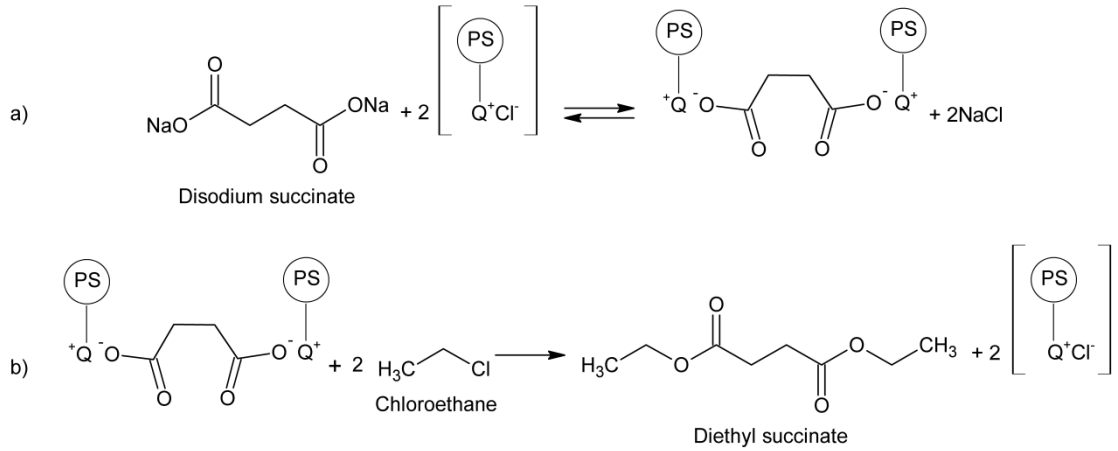

Figure 1. Proposed alternative route for bio-based diethyl succinate. a) Counter-ion removal by means of ion exchange. b) Diethyl ester production by phase transfer catalyzed *O*-alkylation. PS stands for polymer structure (i.e. polystyrene bead).

This new synthetic route has several features, intended to improve the sustainability of the global derivative production process by including the design principles of green chemistry and green engineering (García-Serna et al., 2007). The direct downstream catalysis approach avoids the need for succinic acid purification since the first sorption step can be used as an isolation step from diluted aqueous solutions (for instance, fermentation broth). The resin functional group is regenerated throughout the alkylation reaction, and therefore can be reused in a further sorption cycle. As a consequence, the number of intermediate operations such as precipitation, filtration, acidification and crystallization required in conventional esterification routes is drastically reduced. Furthermore, both sorption and reaction steps might be performed in a single process unit using reliable existent technology, leading to process intensification and therefore reduction in capital costs (Figure 2). The proposed strategy might lead to an improvement of the bio-based succinic acid production, highly relevant for the global bioeconomy scenario.

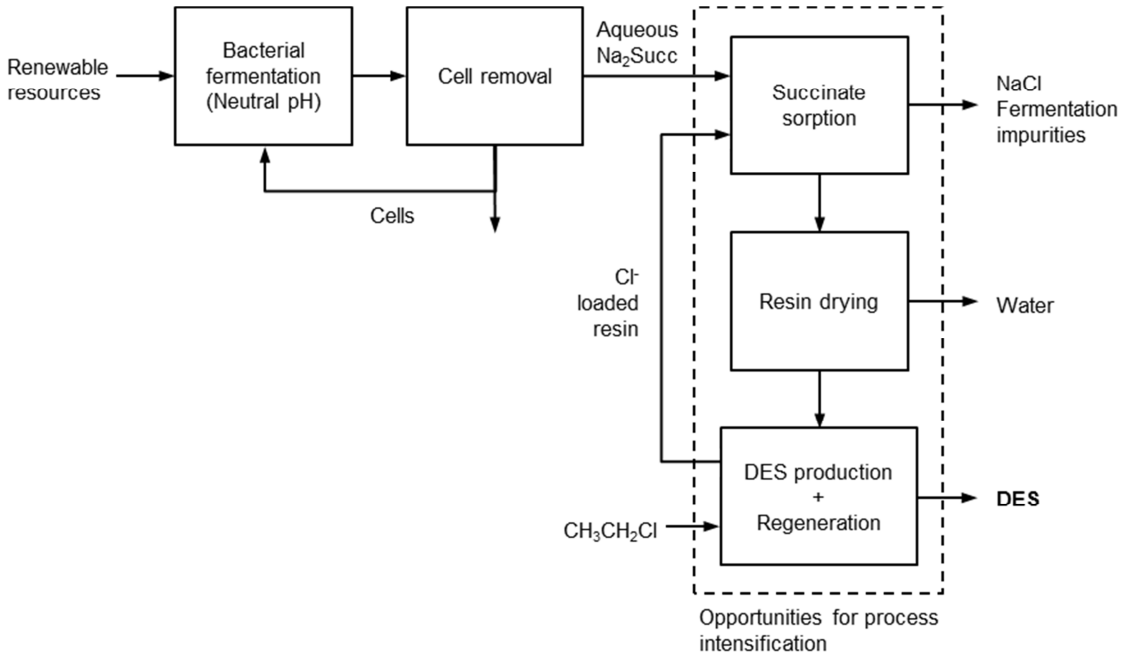

Figure 2. Anticipated process block diagram for the production of diethyl succinate from fermentative succinate by direct downstream catalysis.

4.2 Materials and methods

4.2.1 Materials

Succinic acid (≥99%), chloroethane (≥99,7%) and acetonitrile (≥99.9%) were purchased from Sigma-Aldrich. Sodium hydroxide (≥98%) and hydrochloric acid (37-38%) were supplied by J.T. Baker. Ethanol (≥99.9%) was acquired from Merck. Aqueous solutions were prepared using deionized water from a Milli-Q water purification system. All the succinate solutions were adjusted at pH 7.00 ±0.5 in two steps, first using solid sodium hydroxide to a pH around 6 and to neutral pH with 1 mol/L base solution by means of an Metrohm Titrino automatic titration system. Type I strong anion exchange resins Amberlite IRA-900 (macroporous), Amberlyst A26 (macroporous), Dowex Marathon MSA (macroporous), Dowex Marathon A (gel) and Type II Amberlite IRA-410 (gel) were obtained from Sigma-Aldrich. All the resins were supplied in the chloride form except for Amberlyst A26 which was converted from the hydroxide to chloride form employing the column elution technique according to ASTM standard method (International, 2010). The particle size for the tested resins range from 560 to 820 μm according to supplier especifications.

4.2.2 Sorption isotherms

Static sorption experiments were performed in 30 mL scintillation vials placed horizontally in a New Brunswick Scientific thermostated shaker at 100 rpm and 25°C for 4 h. Previous sorption kinetic trials showed that this time was sufficient to attain equilibrium. In most of the

experiments, 1 g of pre-wetted resin was added to the vial along with 10 mL of succinate solution at the desired concentration. Static saturation capacities were determined by means of mass balance based on initial and equilibrium aqueous succinate concentrations.

4.2.3 Column sorption

Dynamic sorption experiments were carried out in an adjustable height Omnifit glass chromatography column (10 mm internal diameter x 150 mm height). The column was filled using the slurry packing technique and vibrated manually during settling to improve particle distribution. Any bubbles remaining after packing were removed placing the column in an ultrasound bath. The experimental set-up was completed by a Shimadzu LC-8A HPLC pump, a Waters 484 tunable UV absorbance detector set at 210 nm, online effluent pH and conductivity detectors and a Pharmacia FRAC-200 fraction collector. The dynamics of succinate sorption were evaluated as breakthrough experiments. For each run, the equivalent of 2.5 g of dry resin was packed, which corresponds to a bed volume of 9.2 mL for Dowex MSA and Amberlite IRA 900, 8.5 mL for Amberlyst A26, 7.6 mL for Dowex Marathon A and 7.8 for Amberlyte IRA 410. After loading the column was washed with ten bed volumes of deionized water and loaded using a 20 g/L succinate solution at 2 mL/min. Fractions in the column outlet were collected periodically for succinate concentration analysis. Column dynamic capacity was calculated using the breakthrough data by means of integral analysis. When possible, this calculation was also checked by desorption of a loaded resin sample using either sodium nitrate or sodium bisulfate (50 g/L) following the experimental protocol described for static sorption in section 2.2.

4.2.4 Resin pre-treatment for alkylation reactions

Once the resin was saturated in the column operation, it was washed with deionized water until no absorbance signal was observed in the column outlet. After this, the column was unpacked and the resin collected and washed quantitatively three times with five volumes of ethanol using a Millipore Steriflip 60 μm nylon net filtration unit. Finally, the resin was dried in an oven at 72 °C for 24 h and stored in a desiccator until further use.

4.2.5 Alkylation reaction

Alkylation reactions were performed in a 50 mL Büchi glass stirred autoclave reactor placed in a Lauda oil thermostat. The reactor was equipped with a magnetic driven four blade impeller controlled by an overhead motor, thermocouple for temperature control, pressure sensor, high pressure relief valve, nitrogen inlet, reagent addition and sampling ports. For gas-solid reactions, the resin was loaded in a catalyst basket attached to the agitation shaft.

In a typical run, 2 g of dry succinate loaded resin were loaded into the reactor together with 30 mL of acetonitrile and heating and stirring (500 rpm) were started. Once the operation temperature was reached, 3 mL of chloroethane was added to the system at 0 h reaction time. Handling of chloroethane in liquid state was done using a glass gas-tight precision syringe

adapted with a jacket filled with frozen gel. The syringe was kept in a freezer at -20°C until its use. Depending on process temperature, autogenous pressures between 2 and 4 absolute bar were reached. A pressure drop was seen indicating chloroethane consumption. During reaction time, samples of 200 μL were withdrawn at specific times for diethyl succinate analysis. After reaction completion, the vessel was flushed with nitrogen to remove unreacted chloroethane. As a control experiment, a reaction using solid sodium succinate without the presence of any catalyst was carried out at similar conditions.

4.2.6 Analytical Methods

To determine the resin water content, a freshly hydrated sample was dewatered by vacuum filtration and then dried at 105°C for 24 h. Succinic acid concentrations from aqueous samples were analyzed on a Waters HPLC system using a Bio-Rad Aminex HPX-87H column (7.8 x 300 mm) at 60°C. Phosphoric acid (1.5 mmol/L at 0.6 mL/min) was used as an eluent. Quantification was done by UV detection at 210 nm using an external standard.

Succinic acid, monoethyl and diethyl succinate from alkylation reactions were determined using a Waters 2695 HPLC system with a Novapak C18 column (3.9 x 150 mm) maintained at 40°C. A mixture of phosphoric acid (pH=2.0) and acetonitrile (ACN) at 0.6 mL/min was used in gradient mode (0% ACN at t=0 min to 60% ACN at t=20 min to 0% ACN at t=25 min and maintained up to t=30 min). The esters were quantified by UV detection at 210 nm. Succinic acid and diethyl succinate were identified and quantified using their respective calibration standards. Pure standard for diethyl succinate was commercially available (≥99.5%, Sigma Aldrich). Identification and quantification of monoethyl succinate was done using a partially purified standard (90%, Sigma Aldrich). The obtained response factor (1.04 times higher than succinic acid, mass basis) was close to previously reported data (Kolah et al., 2008).

Chloride concentrations were determined using a colorimetric assay based on the improved Fried method (QuantiChrom Cl assay kit, BioAssay Systems, CA, USA). Briefly, 5 μL of the prediluted and acidified (pH 1-3) samples was transferred to wells of a clear bottom 96-well plate, 200 μL of working reagent were added and the plate was incubated for 5 min at room temperature. The optical density was determined at 595 nm and the chloride concentration was calculated using an appropriate calibration curve.

4.3 Results and discussion

4.3.1 Effective succinate binding to quaternary amine ion exchangers occurs at neutral pH

According to its pK_a values, succinate is almost completely dianionic at neutral pH. During sorption at neutral pH conditions, disodium succinate was split into a divalent succinate anion and the respective metal cations by the strong anion exchange resins tested. Both type I and II showed an anion exchange ratio (mol Cl^-/mol succinate) very close to 2 for all the range of concentrations tested (Figure 3). This validates the ion exchange stoichiometry (Figure 1, a) and indicates succinate is sorbed solely by ion exchange mechanism. Although this fact could

appear trivial, it is fundamental to validate the assumption that both carboxylate ends of the succinate molecule are individually bound to quaternary functional groups in the resin. The implications of such sorption behavior on the synthesis of diethyl succinate will be discussed in the coming section.

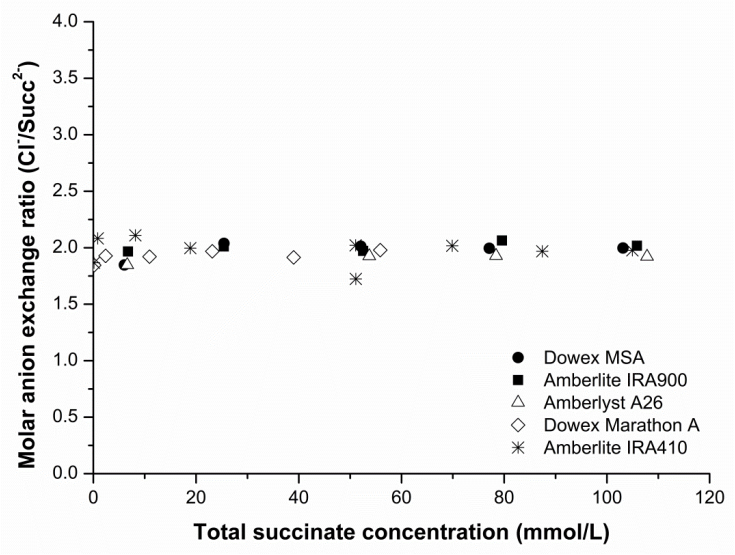

Figure 3. Chloride to succinate molar anion exchange ratio for the selected resins. Data derived from static sorption experiments.

Succinate sorption equilibrium data are presented in figure 4. All the tested resins showed a type I sorption isotherm and reach saturation capacities between 0.12 and 0.14 g/g dry resin. For the sake of comparison, a simple Langmuir model was fitted for Dowex MSA which presented the highest static sorption capacity. The obtained parameters are a saturation loading of 0.14 g/g dry resin and a value of 0.98 L/g for the Langmuir association constant. The equilibrium characteristics of succinate sorption were not greatly affected by either functional group type or structural characteristics. Although Amberlite IRA 410 (gel, type II) showed the lowest saturation capacity (0.12 g/g dry resin) amongst the resins, its association constant (1.21 L/g) was the highest.

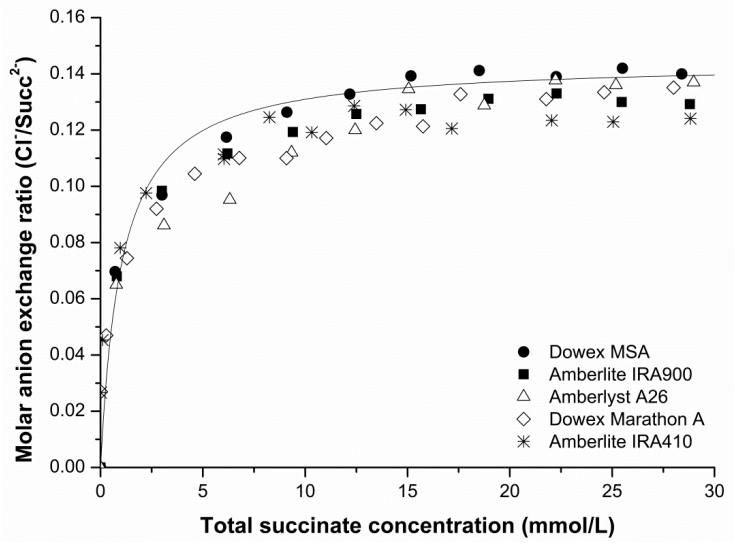

Figure 4. Succinate sorption isotherms for selected strong anion exchangers. All the resins were used in the chloride form. Experiments were performed in duplicate and average values are shown. The coefficient of variation was lower than 0.046 for all the dataset. Solid line represents Langmuir model fitting for Dowex MSA.

The literature on recovery of succinic acid by sorption has focused on low pH applications. In such operations auxiliary phases capture the undissociated acid by means of hydrogen bonding. Weak and strong polymeric anion exchangers have shown comparable loading capacities at low acid concentrations (Davison et al., 2004). Recently, alternative materials have been tested seeking for better desorption and regeneration characteristics; the saturation capacity of zeolites was slightly higher (Efe et al., 2010) whereas functionalized mesoporous silicas (Jun et al., 2007) showed half of the capacity obtained in the present study.

On the contrary, equilibrium data at neutral pH are scarce since acid recovery at such conditions is mainly done by precipitation and ion exchange does not show any process advantage if succinic acid is wanted. As the only reference, a claimed weak anion exchanger presented an unexpected capacity of 0.09 g/g towards succinate at neutral pH (Li et al., 2009). A sorption mechanism was not disclosed, though.

Figure 5 illustrates the dynamic sorption of succinate as breakthrough curves for the selected ion exchange resins. The calculated dynamic column capacities ranged from 0.29 (Amberlite IRA900) to 0.35 g/g dry resin (Dowex Marathon A). The values are in agreement with the fact that, in general, ion exchange capacities are lower for macroporous resins than for gel type resins (i.e. 1.1 eq/L for Dowex MSA and 1.3 eq/L for Dowex Marathon A, manufacturer specifications). It is also evident that these column capacities are higher than the respective static saturation capacities for all the resins. An explanation for this phenomenon originates from the operating mode. In fixed bed, chloride ions initially present in the resin are continuously pushed forward to the column exit by succinate ions and therefore a different equilibrium condition is established. In addition, the wide mass transfer zone found might be related to this fact due to anion competition between succinate and chloride.

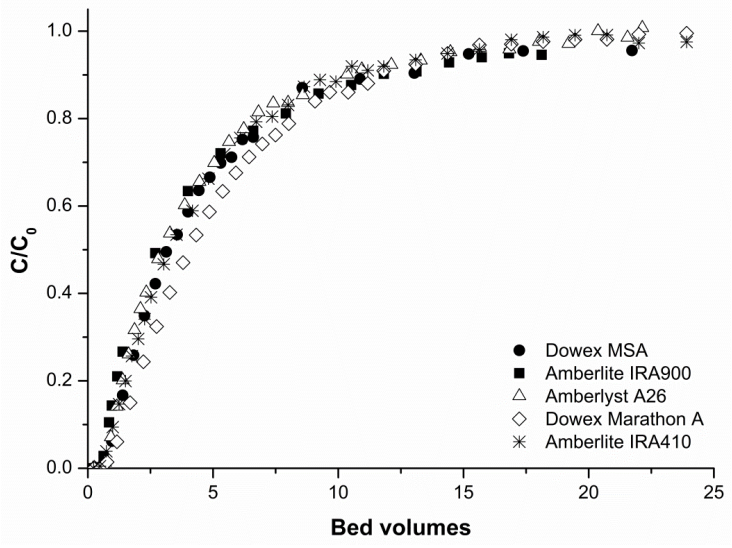

Figure 5. Succinate breakthrough curves in a fixed bed packed column for selected strong anion exchangers. *C* and C_0 are the concentrations in the column outlet and inlet, respectively. Experiments were performed in duplicate and average values are shown. The coefficient of variation was lower than 0.035 for all the dataset.

4.3.2 Diethyl succinate is produced with good yield from sorbed succinate anions

For the first time, diethyl succinate (DES) was synthetized by *O*-alkylation using chloroethane. After resin loading and pre-treatment to remove intraparticle water, the reaction was carried out using the selected resins in acetonitrile as suitable aprotic solvent.

Figure 6 shows diethyl succinate is obtained in good yield after 10 h of reaction at the described experimental conditions. In a control experiment we verified that succinate salts do not react with chloroethane, which indicates that anion exchange resins are a suitable phase transfer catalyst for this synthesis. Carboxylate anions are not very reactive nucleophiles. For an *O*-alkylation to take place, it would be beneficial if this nucleophilicity is increased; the quaternary ammonium groups bound electrostatically to them might play that role. The technical feasibility of using anion exchange resins for the catalysis of such addition reactions has been demonstrated in literature using several monocarboxylic acids and strong alkylating agents (Cainelli and Manescalchi, 1975). In that study, all the alkylating agents using chloride as leaving group (except benzyl chloride) failed to react.

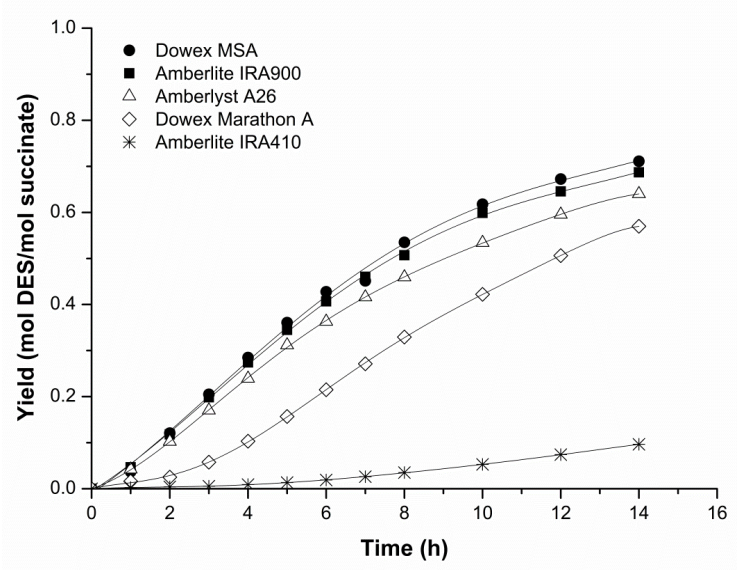

Figure 6. Yield profiles for diethyl succinate synthesis in a solid-liquid-gas system by phase transfer catalysis using strong anion exchangers. Solid lines as guides only. Yields are based on the amount of succinate bound to the resin. Reaction temperature of 60 °C.

As discussed in section 3.1, both succinate carboxylate groups are bound to the resin, which is essential for a diester to be synthesized. Moreover, such feature suggests a two-step reaction mechanism and hence only diethyl ester would be released to the liquid phase, since a monoester would remain bound until ethylation takes place. Simultaneously, the leaving group from the alkylating agent should bind to the resin in order to maintain the required electroneutrality in the solid phase. In our studies no traces of monoester were found in the liquid phase. It should be highlighted that this provides a great downstream advantage because purification of diester from acid or monoester is not required.

Figure 6 also shows that differences among the catalysts tested had a large impact on the reaction rate. Macroporous, type I resins Dowex MSA, Amberlite IRA900 and Amberlyst A26 gave reaction yields exceeding 0.6 mol DES/mol succinate after 14 h of reaction. The two gel resins evaluated presented lower catalytic activity, in particular Amberlite IRA 410 (type II), which gave a final yield as low as 0.1 mol DES/mol succinate. Although it has been described that gel-type resins have better mass transfer properties due to its swelling characteristics and therefore less diffusion resistance (Barbaro and Liguori, 2009, Desikan and Doraiswamy, 1995), macroporous resins possess greater surface area exposed to the solution providing better accessibility to its functional groups. For this type of resins, higher activity and kinetics were found for several applications (Aragon et al., 1993, Regen, 1979). When the comparison

is made over the functional group type, the difference in reactivity is acute. Amberlite IRA410 possesses *N,N*-dimethyl-*N*-(2-hydroxyethyl)ammonium functional groups, whose basicity is lower than trimethylammonium type I groups, which might influence the reaction rate. Moreover, the size of the functional group plays a role in PTC reactions. It is known that the longer the chain linked to the quaternary nitrogen, the less accessible the group and thus the lower reaction rates (Bender, Jepkens, 2010). Finally, type II resins are less thermally stable and therefore might have suffered certain deamination during pre-treatment and reaction procedures.

The promising results obtained using Dowex Marathon MSA and its reported process characteristics, e.g. uniform particle size distribution (mean particle size 640 μm, uniformity coefficient=1.1) ensuring adequate kinetics and mass transfer characteristics and very good physical stability and resistance to organic fouling, allowed us to choose it as a catalyst for further studies and encouraged us to study other factors influencing the kinetics and diester yield. Anion exchange resins are sensible to deamination at high temperatures. Therefore, the impact of temperature was tested at three levels (figure 7) in order to establish its stability to temperature. As anticipated, the reaction rate increased with temperature and yield attained a maximum average value of 0.71 mol DES/mol succinate after 4 h at 80 °C and after just 1 h at 100 °C, indicating that the basic functional group is stable within this temperature range. The reaction rates obtained are good when compared with other synthetic routes. Using regular esterification, similar diethyl ester yields were obtained in a similar period of time using a sulfonated and chlorinated styrene-divinylbenzene resin (Orjuela et al., 2012), after approximately 4.5 h using mesoporous Starbons in aqueous solutions (Budarin et al., 2007), more than 8 h in the presence of montmorilonite clays (Reddy et al., 2005), 10 h using a typical strong acid cation exchanger (Kolah, Asthana, 2008), and more than 50 h using Nafion NR50, a superacid ionomer, assisted by pervaporation (Benedict et al., 2006). The DES production profiles might suggest a first order kinetics, yet several diffusion steps are likely to occur and a more complicated kinetic mechanism is expected (Desikan and Doraiswamy, 1995, Regen, 1979).

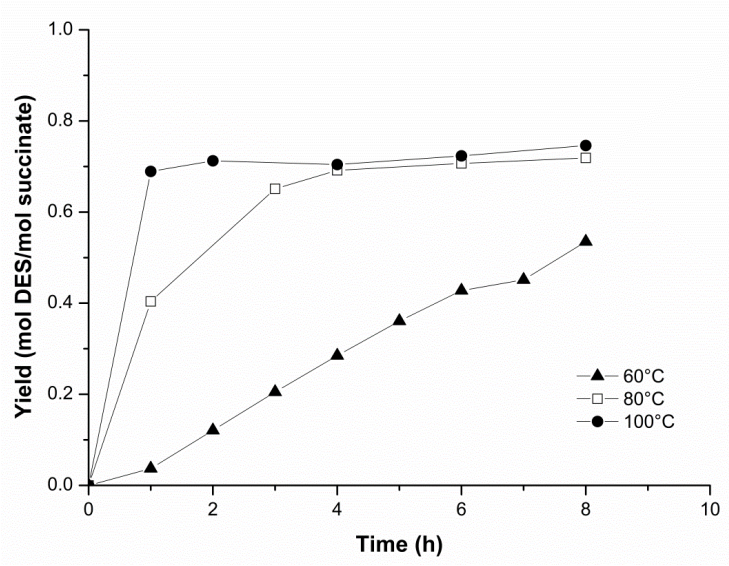

Figure 7. Influence of temperature on the reaction kinetics of diethyl succinate production catalyzed by Dowex MSA.

In the current three-phase system used, chloroethane in the gas phase should dissolve into the liquid solvent phase, diffuse through the solvent and then through the catalytic bead to

react with the activated carboxylate anion. Increasing temperature will not only increase reaction rate constants but also diffusion coefficients, and decrease chloroethane solubility in the liquid. Moreover, stirring rate might have an impact in the reaction rate if under the experimental conditions diffusion limitations are present. Interestingly, it seems the reaction has reached a plateau of maximum yield and is not affected by temperature under the experimental conditions tested. Several factors might be responsible for the incomplete conversion, inherent thermodynamic limitations, the presence of hydration water in the succinate complex or steric hindrance might limit the extent of conversion or promote side-reactions, although none was detected. The influence of water will be briefly discussed in the next section.

4.3.3 Adequate resin stability and feasibility of gas-solid reaction improve process application

Although the selected resin seems to be active up to 100 °C according to figure 7, it is well known that quaternary ammonium groups are temperature sensitive and might suffer deamination under long term exposure to high temperatures (Gelbard, 2005, Naik and Doraiswamy, 1998, Tundo et al., 1989). Several sorption-reaction cycles were carried out in order to quantify the degree of regeneration of the resin and to establish if resin thermal stability is good enough for process application.

It can be observed in figure 8 that both processes maintained performance after five reuse cycles. Average values for dynamic column loading of 0.34±0.019 g succinate/g dry resin and reaction yield 0.71±0.020 mol DES/mol succinate were obtained. In total, the resin was exposed for 160 h to a temperature above 70 °C (including pre-treatment between cycles). During this period the resin activity remained nearly unaltered.

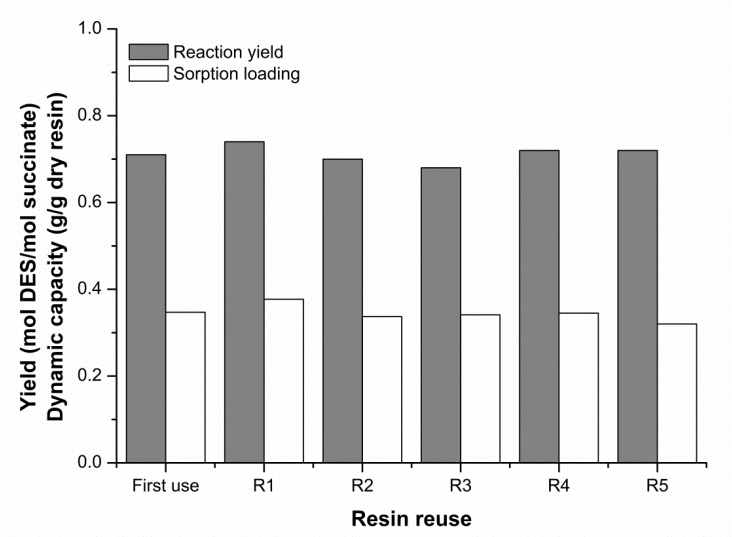

Figure 8. Performance of sorption-reaction cycles using Dowex MSA. 8 h reaction time at 80°C. Other experimental conditions as described before.

The performance of the sorption operation is dependent on the performance of the reaction step, where resin regeneration occurs. It was not expected that the resin would recover its initial dynamic capacity during the cycles since succinate conversion was incomplete. To address this issue, chloride ion elution was determined in each sorption cycle and the average molar exchange ratio for the first three reuse cycles was calculated. An exchange ratio of

1.61±0.14 mol Cl^-/mol succinate was obtained. This value suggest that other species than chloride were present in the resin as counter-ion after the reaction. It is likely that part of the remained succinate reacted only at one of the carboxylate groups and kept bound to the resin as monoethyl succinate. Those monovalent ionic species can be exchanged by succinate anions and hence the column capacity is replenished to operational values in each reuse. As a reference, if all the succinate that was not recovered as DES would be monoalkylated, an exchange ratio of 1.71 mol Cl^-/mol succinate should have been found, and if none would be monoalkylated a ratio of 1.42 mol Cl^-/mol succinate should have been found. The actual value suggests a significant proportion of monoalkylation. An additional factor that might influence and keep the available resin capacity, at least during the first cycles, is the possibility of quaternization of tertiary amine groups initially present in the resin given fabrication limitations. This has been reported to occur for similar applications (Regen, 1979).

Although our main goal is to demonstrate the feasibility of the concept, we are aware that process application will require developments in several reaction engineering aspects. We have addressed two of these; the need for water removal after sorption and the use of a solvent as reaction medium. To assess the first issue, succinate alkylation was tested without resin pre-treatment in an effort to evaluate the impact of water on diethyl succinate yield. In that experiment, the initial mass content of water in the reaction media was estimated in 4.8%, given the resin water content after sorption. A pronounced reduction of the reaction rate was found. After 8 h of reaction, a yield of 0.19 mol DES/mol succinate was obtained, in comparison to 0.71 mol DES/mol succinate upon drying. The role of water during related reactions and its impact on the reaction rate have been widely discussed (Albanese et al., 2001, Zahalka and Sasson, 1984). Up to certain levels, water has beneficial effects increasing anion diffusion, but over a critical amount it will diminish nucleophile reactivity; furthermore chloroethane will slowly hydrolyze in the presence of water, diminishing its concentration and generating hydrochloric acid that might harm the reaction. Water effects during alkylation are case specific and must be studied in detail in further studies.

For the second aspect, to avoid the use of any solvent we tested a gas-solid alkylation. After 8 h, a final reaction yield of 0.35 mol DES/mol succinate was attained, 50% less than when using organic solvent. At the experimental conditions, diethyl succinate is a liquid, so in gas-solid mode it will likely remain embedded in the macroporous resin, creating a static liquid layer and affecting the intraparticle diffusion of chloroethane. The use of other gas regimes or reactor types could enhance mass transfer. Solvent might also be avoided by using pressures beyond the chloroethane vapor pressure at reaction temperature (7.6 bar at 80 °C (Ohe, 1999)). Then the system could operate in liquid-solid mode; removal of excess chloroethane by release of pressure would be straightforward.

4.3.4 Recovery-upgrade integration of succinic acid improves overall atom efficiency

In the current bio-based scheme, the production of succinic acid ester derivatives is the last stage in a chain of three closely interconnected processes. First, a bacterial fermentation process carried out at neutral pH leading to succinate salt; a second stage involves removal of counter-ion (e.g., sodium or calcium) achieved by acidification using mineral acid (Figure 9, reaction a), followed by succinic acid purification and crystallization. Finally succinic acid

crystals are dissolved in a suitable alcohol (for instance, ethanol for ethyl ester production) and esterified via conventional esterification routes (Figure 9, reaction b). Such conversions are performed using homogeneous and heterogeneous acid catalysts and the advantages of the latter are irrelevant in our analysis. Since esterification is an equilibrium-limited reaction, efforts have been done to improve conversion by removing products from the reaction zone, ultimately by means of reactive distillation (Orjuela et al., 2011).

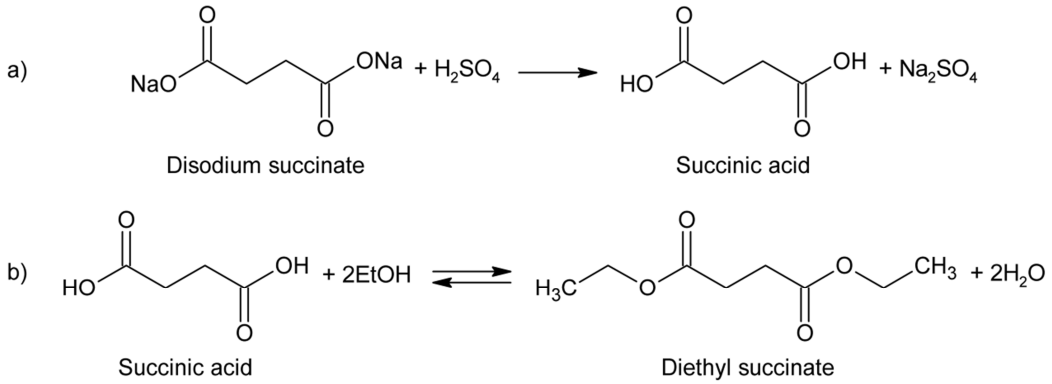

Figure 9. A possible conventional route for diethyl succinate from bio-based disodium succinate. a) Counter-ion removal via homogeneous acidification. b) Overall esterification reaction for diethyl ester production (omitting the intermediate formation of monoethyl succinate)

Aside process advantages, the main attribute of the proposed novel route lies in its conversion efficiency. Table 1 shows calculated atom efficiencies and environmental E-factor over the reactions schemes 1 and 2. Under the light of these indicators it is possible to claim that the alkylation route by direct downstream catalysis is environmentally cleaner than the conventional esterification route. Improvements of that order in the consumption of chemicals (related with atom efficiency) and waste production (E-factor) are relevant for a bulk chemical like succinic acid with a current growing market.

Table 1. Comparison based on green chemistry metrics for diethyl succinate production routes according to reaction stoichiometry. Indicators calculated using standard methodology (Sheldon, 2000).

	Atom efficiency	**E-factor**[a]
Process route	%	(kg waste/kg DES)
Proposed direct downstream catalysis via O-alkylation	60	0.7
Conventional acidification and esterification	49	1.0

[a] Calculated based on a theoretical basis of 100% reaction yield

4.4 Conclusions

Diethyl succinate has been effectively produced for the first time by direct downstream catalysis from neutral succinate solutions under mild reaction conditions. An anion exchange resin was used as a sorbent material and as phase transfer catalyst for the *O*-alkylation of succinate anions. Green chemistry metrics showed clear advantages of the proposed process when compared with a traditional esterification route. The concept has been validated and good yields and excellent kinetics were obtained. The negative impact of water on the reaction yield and the role of solvent were established. Further work is being carried out in our laboratories comprising new modes of reaction without solvent and the use of alternative alkylating agents like dimethyl carbonate.

4.5 Acknowledgments

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5

Green upgrading of succinate using dimethyl carbonate for a better integration with fermentative production

In this work we present a new process alternative comprising bio-based succinate production, its recovery by ion exchange and further upgrading to dimethyl succinate via *O*-alkylation by direct downstream catalysis. The use of dimethyl carbonate as alkylating agent permits the *in-process* generation of a mobile bicarbonate anion, playing the role of counter-ion during succinate capture and then transported upstream where is used as inorganic carbon source and neutralizing agent required for most bacterial succinate fermentations. Succinate recovery using a strong anion exchange resin in the bicarbonate form presented good equilibrium characteristics and column performance. Subsequently, dimethyl succinate was produced in high yield (96%) using dimethyl carbonate as a solvent and reagent and the kinetics of the system were described by a pseudo-first order model. Throughout the reaction and in the presence of water, it was verified that the resin is effectively regenerated to its bicarbonate form maintaining its ion exchange capacity. As a main advantage, the validated concept leads to an improved bio-based process for succinate to ester derivatives where no stoichiometric waste is produced, contrary to conventional acidification-esterification operations.

Keywords: Succinic acid, Dimethyl succinate, Dimethyl carbonate, Ion exchange, Alkylation.

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5.1 Introduction

Succinic acid is an attractive renewable platform chemical due to its chemical functionality and valuable derivatives (Bozell and Petersen, 2010). Among them, succinate esters are considered to be precursors for many petrochemical products and can be used as a monomer for a wide range of polymers (Xu and Guo, 2010). To realize such platform potential, research efforts have focused on its biotechnological production (Jang et al., 2012, Song and Lee, 2006, Zeikus et al., 1999), downstream processing (Cheng et al., 2012a, Kurzrock and Weuster-Botz, 2010) and chemical modification (Cukalovic and Stevens, 2008, Delhomme et al., 2009). Although the results of such advances are applied by many industrial enterprises around the world (Engel et al., 2008, Kurzrock and Weuster-Botz, 2010, McKinlay et al., 2007, Raab et al., 2010), there are still many challenges concerning undesired salt production (Werpy and Petersen, 2004). Waste production and chemicals consumption can still be reduced significantly for improving the sustainability of a succinate-producing biorefinery.

Bio-based production of succinic acid using bacteria runs most efficiently at neutral pH. The base addition required to maintain neutral pH leads to production of divalent succinate salt (Lee et al., 2006, Litsanov et al., 2012, Okino et al., 2008, Wang et al., 2011a). The salt should be converted back into the acid and purified thoroughly, involving the consumption of mineral acid and stoichiometric production of waste salt, before traditional methods can be applied for the upgrading of succinic acid to derivatives, e.g. esters (Orjuela et al., 2012), or polymers. Dimethyl succinate (DMS) is a promising alternative to petrochemical dibasic esters with direct applications as solvent and polymer additive and will be produced shortly from bio-based succinic acid (Wang et al., 2011b). As a polymer platform, DMS offers advantages over succinic acid since can be polymerized using current transesterification polymerization methods, which are simpler than the direct melt polymerization methods required for succinic acid (Bechthold et al., 2008, Shirahama et al., 2001, Xu and Guo, 2010). To

achieve this, DMS should be produced efficiently and it is believed that integration of green chemical transformation routes into succinate recovery may improve production economics and efficiency (H. Clark et al., 2009).

In a previous publication (López-Garzón et al., 2012) we applied the concept of direct downstream catalysis to integrate recovery and chemical upgrading of succinate without intermediate acidification. In that study, we proposed and validated experimentally a new route for diethyl succinate production from a succinate salt via *O*-alkylation using chloroethane as alkylating agent and a commercial macroporous strong anion exchange as both sorbent and catalyst (López-Garzón, Ottens, 2012). Although the calculated overall atom efficiency and E-factor demonstrated the advantages of the integrated process over the conventional acidification-esterification route, the halogenated alkylating agent is not considered to be green and led to chloride salt as by-product, reducing the attractiveness of that particular reaction system.

We believe that the above mentioned issues can be overcome by the use of dimethyl carbonate (DMC) as alkylating agent. DMC is a nontoxic, biodegradable solvent currently produced from syngas and methanol (Romano et al., 1982, Romano et al., 1980) and in the future from carbon dioxide and hydrogen (Aljundi et al., 2005) which makes it a true green reagent. Compared to other alkylating agents, it is efficient in terms of atom economy and waste generation, producing carbon dioxide and methanol as by-products (Selva and Perosa, 2008), in which the latter may be recycled to its synthesis. In spite of the above mentioned benign characteristics, the reactivity of DMC is low and depends on reaction conditions, undergoing methylation reactions with a large number of nucleophilic substrates (Memoli et al., 2001) at temperatures ranging from 90 to 175 °C depending on the catalyst used (Shaikh and Sivaram, 1996, Shieh et al., 2002, Tundo and Selva, 2002). Under the direct downstream catalysis approach, dimethyl succinate formation using DMC could permit cascade up integration to fermentation, leading to an improved bio-based succinate production process. However, the feasibility of such reaction system has not been reported in literature and should be demonstrated first.

The objective of this work is therefore to verify the main reaction features allowing the realization of a new conceptual process. The alkylation stoichiometry and in particular the nature of the resin counter-ion after alkylation was addressed. Furthermore, it was established that quaternary ammonium functional groups such as found in commercial strong anion exchange resins were able to act as a catalyst under mild temperature conditions. The temperature effects on the reaction kinetics were interpreted by a pseudo-first order kinetic model. Finally, based on our previous experience (López-Garzón, Ottens, 2012), the effects of water on methylation yield and rate were determined.

5.2 Process conception

According to the state of the art description for reactions using DMC, it is possible to assume that carbon dioxide-related species, i.e. carbonates, produced during alkylation could play the role of mobile anion within our proposed process. Having that in mind, it is possible to describe the new integrated route as consisting of two reactive stages. Firstly, the succinate anion is captured from an aqueous solution such as produced by fermentation by means of a

strong anion exchange resin in a (bi)carbonate form (Figure 1) releasing the respective (bi)carbonate salt. Next, sorbed succinate is *O*-alkylated at both its carboxylate groups by DMC. The reaction is catalyzed by the quaternary ammonium functional groups (Q^+) to which succinate anion is electrostatically bound. As a result of the reaction, dimethyl succinate is formed whereas the anion exchange material is left in a proper ionic form in order to keep electroneutrality. During this stage, several reactions might occur as depicted in Figure 2. In the absence of water, the methoxycarbonate anion (DMC leaving group) could remain attached as a whole to the basic functional group (Figure 2a) in a similar manner as reported for *N*-methylations (Holbrey et al., 2010). Although not reported before, methoxycarbonate might also react further and participate in a second methylation reaction yielding a resin in a carbonate form (Figure 2b). In the presence of water, the decomposition of the bound methoxycarbonate anion could lead to the formation of bicarbonate anion and methanol with the former remaining bound to the resin (Figure 2c). Moreover, water might hydrolyze DMC in a side reaction (Figure 2d).

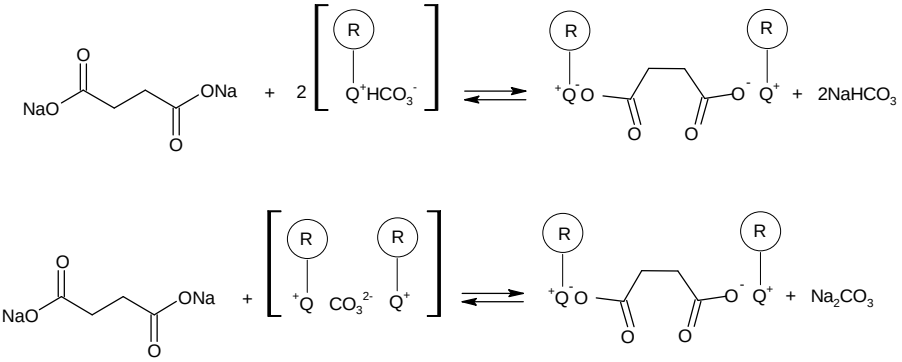

Figure 1. Ion exchange stoichiometry for the sorption of disodium succinate from neutral pH succinate solutions by a strong anion exchange resin in the (bi)carbonate form.

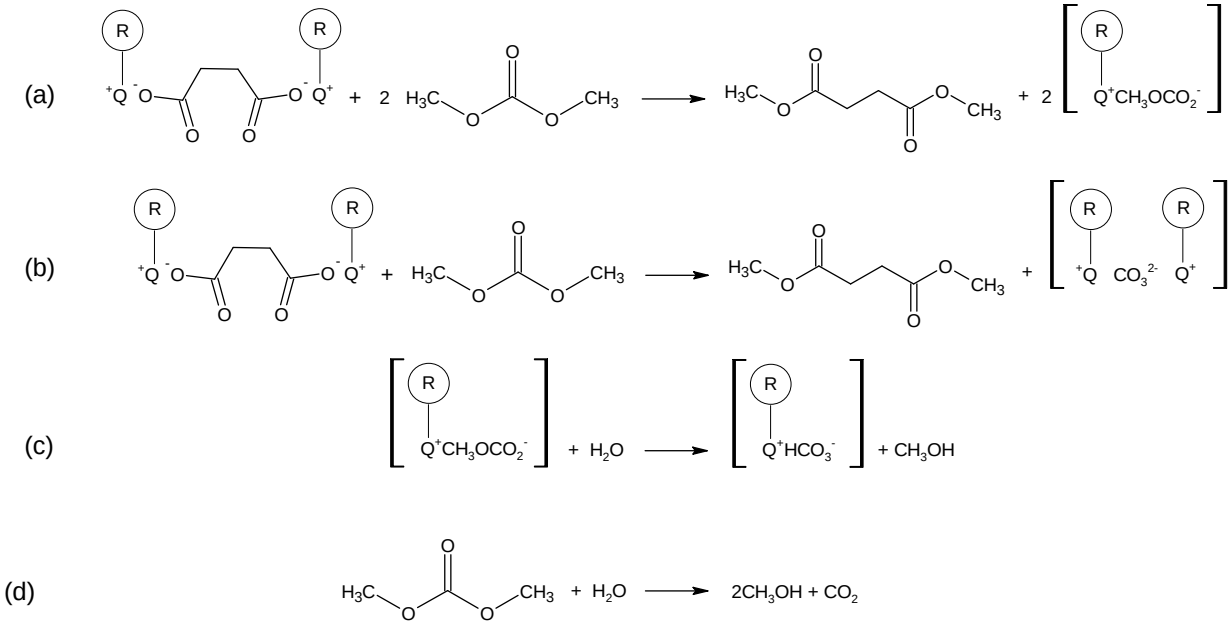

Figure 2. Proposed reaction stoichiometries involved in the production of dimethyl succinate by *O*-alkylation using dimethyl carbonate as alkylating a gent.

It is possible to foresee the impact of both ion exchange sorption and *O*-alkylation DMC-based stoichiometries on the sustainability of the overall bio-based dimethyl succinate process. From a recovery perspective the anion exchange material is regenerated in the (bi)carbonate form through the alkylation process. Therefore it can be used in a new succinate sorption cycle. Then, the (bi)carbonate anion is released in aqueous solution and its use can be extended further to the fermentation process, playing the role of neutralizing agent required for maintaining optimal production pH, as described elsewhere (Andersson et al., 2009, Cheng et al., 2012b). Additionally, the best bacterial succinate fermentations require carbonate species as carbon source in addition to carbohydrates to achieve optimum yields (Cheng, Zhao, 2012b). In such cases, the (bi)carbonate obtained from DMC will also provide (at least partially) the necessary carbon for biosynthesis of succinate. A similar combined effect has been reported (Zou et al., 2011), avoiding the need of gaseous carbon dioxide supply. The complete block diagram for this alternative process can be seen in Figure 3.

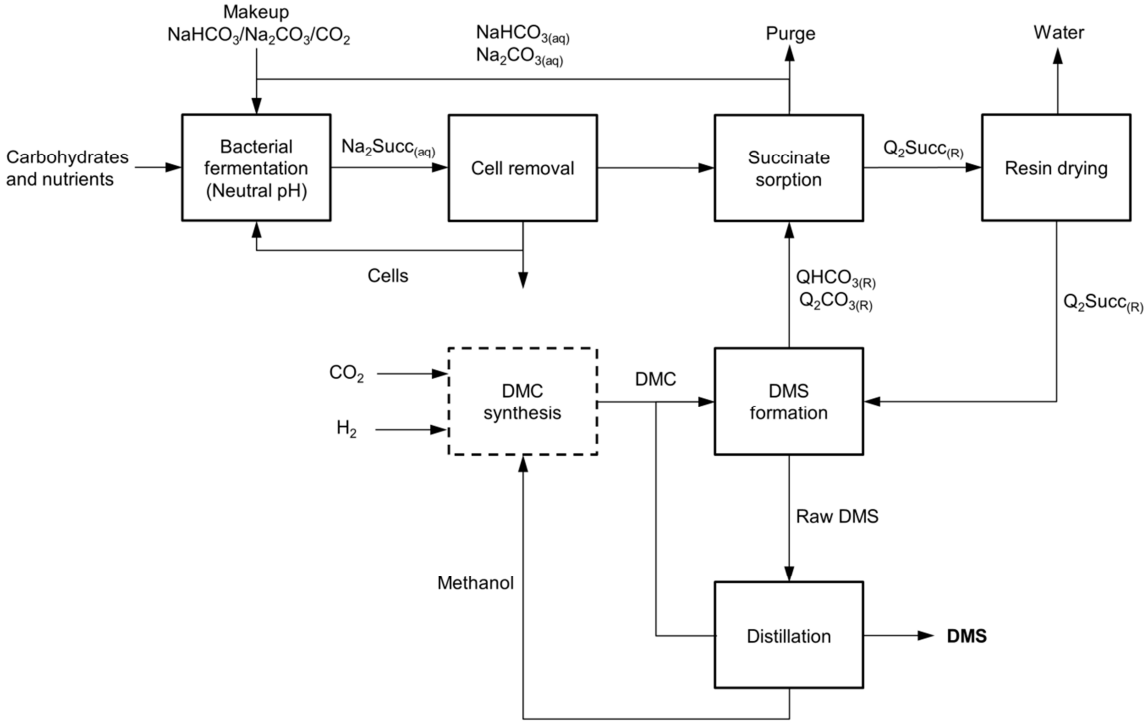

Figure 3. Process block diagram for the integrated chemical upgrading of succinate via direct downstream catalysis using dimethyl carbonate (DMC). Makeup and purge streams are case dependent. DMC synthesis has been included to demonstrate complete integration possibilities.

Our process alternative has various advantages when compared to a conventional acidification-esterification route (Figure 4). The number of operations has been reduced and new intensification opportunities are possible since succinate sorption, resin drying and DMS production operations may be carried out in the same process unit. Moreover, the chemicals consumption and waste production are also reduced as alkylation by-products derived from the decomposition of the DMC leaving group can be recycled, maximizing the carbon usage. Overall, a process without stoichiometric waste production has been conceived.

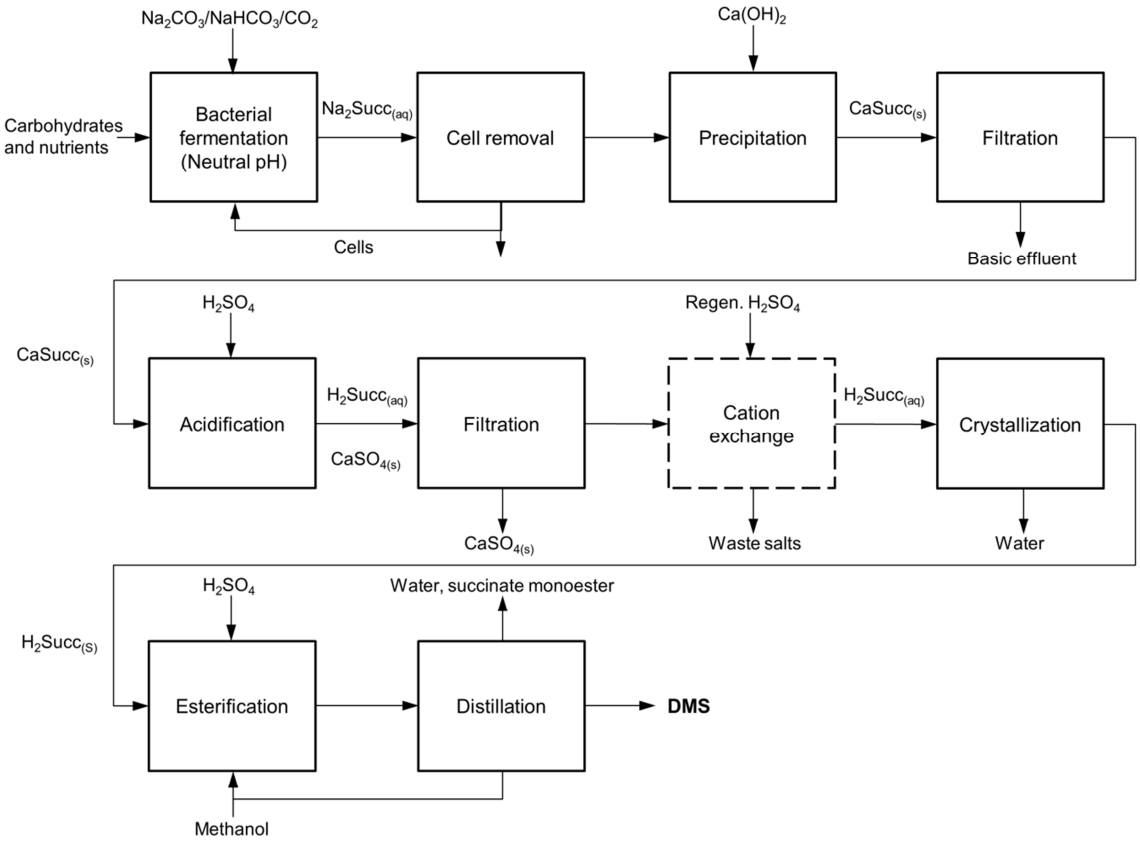

Figure 4. Possible process diagram for a conventional dimethyl succinate production process from bio-based succinate, partially based on literature (Kurzrock and Weuster-Botz, 2010). Note the stoichiometric consumption of bases, acids and carbon dioxide and the production of waste salts such as gypsum. The dashed block is optional.

5.3 Materials and methods

5.3.1 Materials

All chemicals were analytical grade (Sigma-Aldrich) and were used without any purification. Aqueous solutions were prepared using deionized water from a Mili-Q water purification system (Millipore). Succinate solutions were adjusted to pH 7.00 ±1.0 in two steps, first using solid sodium hydroxide to a pH around 6 and to neutral pH with 1 mol/L aqueous NaOH solution. In previous studies (López-Garzón, Ottens, 2012), the resin Dowex Marathon MSA was selected due to its capacity and catalytic activity. The resin is a strong anion exchanger, with a type I quaternary ammonium functional group, macroporous structure and monodisperse bead size. According to its commercial specification, it has a mean particle size of 640±50 μm and a minimum total ion exchange capacity of 1.1 eq/L. The resin was obtained in the chloride form (Sigma-Aldrich) and converted to the required ionic form by the column elution technique.

5.3.2 Ion exchange capacity determination

The total ion exchange capacity of resin was determined by following the ASTM standard method for anion exchange materials (ASTM, 2010).

5.3.3 Succinate sorption isotherms

Static sorption experiments were performed as reported before (López-Garzón, Ottens, 2012). After equilibrium was attained, the aqueous solution pH was determined using a pH meter.

5.3.4 Succinate column sorption

Dynamic sorption experiments were carried out as described previously (López-Garzón, Ottens, 2012). Due to variable swelling degree for each counter-ion for Dowex Marathon MSA, the same resin dry mass (2.5 g) led to different column volumes. Empty column bed volumes for HCO_3^- and CO_3^{2-} forms were 9.4×10^{-3} and 8.4×10^{-3} L, respectively. Using acetone as a tracer (10% aqueous solution) and by analyzing the peak of a 100 μL pulse, the total bed porosity for HCO_3^- and CO_3^{2-} forms were estimated at 0.85 and 0.89 respectively. Breakthrough experiments were carried out using an inlet concentration of 20 g/L succinate at a flow rate of 9.4×10^{-3} L/min.

5.3.5 Resin washing and drying

Alkylation reactions and quantitative desorption determinations require dry ion exchange resin. For alkylation experiments the resin was loaded in a column operation as described above. Once the resin was saturated, it was washed with deionized water until no absorbance signal was observed in the column outlet. After this, the column was unpacked and the resin collected and washed quantitatively three times with five volumes of methanol using a Millipore Steriflip 60 μm nylon net filtration unit. The resin was dried in an oven at 72 °C for 24 h and transferred to a desiccator where vacuum (31 kPa) was applied during 4 h. For quantitative desorption assays, the washing and drying protocol was followed as described and slightly adapted for resin samples from alkylation reactions which were washed directly with methanol instead.

5.3.6 O-Alkylation reactions

Initial alkylation reactions were carried out in closed glass tubes. Succinate loaded resin (0.2 g) along with DMC in acetonitrile as solvent (5 g) were put in glass tubes and then placed in a heating block at 100 °C for 15 h without agitation. Initial and final samples were analyzed for dimethyl carbonate conversion and dimethyl succinate and methanol production. Initial water concentration was also determined. For further studies, alkylation reactions were

performed in a 50 mL Büchi glass stirred autoclave reactor placed in a Lauda oil thermostat. The reactor was equipped with a magnetically driven four blade impeller controlled by an overhead motor, thermocouple for temperature control, pressure sensor, high pressure relief valve, nitrogen inlet, reagent addition and sampling ports. For most reactions, the resin was loaded in a catalyst addition device and released applying nitrogen pressure.

In a typical run, 1 g of pre-treated succinate-loaded resin (0.24 g succinate/g total dry resin) was placed in the catalyst addition device and 30 g of DMC was added to the reactor vessel. The vessel was then purged 10 times with N_2 to achieve an inert atmosphere. Agitation was set at 750 rpm. The reactor was then brought to reaction temperature. Once stable, resin was released to start the reaction. During reaction, liquid sampling and weighing of samples was performed at regular intervals for dimethyl succinate, methanol and water analysis. For most reactions, an increase of pressure was noticed during reaction. After the reaction, the reactor was cooled down to room temperature and weighed to improve mass balance calculations.

To achieve low water contents, water was removed *ex-situ* by means of adsorption onto molecular sieves at room temperature. In such experiments, 1 g of succinate-loaded resin was charged along with 50 g DMC into the reaction vessel. Agitation was started and the liquid phase was recirculated through two stainless steel columns (1.0 cm internal diameter × 15 cm length) packed each with 10 g activated 3Å molecular sieve pellets (230 °C, 48 h) by means of a peristaltic pump during 12 h. After water removal, the reactor was heated to reaction temperature (for about 12 min) and sampling was started.

5.3.7 Resin desorption

To verify resin loading and counter-ion nature, desorption was carried out as column operation. A known amount of dry resin sample, about 1 g, was wetted in deionized water and packed into a glass column (Bio-Rad, 0.7 cm internal diameter × 10 cm length). The column was eluted with a displacer salt solution at 2 mL/min until 100 mL solution was collected. For succinate loading determinations, sodium bisulfate (50 g/L) solution was used as displacer. Resin samples from alkylation reactions were eluted using sodium nitrate (50 g/L) to avoid carbon dioxide evolution.

5.3.8 Analytical methods

Succinic acid concentrations from aqueous samples were analyzed on a Waters HPLC system using a Bio-Rad Aminex HPX-87H column (7.8 x 300 mm) at 60 °C. Phosphoric acid (1.5 mmol/L at 0.6 mL/min) was used as an eluent. Quantification was done by UV detection at 210 nm using an external standard. Reactions carried out in acetonitrile were analyzed by gas chromatography on an Agilent 6890N system equipped with a CP-PoraPLOT Q column (25 m length × 0.32 mm internal diameter, 10 μm film, 2.5 m particle trap) and a flame ionization detector. Helium was used as carrier gas. An injection size of 1 μL was used with a split ratio of 20. The injector temperature was 200 °C. The column temperature program was initiated at 60 °C and a 10 °C/min temperature ramp was used up to 150 °C followed by a second ramp of 20 °C/min up to 240 °C. Methanol, DMC and dimethyl succinate were

identified by comparison with known standards. Reactions using DMC as solvent and reagent were analyzed in the same GC system using a HP-Innowax column (30 m length × 0.25 mm internal diameter, 0.25 μm film). Injection and detector conditions were maintained. The column temperature program was initiated at 60 °C and maintained for 0.5 min, then a 10 °C/min temperature ramp was used up to 220 °C with a final holding time of 5 min. Dimethyl succinate was identified by mass spectrometry. In all the cases quantification was done using Anisole as internal standard and methanol concentrations were corrected for methanol initially present in the DMC. Water concentration in alkylation reactions was determined by Karl Fischer titration using a Metrohm 831 KF coulometer. Carbonates were quantified as inorganic carbon by Total Carbon Analysis in a Shimadzu TOC 5050A system.

5.4 Results and discussion

5.4.1 (Bi)carbonate counter-ions provide better equilibrium characteristics for ion exchange recovery of succinate.

The design of the succinate ion exchange recovery operation should take into account, among several factors, the resin counter-ion as its nature affects the selectivity towards the dicarboxylate. Furthermore, resin counter-ion is determined by the chosen regeneration/desorption strategy. In the proposed route, the exchanger is regenerated during the ester formation and is left in a (bi)carbonate form. To assess the effects of (bi)carbonate on succinate capture, static sorption and dynamic column breakthrough experiments were carried out using Dowex Marathon MSA, a strong anion exchange resin, in the expected ionic form. The resin was characterized for total ion exchange capacity and an experimental value of 4.25±0.06 mEq/g dry resin was found. The results derived from the static sorption experiments can be interpreted by means of a constant separation factor isotherm, expressed as:

$$q_{e,i} = \frac{K_i q_{sat,i} C_i}{C_{sat,i} + (K_i - 1) C_i} \quad (1)$$

Where for species *i*, $q_{e,i}$ is the exchange capacity (g_i/g dry resin), C_i the equilibrium aqueous concentration (g_i/L), K_i the separation factor and $q_{sat,i}$ and $C_{sat,i}$ the exchange capacity and aqueous concentration at saturation respectively. The derivation of Eq. 1 is based on the mass action law and can be found elsewhere (Arévalo et al., 2000, Perry and Green, 2008).

As can be seen in figure 5a, the isotherm fits very well the experimental equilibrium data for hydroxide, carbonate and bicarbonate forms of the resin. The shape of the isotherms indicates that succinate sorption is favorable for the tested counter-ions. The parameters for the isotherm were determined and are summarized in table 1. The results show that both exchange saturation capacity and separation factor are functions of the counter-ion basicity, with a direct dependence of the former. The separation factor was the highest for the hydroxide form, followed by its bicarbonate similar, which is beneficial for succinate capture at low aqueous concentrations. Static sorption capacities of 0.22 and 0.17 g succinate/g dry resin were determined for carbonate and bicarbonate forms of the resin. It has been estimated

that a sorption-based downstream process for succinic acid will need a sorption capacity higher than 0.05 g/g to be feasible (Davison et al., 2004). The obtained capacities for all the counterions tested greatly exceed that minimum and are higher than those observed for other materials at neutral pH (Jun et al., 2007, Li et al., 2009).

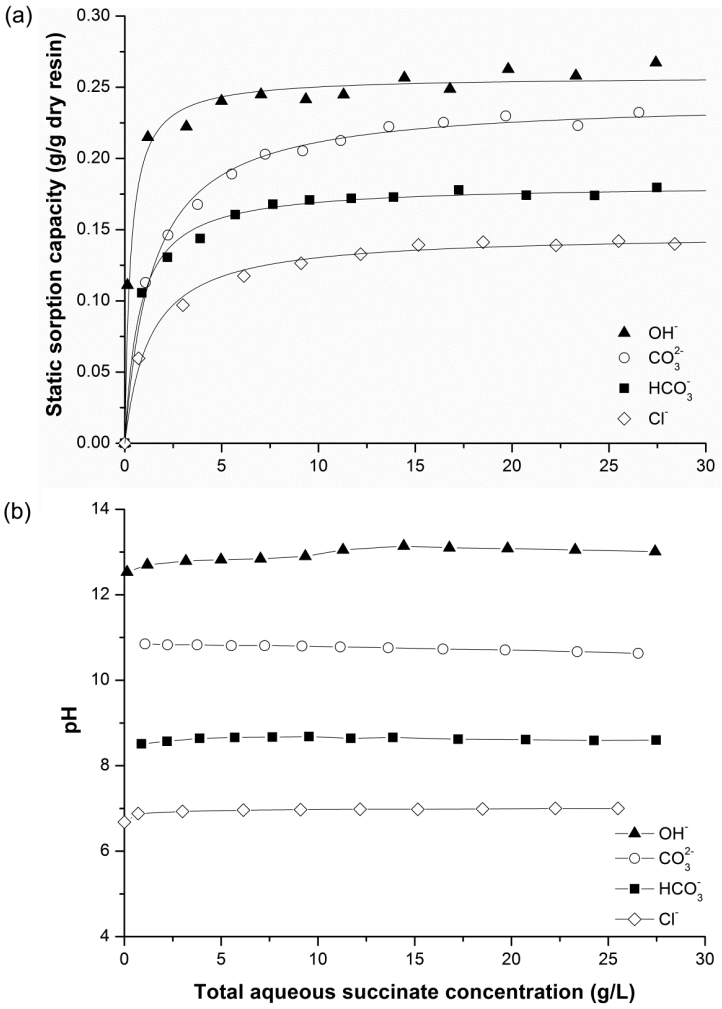

Figure 5. Succinate sorption equilibrium dependence on resin counter-ion at neutral pH. Hydroxide and chloride ions are included for comparison. Chloride data obtained from published data (López-Garzón, Ottens, 2012). (a) Isotherm equilibrium data. Solid lines are model fits of a constant separation factor isotherm regressed by a non-linear method. The calculated correlation coefficient was higher than 0.99 in all the cases. (b) Equilibrium pH for succinate sorption isotherms.

Table 1. Constant separation factor isotherm parameters for succinate sorption using different ionic forms of the resin.

Resin ionic form	K_{succ}	$q_{sat,\ succ}$ (g succinate/g dry resin)	$C_{sat,\ succ}$ (g/L)
OH^-	19.9	0.25	5.58
CO_3^{2-}	11.5	0.22	14.8
HCO_3^-	14.8	0.17	10.3
Cl^-	13.8	0.14	16.4

The equilibrium results also showed that the tested ionic forms raised the aqueous pH up to values in accordance with their basicity (Figure 5b). Although, the sorption equilibrium for

the analyzed counter-ions is less favorable than that encountered for the hydroxide form of the resin, it should be realized that none of the desorption methods currently available will yield a hydroxide form of the resin and a succinate ester instead of a salt. These equilibrium results indicate that an ion exchange recovery operation in which a (bi)carbonate resin is used improves the sorption step as compared with our previous case, in which chloride was used as counter-ion (López-Garzón, Ottens, 2012).

The determination of the dynamic sorption behavior of succinate for the expected ionic forms of the resin is necessary for further process evaluation. Figure 6 shows the succinate breakthrough profile for the resin in bicarbonate form. The succinate breakthrough occurs at approximately 1.6 bed volumes and the column is exhausted after 11 bed volumes. During that period, as ion exchange takes place, bicarbonate is eluted from the column resulting in a pH increase of the outlet stream. The observed elution profile is consistent with the pH equilibrium data validates the first part of our process concept since, if integrated with a fermentation operation as suggested in Figure 3, a supply of inorganic carbon species and neutralizing base would be provided to the fermentation.

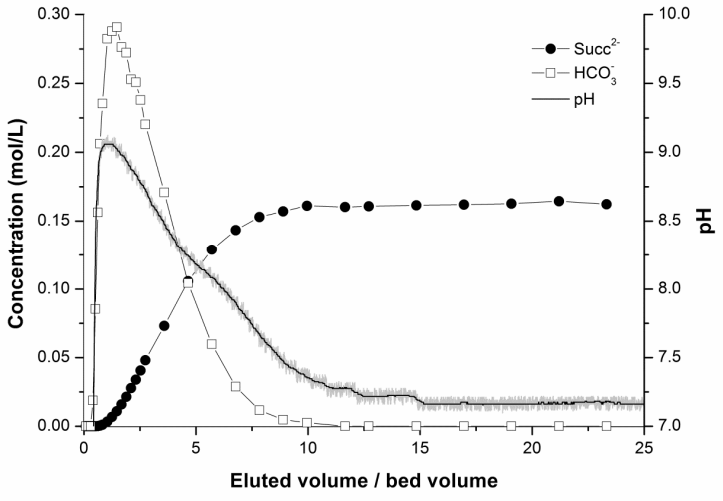

Figure 6. Dynamic succinate sorption as breakthrough profile using Dowex Marathon MSA in bicarbonate form.

Regarding the column performance, an integral analysis of the breakthrough data was done to calculate the column total dynamic capacity (g succinate/g dry resin), $q_{d,succ}$, using the following equation:

$$q_{d,succ} = \left(F \int_0^{t_{sat}} \left(C_{succ,in} - C_{succ,out} \right) dt - C_{succ,in} \times V_{ECB} \times \varepsilon_b \right) \frac{1}{W} \qquad (2)$$

F is the volume flow rate through the column (L/min), $C_{succ,in}$ and $C_{succ,out}$ the concentrations of succinate (g/L) at the column inlet and outlet respectively, V_{ECB} the empty column bed volume (L), ε_b the total bed porosity and W the dry mass of resin in the column (g). A total dynamic capacity (at exhaustion) of 0.26 g succinate/g dry resin was calculated from the integral analysis of the data. Such determined capacity exceeds the expected capacity if sorption by pure ion exchange is considered (estimated for the resin in bicarbonate form as 0.24 g succinate/g dry resin). An explanation for this discrepancy might be related with the different degree of swelling of the resin loaded with succinate, impeding a more accurate

estimation as the total bed porosity would be different. Knowing the amount of succinate effectively bound is important since later it will compromise diester yield calculations, therefore its magnitude was checked. An integral analysis of the eluted inorganic carbon can be used to check the column saturation capacity. The total released inorganic carbon, assumed as bicarbonate, was determined as 9.89 mmol. Given the ion exchange stoichiometry, the amount of sorbed succinate can be calculated as 4.94 mmol resulting in a dynamic sorption capacity of 0.24 g succinate/g dry resin, confirming succinate sorption solely by ion exchange.

In the case of the resin in carbonate form, a similar breakthrough behavior was observed (Figure 7). As in the bicarbonate case, discrepancies in the calculated capacity were found, nevertheless a calculated dynamic column capacity of 0.26 g succinate/g dry resin was obtained.

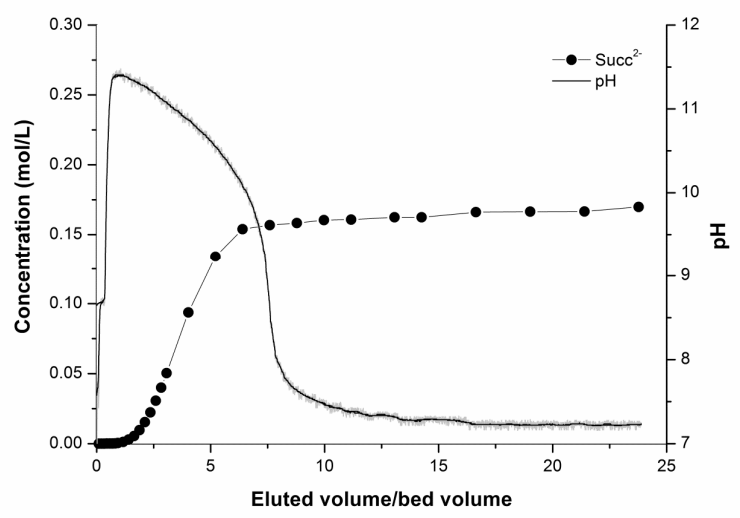

Figure 7. Dynamic succinate sorption as breakthrough profile using Dowex Marathon MSA in carbonate form.

5.4.2 Dimethyl succinate production via direct downstream catalysis is feasible using dimethyl carbonate

To demonstrate the second stage of the novel process, the reaction characteristics of the alkylation of sorbed succinate using DMC should be addressed. After resin drying, *O*-alkylation experiments were carried out at different ratios of DMC to sorbed succinate. Acetonitrile was selected as a solvent since its polar and aprotic characteristics are beneficial for nucleophilic substitution reactions, the expected mechanism when DMC acts as a methylating agent (Tundo and Selva, 2002). Table 2 shows that reaction has indeed occurred and dimethyl succinate has been produced for all the tested DMC to succinate ratios, proving the effectiveness of the quaternary ammonium functional group as a catalyst. The result is promising since typically DMC is active as methylation agent at high temperatures, as discussed in section 1.

At the tested experimental conditions, methanol was observed as main by-product and no additional succinate-derived products were detected. Methanol can be produced from methoxycarbonate anion hydrolysis or from DMC hydrolysis (Figure 2 c,d). As water was present in all the experiments at initial levels of about 0.1%, both reactions could occur. Low DMC conversions and moderate dimethyl succinate yields are likely the result of a diffusion-

limited system, as agitation was absent. Nevertheless, the results strongly indicate that using DMC as solvent and alkylating agent will lead to higher yields and selectivities, as presented subsequently.

Table 2. Dimethyl succinate (DMS) production by *O*-alkylation as a function of the DMC concentration. Non-stirred reactions at 100 °C.

Reactants ratio (mol DMC/mol succ)	**DMC conversion** (%)	**DMS yield on initial succinate** (mol DMS/mol succ) (%)	**Selectivity**[a] (mol DMS/mol MeOH) (%)
2	5.09	0.70	1.19
4	4.72	0.74	4.03
10	4.26	6.23	7.24
20	4.58	17.0	13.8
30	2.75	19.9	14.9

[a] DMS selectivity based on DMC. Calculation based on the total methanol produced.

5.4.3 Faster O-alkylation kinetics and high dimethyl succinate yields are obtained using DMC as a solvent and reagent

Kinetics of dimethyl succinate formation via *O*-alkylation from sorbed succinate were determined at three different temperatures (80, 100 and 120 °C) using DMC as both reaction solvent and reagent. Figure 8 shows a yield of 0.92 mol DMS/mol succinate after five hours at 120 °C. Slower reactions rates were obtained at lower temperatures. Interestingly, dimethyl succinate was formed even at 80 °C, below the boiling point of DMC, with a yield of 0.2 mol DMS/mol succinate after eight hours of reaction. No monoester was detected at any condition, indicating a two-step reaction mechanism in which only the diester is released from the resin after methylation. The monoester is formed as an anionic form and will remain bound, facilitating conversion to diester. These results prove our catalytic system to be effective, even at relatively low temperatures for this type of reaction. In similar alkylation studies, good reaction rates were achieved at low reaction temperatures only using superbases such as 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as a catalyst at 90 °C (Shieh, Dell, 2002).

Although the resin had been dried thoroughly, some intraparticle water remained and was released during the reaction (Figure 9). A water concentration of about 0.06 mmol/g reaction liquid was found after 2 min of reaction at 120 °C and slower release rates were observed at lower temperatures. Throughout the reaction at the tested temperatures, water was consumed and methanol produced in amounts higher than expected only from methoxycarbonate hydrolysis reaction (Figure 2c), suggesting the occurrence of DMC hydrolysis (Figure 2d).

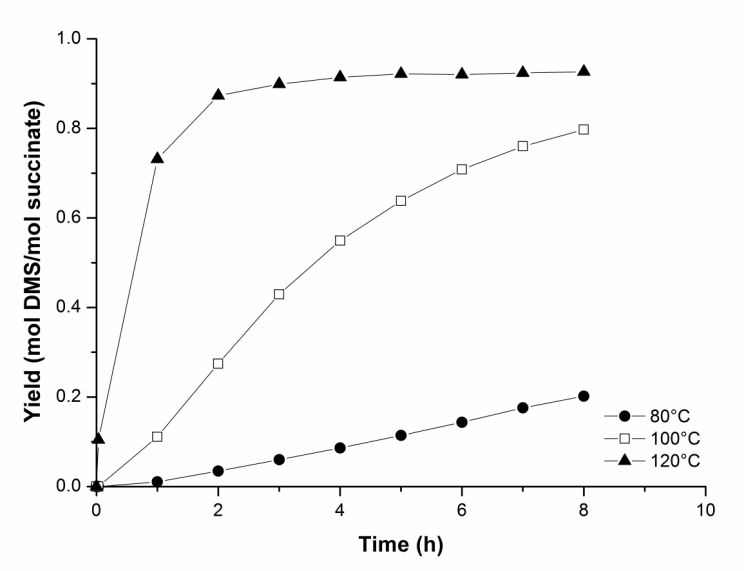

Figure 8. Effect of reaction temperature on the reaction kinetics of dimethyl succinate formation. An anion exchange resin (1 g) loaded with succinate (0.24 g; 2.1 mmol) was used in DMC (30 g).

At this stage, it is desirable to gain a better insight about the kinetic behavior of the proposed reactive system by assuming a kinetic model. In such model, an overall dimethyl succinate reaction (Figure 2, a,b,c) is considered. In the performed experiments, DMC was used in high excess with respect to the sorbed succinate. Thus, it can be considered that its change in concentration during the course of reaction was negligible. Assuming the overall reaction as a pseudo-first order reaction, the rate of sorbed succinate conversion is:

$$r = \frac{-\mathrm{d}W_{Q_2Succ}}{\mathrm{d}t} = kW_{Q_2Succ} \tag{3}$$

W_{Q_2Succ} is the mole of sorbed succinate referred to the mass of resin free of counter-ion (mole succinate/g resin free counter-ion). The apparent kinetic constant *k* reflects the reactivity of the starting materials and steric accessibility of reactants. It should be stated that the process occurring in the solid-phase reaction is more complicated and involves solid-phase sorption and desorption phenomena, reaction, diffusion and interaction processes. The development of a model accounting for all those processes is not the intention of this part of the work.

Integration of the rate expression (Eq. 3) gives:

$$\ln W_{Q_2Succ,0} - \ln W_{Q_2Succ,t} = kt \tag{4}$$

In addition, the produced amount of dimethyl succinate can be expressed per mass of resin, therefore:

$$W_{Q_2Succ,0} - W_{Q_2Succ,t} = W_{DMS,t} \tag{5}$$

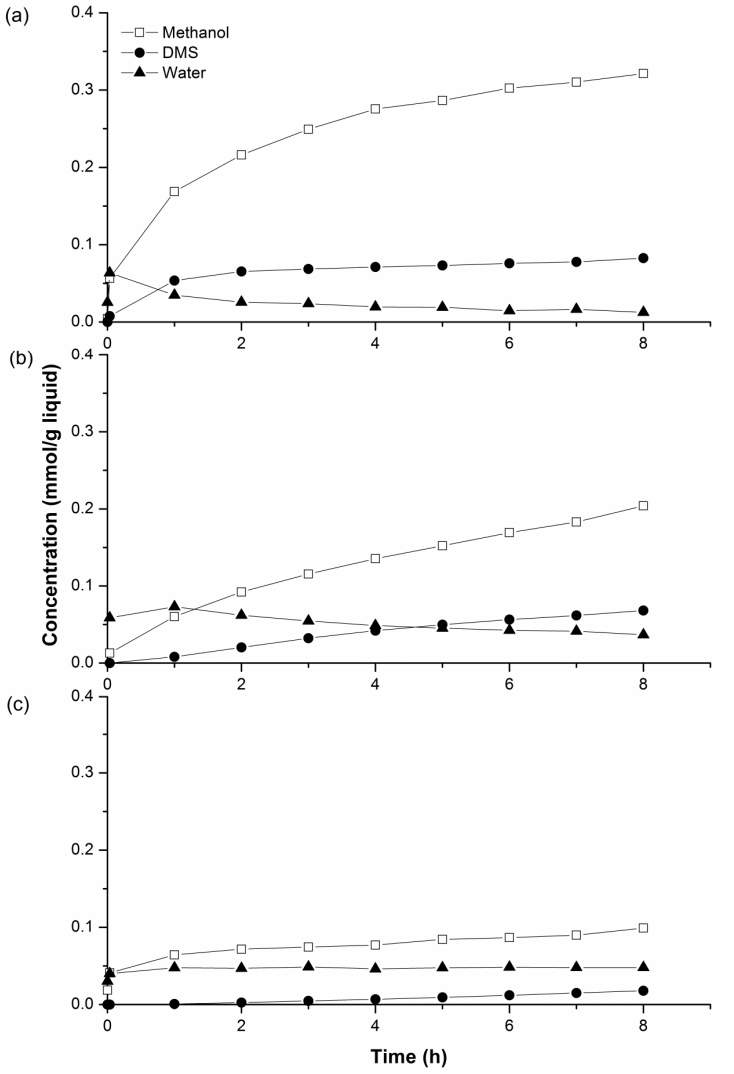

Figure 9. Reaction species kinetic profiles for dimethyl succinate formation at different temperatures. (a) 120 °C (b) 100 °C (c) 80 °C

Leading to:

$$\frac{W_{DMS,t}}{W_{Q_2Succ,0}} = Y_{DMS/Succ} = 1 - e^{-kt} \quad (6)$$

Where $Y_{DMS/Succ}$ is the DMS reaction yield. The apparent rate constant k can be obtained by a linear fit of Equation 4 through the origin. A good fit between the model and the experimental data was obtained (Figure 10) and the apparent rate constants were calculated for 80, 100 and 120 °C as 3.83×10^{-6}, 5.56×10^{-5} and 3.02×10^{-4} s^{-1} respectively.

Since the apparent reaction rate constants were determined at different temperatures, the apparent activation energy for the solid-phase reaction can be calculated. In an Arrhenius plot (Figure 11) a good linearity was found between ln k and $1/T$ over the temperature range studied. The apparent activation energy obtained was 130 kJ/mol with a pre-exponential factor of 2.2×10^{13} s^{-1}. Such a relatively large apparent activation energy found denotes that the rate is limited by the chemical reaction itself, and not by other factors like diffusion. There is not much information in the literature regarding the synthesis of dimethyl succinate since all

the efforts have been focused on the production of diethyl succinate from succinic acid and ethanol via esterification. One of the most active catalysts for such system is Starbon®, which exhibited an activity of 3.2×10^{-4} s^{-1} at 80 °C (Budarin et al., 2007), comparable to the rate found in our system at 120 °C. In cases where DMC has been used for esterification reactions of aromatic carboxylic acids using zeolites as a catalyst, much slower reaction rates at higher temperatures were obtained (Kirumakki et al., 2003). For salicylic acid, rate constants between 0.23 and 7×10^{-6} s^{-1} at 120 °C were reported (Kirumakki et al., 2002), much lower than obtained in the present study.

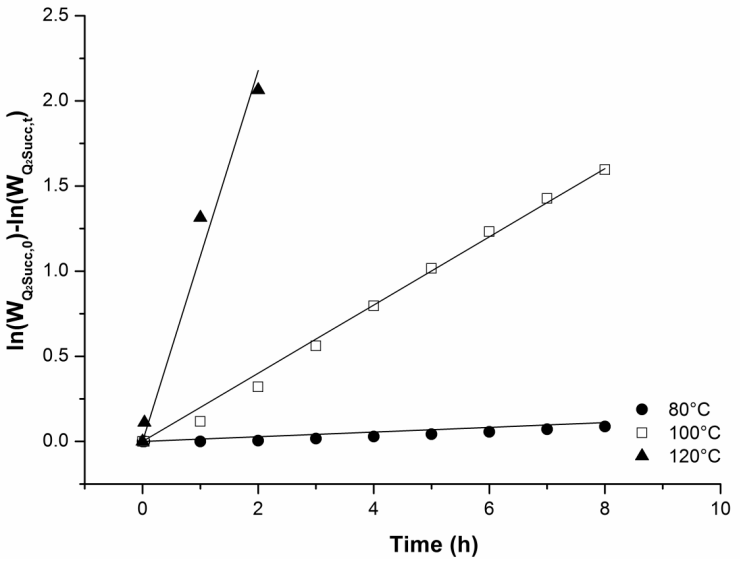

Figure 10. Determination of the apparent kinetic constants for dimethyl succinate production by direct downstream catalysis at different temperatures. R^2 was higher than 0.99 for all the fittings.

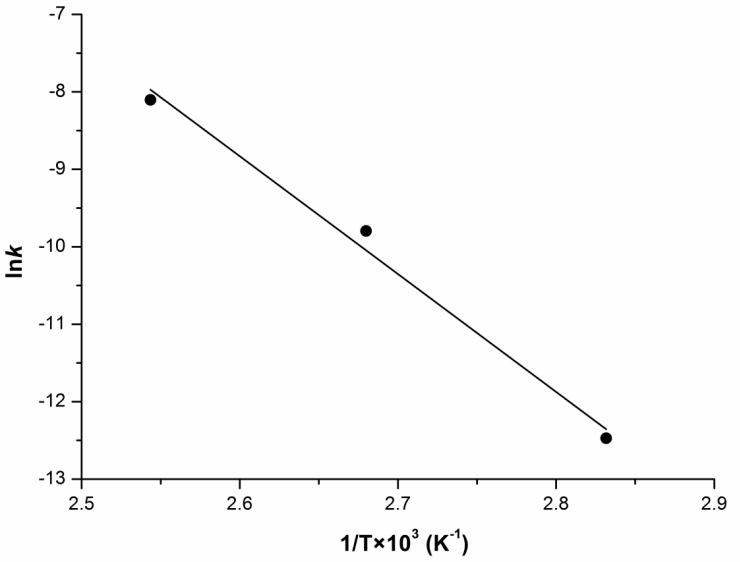

Figure 11. Arrhenius plot for determining the apparent activation energy of the system. Linear fitting with R^2=0.991.

5.4.4 Proposed integrated process is feasible as resin is regenerated to its bicarbonate form through diester formation

From the reaction carried out at 120 °C, samples of resin after reaction were used for determination of total ion exchange capacity and desorbed to quantify the total carbon bound

to the resin in order to elucidate the nature of the resin counter-ion. While executing the total ion exchange determination, a strong evolution of gas was noticed during the resin elution with hydrochloric acid, which might be an indication of the presence of carbonate species. The determined total ion exchange capacity was 4.02±0.07 mEq/g dry resin. Although the new exchange capacity was on average 5% lower than for fresh resin, it is clear that the resin has been regenerated through the alkylation reaction and can be used in a new sorption cycle. For total carbon analysis, the post-reaction resin was eluted using aqueous sodium nitrate (a neutral salt) producing a basic effluent (pH = 8.5). Considering the nature of the inorganic carbon species to be bicarbonate, 3.29±0.30 mmol/ g dry resin was desorbed. This analysis is inconclusive since it does not fit a 1:1 exchange stoichiometry expected for bicarbonate. Although no gas evolution was noticed during the desorption, losses in the form of carbon dioxide could have affected the determination. Despite such difficulties, the reported evidence strongly suggests that in the presence of water, the anion exchange resin is regenerated to the bicarbonate form during the methylation of succinate by DMC.

Our previous studies on direct downstream catalysis have shown that, once the resin has been regenerated, it can be reused without major activity loses (López-Garzón, Ottens, 2012). In the new proposed reaction mode, higher temperatures are needed for achieving comparable ester formation kinetics (100 °C against 80 °C) which might lead to resin defunctionalization in long term operation. Thermal stability of strong anion exchangers with quaternary ammonium groups is highly dependent on the counterion type, nonetheless it has been found that bicarbonate and carbonate forms of these kind of resins are far more stable than the hydroxide form (Baumann, 1960), improving the application perspective of the proposed alternative. As the main aim of this study was to demonstrate the feasibility of the process integration, a complete thermal stability study meant to assess the influence of such variable on the economic performance of the process was outside the scope of this study. Although the selected resin proved to be active up to 120 °C, we anticipate that resins functionalized with guanidinium moieties could also be used as such groups have shown superior thermal stability in other catalytic applications(Senet, 2000, Xie et al., 2005, Zhang et al., 2010).

5.4.5 Low water concentrations improve reaction performance

Avoiding undesired methanol generation requires a reaction medium without the presence of water. An attempt to approach such a condition was carried out by *ex situ* water removal using molecular sieves prior to reaction. Figure 12 shows the yield and reaction species profiles for this experiment at 100 °C. The alkylation rate was increased by about 60%, and 96% yield of DMS was reached after 6 h. Although the initial water level was diminished, the amount of water was almost stoichiometric to succinate and still promoted the production of methanol. However, under these conditions methanol production was slightly less than expected assuming only methoxycarbonate hydrolysis (Figure 2c). These findings do not indicate which of the considered alkylation reactions (Figure 2a,b) is occurring neither to what extent. Water is present in the ion exchanger likely as solvation water bound to the functional groups and its complete removal would be difficult to achieve. If kept at its minimum, the production of methanol would not compromise the process feasibility, as methanol is easily separated and recycled to the DMC synthesis.

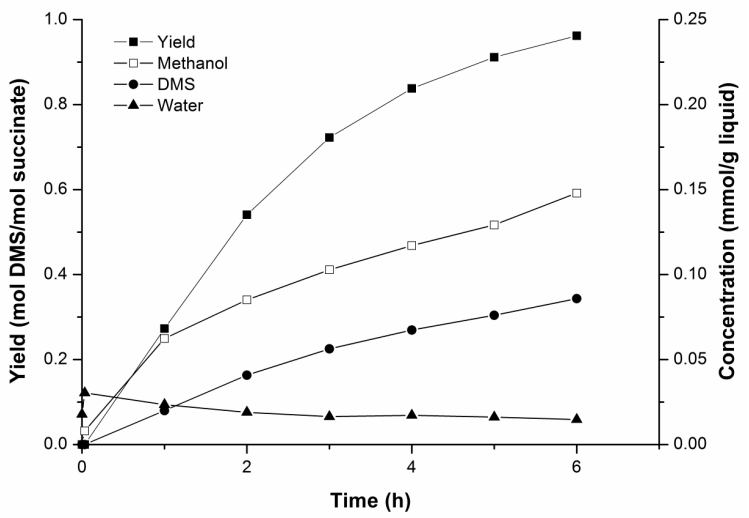

Figure 12. Dimethyl succinate formation kinetics (yield and reaction species concentration) at low initial water concentration at 100 °C.

5.5 Conclusions

We have developed a new route for the production of dimethyl succinate via direct downstream catalysis using dimethyl carbonate as alkylating agent. The proposed process integration leads to a bio-based succinate production scenario avoiding the stoichiometric production of waste salts. A commercial strong anion exchange resin was used as a succinate sorbent and catalyst in the alkylation reaction. A reaction yield of 0.96 mol dimethyl succinate/mol succinate was achieved under mild conditions. The carbon economy of the overall process is maximized as the carbon dioxide produced during the alkylation reaction is retained by the anion exchanger and redirected upstream to the succinate fermentation. Further work is being carried out in our laboratory to assess the impact of fermentation impurities on the process performance. The extension of our approach to other bio-based chemical building blocks such as 2,5-furandicarboxylic acid is currently being pursued.

5.6 Acknowledgments

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6

Ester production from bio-based dicarboxylates *via* direct downstream catalysis: succinate and 2,5-furandicarboxylate dimethyl esters

Two culture broths, containing succinate produced *de novo* by *Corynebacter glutamicum* and 2,5-furandicarboxylate by whole cell biotransformation of 5-(hydroxymethyl)furfural (HMF) by a recombinant *Pseudomonas putida,* were used for dimethyl ester production. For anion exchange, they were characterized for competing organic anions (i.e., other carboxylates) and inorganic anions (phosphate, sulfate and chloride). These affect capturing of the target dicarboxylate via sorption. For the analysis of the sorption process, independent multicomponent column experiments using mimicked mixtures of the respective target building block with organic anions, inorganic anions and actual fermentation broth were performed. In the case of succinate, breakthrough profiles and column capacities showed that α-ketoglutarate, malate and other fermentation impurities reduced sorption capacity. For 2,5-furandicarboxylate the effect of impurities in sorption was less pronounced, with residual HMF eluting without any apparent ionic interaction. After sorption, upgrading via alkylation from mimicked and bio-based broth was successfully carried out producing the respective succinate and 2,5-furandicarboxylate dimethyl esters. Yield towards dimethyl succinate was reduced from 0.98 to 0.66 mol ester/mol carboxylate due to the presence of fermentation impurities, which were also esterified in good yields. Dimethyl 2,5-furandicarboxylate final yield ranged between 0.75-0.77 mol ester/mol carboxylate for both pure and raw bio-based sorbed furandicarboxylate. Esterification kinetics correlate well with the acidity of the carboxylates and impurities.

Keywords: Succinic acid, 2,5-furandicarboxylic acid, dimethyl carbonate, bio-based building blocks

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6.1 Introduction

Although many carboxylates can be produced conveniently by fermentation, their recovery is not straightforward (López-Garzón and Straathof, 2014). A new option has recently been proposed (López-Garzón et al., 2014), wherein upon sorption of aqueous carboxylates from fermentation broth using quaternary anion-exchange resins, the sorbed carboxylate may be converted in dimethyl carbonate as solvent and reactant. This may lead to methyl carboxylate and regenerated resin in one step. This integrated direct downstream catalysis has proven to be successful when using synthetic sodium succinate solutions. However, it is not clear if it can be applied with real fermentation broth, containing many impurities, and with other carboxylates.

In this study, the proposed integrated method will be studied using succinate from *C. glutamicum* broth (Litsanov et al., 2012) and 2,5-furandicarboxylic acid (FDCA) from *P. putida* broth (Koopman et al., 2010). The resulting dimethyl esters are potentially interesting as building blocks (Jong et al., 2012, Orjuela et al., 2011). In particular, dimethyl 2,5-furandicaboxylate is a more attractive monomer for the production of polyethylene 2,5-furandicarboxylate (PEF), a drop-in replacement of polyethylene terephthalate (PET), than its diacid precursor (Jong, Dam, 2012).

6.2 Materials and methods

6.2.1 Materials

All chemicals used were analytical grade. A strong anion exchange resin, Dowex Marathon MSA (macroporous), was obtained in the chloride form and converted to the bicarbonate form prior to utilization. Samples of bio-based succinate and 2,5-

furandicarboxylate in crude fermentation broth were obtained from Research Centre Jülich (Litsanov, Brocker, 2012) and Bird Engineering (Koopman, Wierckx, 2010) (now Corbion), respectively. The used succinate broth was produced by a batch fermentation using *C. glutamicum* BOL-1/pAN6-pycP458S and a similar fermentation protocol and cultivation media as Litsanov et al (Litsanov, Brocker, 2012). Briefly, both cell cultivation and anaerobic succinate production were combined in a single fermentation via a controlled dissolved oxygen (D.O.) ramp. Cells were grown aerobically during 10 h until 30% D.O. was reached, followed by a linear ramp to anaerobic conditions (0% D.O.) for 6 h. Total cultivation time was 62 h and final succinate titer was 26 g/L. The harvested broth was centrifuged, filtered, frozen and shipped and subsequently thawed just before its use in these studies. Moreover, a mimicked mixture using the major carboxylate by-products found in a high producing succinate fed-batch fermentation using the strain BOL-3/pAN6-gap was prepared based on the composition reported by Litsanov et al (2012). In the case of FDCA, the raw mixture was prepared by dissolving a partially purified FDCA sample with a declared purity of 67%, containing HMF and HMF acid as impurities. Pure and partially purified FDCA were recovered and purified based on existing methods (Koopman, Wierckx, 2010).

For comparison purposes, a dimethyl 2,5-furandicarboxylate (dmFDCA) standard was synthetized via Fischer esterification adapted from an existing protocol (Gubbels et al., 2013). Pure FDCA from Bird Engineering (3 g, 19.2 mmol) was reacted with excess of methanol (32 g, 1 mol) using hydrochloric acid (1.5 mL) as a catalyst. The reaction was carried out under reflux for 18 h. After reaction, the catalyst was neutralized by addition of 50 mL of 0.5 mol/L methanolic KOH solution. Methanol was evaporated and the solid product dissolved in chloroform. The solution was filtered and washed with deionized water. Subsequently traces of water were removed using brine and magnesium sulfate. The filtered solution was evaporated and the solids recrystallized from acetone obtaining white crystals. Yield: 74%. Purity was >99% according to absence of contaminant peaks in HPLC and NMR. ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): 3.93 (s, 6H), 7.22 (d, 2H). ^{13}C-NMR (400 MHz, $CDCl_3$, δ, ppm): 52.36 (OCH_3), 118.48 (furan ring C3 and C4), 146.62 (furan ring C2 and C5), 158.31 (C=O).

6.2.2 Fermentation broth characterization

Samples of succinate and FDCA fermentation broths were characterized in physical appearance and composition of relevant impurities. Succinate broth was obtained free of suspended solids whereas raw FDCA broth contained cells and other solids which were removed by centrifugation (5000 rpm, 20 min) and a sequence of filtration steps. Using water, broth was diluted according to the column size, and thus its theoretical capacity, to be able to analyze the sorption profile. No further additional pretreatment was done prior to sorption experiments. Further broth characterization comprised the determination of organic anionic by-products and inorganic anions from nutrient salts.

6.2.3 Dicarboxylate recovery by column sorption

Dynamic sorption experiments were carried out in a similar way as in previous studies (López-Garzón et al., 2012). Briefly, a Bio-Rad column (1 cm internal diameter x 27 cm height)

was packed with anion exchange resin, resulting in a 21 mL bed volume corresponding to 16 g wet resin (5.7 g dry resin). Carboxylate solutions were pumped at a 2 mL/min flow at 25 °C and 1 mL fractions were collected for analysis. Furandicarboxylate sorption was done in a shorter column (1 cm internal diameter x 6.2 cm height) with a 5 mL column bed corresponding to a 3.8 g wet resin (1.4 g dry resin).

6.2.4 Ester formation by O-alkylation experiments

Main ester formation experiments were performed as reported previously (López-Garzón, van der Wielen, 2014). Typically, in a stirred autoclave reactor, 30 g of dimethyl carbonate were added and 1 g of dry loaded resin was held in the solid addition device until reaction temperature (100 °C) was reached. After releasing of the resin into the vessel, samples were taken periodically for ester quantification. Ester formation experiments for individual impurities were performed in agitated glass closed tubes heated using an oil bath. A given amount of resin in the bicarbonate form was loaded batch-wise with carboxylate, then washed and dried, finally placed in the agitated tube and reacted with dimethyl carbonate.

6.2.5 Analytical methods

Organic acids present in succinate broth, mimicked mixture and column sorption eluent were determined by an established ion exchange HPLC method (López-Garzón, Ottens, 2012). Concentrations of relevant organic impurities present in FDCA broth and column sorption eluent were determined using a RP-HPLC method based on the method developed by Koopman et al.(2010) Inorganic anions such as phosphate, sulfate and chloride were measured spectrophotometrically using respective commercial cuvette tests from Hach-Lange.

Methyl esters of succinate and other carboxylates from succinate fermentation broth and mimicked mixture were determined by gas chromatography as reported before(López-Garzón, van der Wielen, 2014) using anisole as internal standard and commercial methyl ester standards from Sigma. Dimethyl FDCA was determined using the same RP-HPLC method as for the acid, properly adjusting the running time and using the synthesized diester, as described above, as the quantification standard. The identity of dimethyl FDCA produced by alkylation was confirmed by ^{1}H-NMR and ^{13}C-NMR as previously described (Gubbels, Jasinska-Walc, 2013, Thiyagarajan et al., 2013).

6.3 Results and discussion

6.3.1 Fermentation broth characterization and definition of mimicked mixtures

Succinate fermentation broth (Litsanov, Brocker, 2012) had a translucent purple to brown appearance without the presence of any solids. The color is attributed to the presence of protocatechuic acid (3,4-dihydroxybenzoic acid), used as a micronutrient for *C. glutamicum*.

The broth containing 2,5-furandicarboxylate had a very dark brown color and contained solids in suspension, which were effectively removed by centrifugation and filtration.

Succinate and FDCA raw broths may contain other carboxylates that may decrease the sorption capacity towards the target dicarboxylate, which indicates the importance of their identification and quantification prior to recovery using sorption. As mentioned in the previous section, the reported final composition of a fed-batch cultivation for a similar succinate fermentation (Litsanov, Brocker, 2012) was chosen to compose the mimicked mixture used in the current study. Table 1 summarizes the composition of the three cases.

Table 1. Composition of a representative succinate fermentation final broth and the mimicked mixture used in sorption experiments. All broths were adjusted to neutral pH.

	Reported composition (Litsanov, Brocker, 2012)		**Mimicked mixture**	**Diluted fermentation broth**
Component	mM	g/L	g/L	g/L
Succinic	1135	134.0	10.0	10.0
Pyruvic	6	0.53	0.04	0.12
Acetic	20	1.20	0.09	0.10
α-Ketoglutaric	35	5.11	0.38	0.05
Malic	33	4.42	0.33	---
Fumaric	13	1.51	0.11	0.02

In the case of 2,5-furandicarboxylate, the final broth contained several HMF-related compounds co-produced during the biotransformation (Table 2). The partially purified sample, used in this case as the equivalent mimicked mixture, and broth were diluted as done in the succinate case. Given the very low concentrations of residual furanic compounds in the partially purified compounds, all of them were below quantification limits after dilution and thus reported as not detected.

Inorganic anions, present from unconsumed salts in the medium, will influence the carboxylate sorption equilibrium. For succinate production, most of the salts used in the medium were sulfate salts, especially ammonium sulfate as nitrogen source. Phosphates and chlorides were expected in lower amounts. Whole cell bioconversion of HMF to FDCA was done using culture conditions based on a described protocol (Koopman, Wierckx, 2010) in which a phosphate buffer system was used and ammonium sulfate as nitrogen source. Other micronutrient salts contribute with additional sulfate and chloride anions. The concentration of such inorganic anions in the raw broths was determined for each case and presented in Table 3. The levels encountered for both cases are in line with the values expected on basis of the initial medium composition.

Table 2. Composition of a representative raw and diluted FDCA fermentation broth

	Reported composition[a]		Mimicked mixture[b]	Diluted biotransformation broth
Component	mM	g/L	g/L	g/L
FDCA	472.42	73.74	9.3	10.0
HMF acid[c]	142.10	4.53	n.d.	0.60
FFA[d]	140.09	2.92	n.d.	n.d.
HMF	12.11	8.34	n.d.	1.10

n.d.: Not detected. Limit of detection < 0.5 g/L
[a] Data provided by Bird Engineering
[b] Prepared using a partially purified FDCA sample of 67%
[c] 5-Hydroxymethyl-2-furancarboxylic acid
[d] 5-Formyl furoic acid

Table 3. Main inorganic anions present in succinate and FDCA final broths

Fermentation broth	PO_4^{3-}	SO_4^{2-}	Cl^-
	g/L	g/L	g/L
Succinate	0.88±0.02	11.53±0.07	0.09±0.01
FDCA	2.09±0.02	5.30±0.30	0.22±0.01

6.3.2 Upgrading of bio-based succinate via direct downstream catalysis

Part I: Dynamic sorption

Sorption studies were done using the mimicked mixture and diluted fermentation broth. Although the succinate titers are low, the molar ratio between the different components was kept, resulting in an experiment that is intended to reflect the real concentrated case in terms of component separation.

Figures 1 and 2 show the elution profile of the carboxylates as breakthrough curves for both cases. Normalized outlet concentrations allow a better analysis of the system behavior. As expected, monocarboxylic acids (acetate and pyruvate) are less retained than dicarboxylic acids. As ion exchange is the main interaction mechanism present, selectivity rules according to anion valence apply. In the mimicked case, Figure 1, succinate breakthrough occurred at approximate 76 mL and showed a narrow mass transfer zone saturating the column until 208 mL. Later in the run, α-ketoglutarate and fumarate eluted slowly and did not reach the feed concentration by the end of the experiment after 640 mL (30 bed volumes, BV, 5.3h). The

elution profiles of the dicarboxylates are consistent with trends observed in several studies(López-Garzón and Straathof, 2014) where the selectivity order was established as fumarate > succinate ≥ malate for strong anion exchangers.

(a)

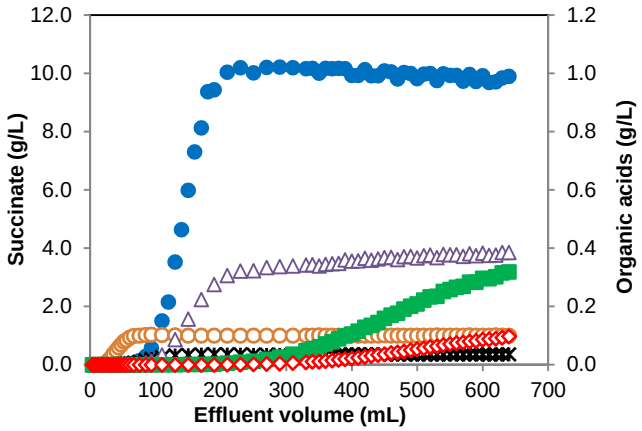
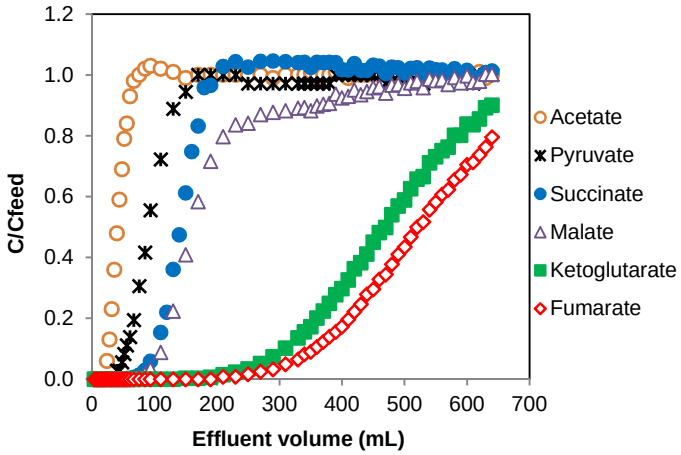


Figure 1. Multicomponent column sorption experiments for succinate using mimicked mixture (a). Normalized profiles for each component are presented in (b). The elution profiles were constructed by overlaying two sets of data from independent experiments.

(b)

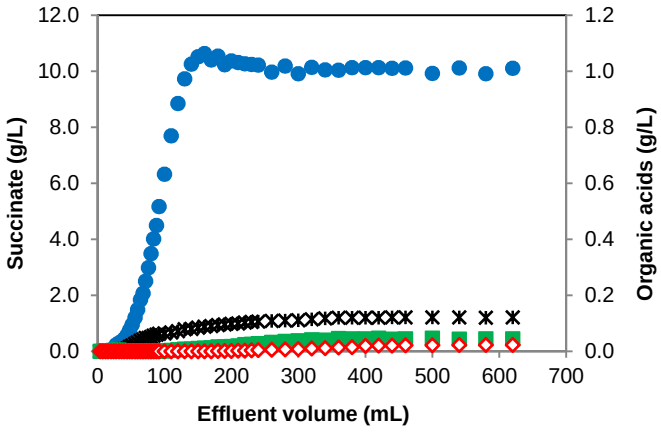


Acetate, pyruvate and succinate showed chromatographic peaking resulting in elution concentrations higher than feed concentrations. The reason of this can be attributed to the high affinity towards α-ketoglutarate and fumarate, which compete for exchange sites already occupied resulting in desorption of those species. In a longer run it is expected than the inlet and outlet concentrations will be equal, meaning that the column is exhausted and in equilibrium with the feed solution.

Similar behavior was observed in the dynamic sorption runs with fermentation broth presented in Figure 2. An earlier succinate breakthrough at 25 mL was observed reaching succinate saturation after 132 mL. All other species started to elute faster when compared to the mimicked mixture. A cause for this could be the presence of inorganic anions, competing for exchange sites. Chromatographic peaking was less pronounced in this case though, resulting in less succinate desorption.

As all the carboxylates adsorb to a certain extent, the column capacity towards succinate was reduced. Sorption capacities for each component were evaluated by integral analysis at succinate saturation and at the end of the run. The results of these calculations are presented in table 4.

(a)



(b)

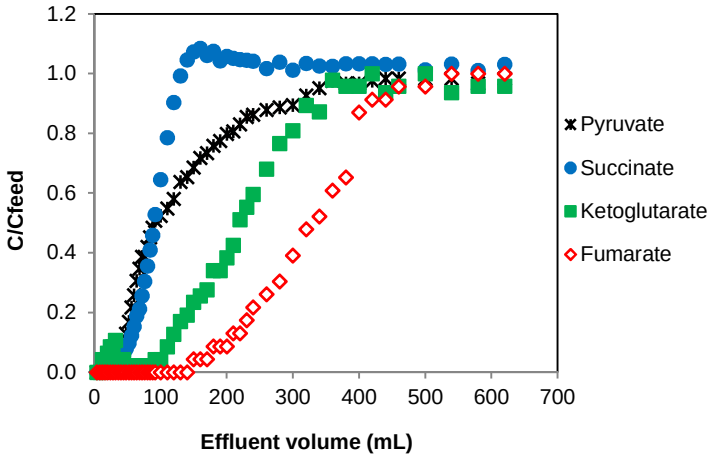


Figure 2. Multicomponent column sorption experiments for bio-based succinate using diluted fermentation broth (a). Normalized profiles for each component are presented in (b). The elution profiles were constructed by overlaying two sets of data from independent experiments. Profiles for malate and acetate present in fermentation broth were not determined as they were not properly resolved by HPLC.

Table 4. Sorption capacities for organic anions at succinate saturation and at the end of the column run.

	Sorption capacity (g carboxylate/g dry resin)			
	Mimicked mixture		Fermentation broth	
Component	Succ. Sat.	End	Succ. Sat.	End
Succinate	0.210	0.184	0.120	0.108
α-Ketoglutarate	0.013	0.031	0.009	0.002
Malate	0.009	0.012	---	---
Fumarate	0.008	0.011	5.0×10^{-4}	0.001
Pyruvate	5.0×10^{-4}	5.0×10^{-4}	0.0016	0.003
Acetate	4.0×10^{-4}	4.0×10^{-4}	---	---

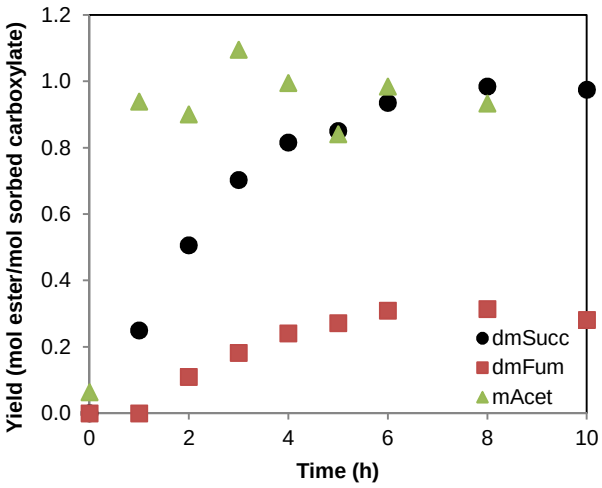
In both studied cases, a reduction in succinate capacity compared with the single component case (0.24 g succinate/g dry resin) was observed. For the fermentation broth, the decrease is as much as 50%. During such run, darkening of the resin was noticed as an indication that other species might be interfering. Although it is not clear whether such colored compounds are bound by ion exchange, as they were only desorbed by acid treatment (not by salt displacement), they are likely to influence the sorption by fouling the resin impeding accessibility to functional sites.

Part II: Ester formation by O-alkylation

After loading using either mimicked mixture or fermentation broth, the resin was used in alkylation experiments with dimethyl carbonate as solvent and alkylating agent. The reaction mechanism is expected to follow the reaction stoichiometry proposed previously,(López-Garzón, van der Wielen, 2014) where sorbed carboxylates are methylated in the presence of water yielding a methyl or dimethyl ester in the case of mono and dicarboxylates, respectively. An additional feature of the reaction is the regeneration of the anion exchange resin to the bicarbonate form and methanol formation. Figure 3 shows the obtained yield profiles for both cases. In a first instance (Fig. 3a), dimethyl succinate, dimethyl fumarate and methyl acetate were produced in good yields. Dimethyl succinate formation kinetics resembles the case in which succinate is present as the only counter-ion in the resin, seen in previous studies.(López-Garzón, van der Wielen, 2014) In the case of the sorbed succinate from fermentation broth (Fig. 3b), only partial conversion towards dimethyl succinate was found. The reason for this incomplete conversion was not clear, and might be related to other species being sorbed, and their influence on the catalytic activity of quaternary ammonium site. Therefore, the conversion of other carboxylates was determined and interestingly, a high yield of methyl acetate was determined and traces or dimethyl fumarate were observed.

In none of the experiments, dimethyl α-ketoglutarate, dimethyl malate and methyl pyruvate were detected as products. To elaborate a feasible hypothesis about this observation, experiments where the resin was loaded solely with the carboxylate impurities were carried out. Table 5 shows that, as expected, esters of succinate (control experiment), acetate and fumarate were produced in good yield. Esters of the ketoglutarate and pyruvate were not detected and led to a dark coloration of the resin after reaction, indicating a possible decomposition of those carboxylates at the tested reaction conditions. Interestingly, *malate* was mainly converted to *dimethyl fumarate* rather than the expected dimethyl malate. Malic acid is an alpha-hydroxy acid that can undergo dehydration at the alpha carbon yielding fumaric acid. Although it is known that esters are better substrates for such reaction, it occurs in the presence of an acid as catalyst. The mechanism of reaction in our particular case is not understood. It was noted that such reaction might be of industrial interest if the same mechanism would prevail in the case of lactate, for which methyl acrylate would be obtained. However, preliminary experiments pointed at formation of oligomers of unclear composition rather than at methyl acrylate.

(a)



(b)

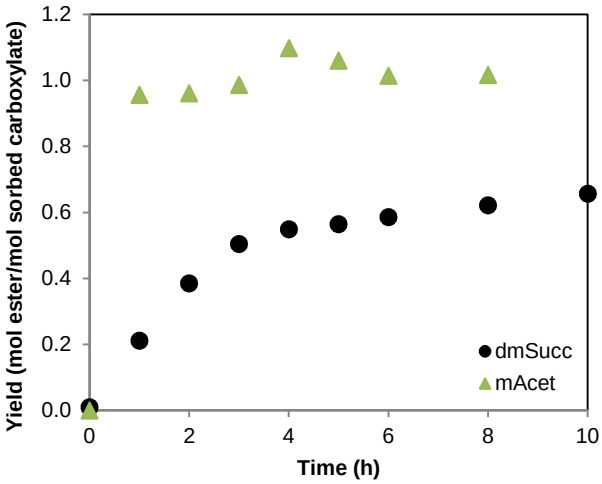


Figure 3. Formation of esters (dmSucc, dimethyl succinate; dmFum, dimethyl fumarate; mAcet, methyl acetate) from sorbed carboxylate species using final loaded resin from mimicked mixture (a) and fermentation broth (b).

Table 5. Resin loading and ester yield for carboxylate impurities tested individually. Reaction conditions 100 °C, 500 rpm, 10 h

Component	**Resin loading**	**Ester yield**
	g/g dry resin	mol ester/mol sorbed carboxylate
Succinate	0.16	1.03
Acetate	0.11	1.00
Fumarate	0.19	0.44
Malate	*0.18*	*0.10 (0.33[a])*
Pyruvate	0.17	0.00
α-Ketoglutarate	0.23	0.00

[a]Yield for dimethyl fumarate based on sorbed malate

Observed reaction yields and rates are correlated. Their magnitude can be partly attributed to the strength of the interaction carboxylate-quaternary amine, being higher for more acidic carboxylic acids (Table 6). The presence of other impurities, and even the carboxylate concentration in the resin bead could also be affecting factors.

Table 6. Acid dissociation constant of selected carboxylates.(Weast, 1979)

Acid name	p$K_{a,1}$	p$K_{a,2}$
Acetic	4.75	---
Succinic	4.16	5.61
α-Ketoglutaric	3.90	
Malic	3.40	5.11
Fumaric	3.03	4.44
Pyruvic	2.50	---
Furandicarboxylic[a]	2.69	4.13

[a](Ferraz and Do Amaral, 1976)

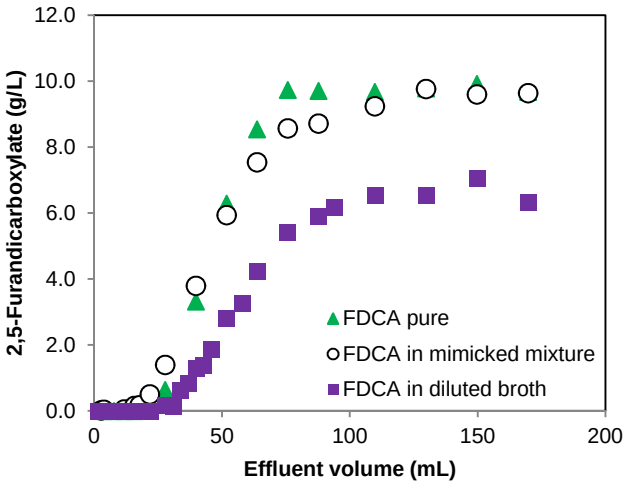
6.3.3 Upgrading of bio-based 2,5-Furandicarboxylate via direct downstream catalysis

Part I: Dynamic sorption

Similarly to the succinate case, column loading experiments were carried out using three different 2,5-furandicarboxylate feeds. Figure 4a shows the sorption comparison between breakthrough curves for $FDCA^{2-}$ pure, in the mimicked mixture based on a partially purified sample and in diluted bioconversion broth. A certain reduction can be observed in FDCA loading capacity as the feed mixture complexity is increased. Table 7 summarizes the calculated capacities for the three cases, in which a maximum capacity of 0.30 g $FDCA^{2-}$/g dry resin is achieved in the pure case, dropping 17% in the case of the diluted bio-based FDCA. Such reduction in capture capacity is less pronounced than in the succinate case (50%), however it cannot be justified by the sorption of the main furanic-related impurities. Figure 4b shows normalized breakthrough curves for FDCA, HMF acid and HMF, being the former initially sorbed but almost fully desorbed by competition with FDCA, seen as chromatographic peaking. HMF eluted at the empty bed volume and was not sorbed at the run conditions, indicating no major ionic or hydrophobic interactions between the compound and the resin.

The reduction seen for the diluted broth is likely to be caused by sorption of competing inorganic anions and fouling of the resin by other compounds not determined in this study. These sorption results are promising for FDCA, and may indicate a greater feasibility of ion exchange sorption as primary recovery.

(a)



(b)

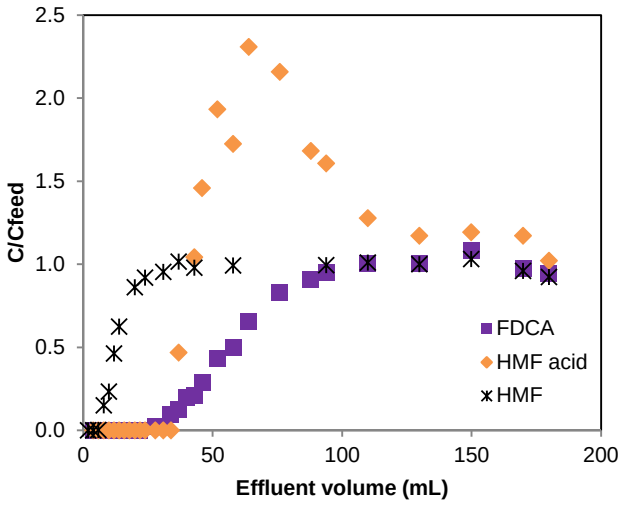


Figure 4. 2,5-Furandicarboxylate breakthrough curves from different mixtures (a) FDCA breakthrough from pure, mimicked mixture and diluted biotransformation broth. (b) Normalized multicomponent breakthrough of FDCA and related impurities in diluted biotransformation broth.

Table 7. Sorption capacities for FDCA and related impurities at the end of the column run for the three cases studied.

	Sorption capacity (g carboxylate/g dry resin)		
Component	FDCA pure	Mimicked mixture	Diluted bioconversion broth
FDCA	0.30	0.29	0.25
HMF acid	--	--	1.0×10^{-4}
HMF	--	--	Not sorbed

Part II: Ester formation by O-alkylation

As demonstrated before, succinate and other carboxylates can be upgraded to esters via *O*-alkylation. However, it was also seen that stronger carboxylates are alkylated at a slower reaction rate. To demonstrate the feasibility of this transformation for FDCA, a more acidic dicarboxylic acid, alkylation experiments with dimethyl carbonate were carried out in a similar manner as for succinate, using FDCA-loaded resins from the three mentioned mixtures. The reaction stoichiometries for the sorption and upgrading of FDCA are assumed to be analogous to those suggested for the succinate case and are presented in Figure 5a and 5b, respectively. Briefly, during sorption at neutral pH the divalent furandicarboxylate anion will be captured, occupying two exchange sites and subsequently alkylated producing the respective dimethyl ester. As a result of both processes, the resin is regenerated to the bicarbonate form with stoichiometric amounts of the respective bicarbonate salt and methanol as by-products. As discussed in previous studies, such by-products can be recycled within an integrated process comprising fermentation and upgrading.(López-Garzón, van der Wielen, 2014)

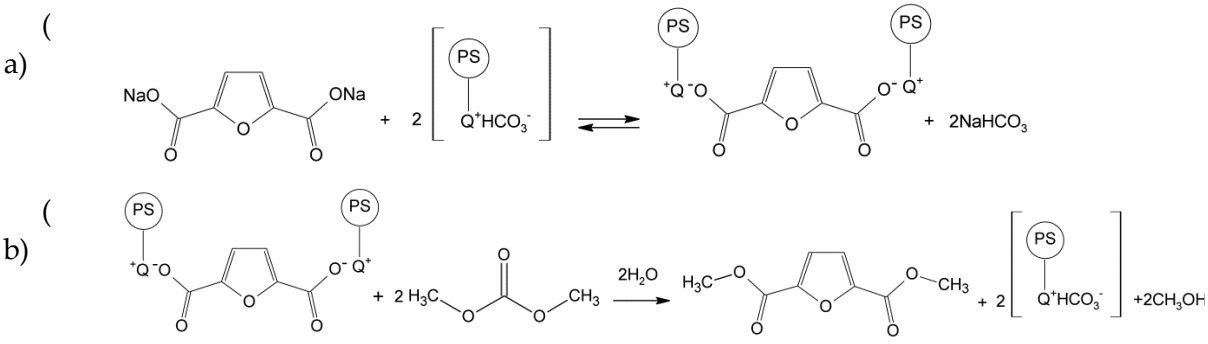


Figure 5. Proposed overall stoichiometry for the formation of dmFDCA via direct downstream catalysis. (a) FDCA sorption stoichiometry. (b) Production of dmFDCA by *O*-alkylation using dimethyl carbonate as alkylating agent. PS is polystyrene resin, Q is quaternary ammonium.

After initial short reaction trials, the reaction product was analyzed in RP-HPLC by comparing the retention time of the compound, corresponding well with dmFDCA standard prepared by Fischer esterification. Further identity verification was done using NMR after work up of the product of direct downstream catalysis.

Sorbed 2,5-furandicarboxylate from the three cases evaluated was used to determine the rate of formation of its dimethyl ester. As can be seen in Figure 6, alkylation of FDCA from a purified mixture yielded 0.77 mol dmFDCA/mol FDCA after 40 h. Compared to succinate, the reaction kinetics are very slow, in agreement with the discussed pK_a reasoning in the previous section. In contrast to the results seen in the case of succinate, ester formation kinetics and yields were not significantly decreased when raw bio-based solutions were used, suggesting the importance of optimizing fermentation conditions towards residual salt concentration and

by-products. Higher temperatures would make the process more attractive for this carboxylate, resin stability being a major hurdle for this improvement.

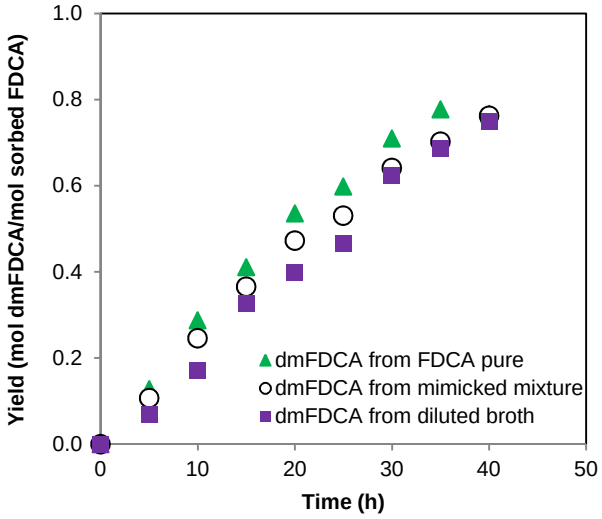


Figure 6. Dimethyl furandicarboxylate (dmFDCA) formation yield from sorbed FDCA from pure solution, mimicked mixture and diluted bioconversion broth.

6.4 Conclusions

The application of the direct downstream catalysis concept to bio-based succinate and 2,5-furandicarboxylate is promising and a potential processing alternative for carboxylates produced by fermentation, resulting in the production of diesters without the need of prior carboxylate purification. Ion exchange as a capturing step for raw dicarboxylates can be efficient, as demonstrated for 2,5-furandicarboxylate for which a capacity of 0.3 g $FDCA^{2-}$/g dry resin was achieved. The presence of competing anions, which reduce the sorption capacity, has to be minimized by optimization of fermentation conditions.

The main characteristics of the reaction system used for *O*-alkylation, described initially for succinate, were also applicable to 2,5-furandicarboxylate and appear to be general to other carboxylates. A reaction feature such as dehydration of certain alpha-hydroxyacids, such as malic acid, during ester formation was observed and could be of important relevance if extended to other substrates. In general, the reaction rate is relatively slow, especially for carboxylic acids with a low pK_a. Further evaluation of this integration concept for bio-based carboxylates could pave the development of fully sustainable building blocks produced by biological transformations.

6.5 Acknowledgements

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Summary

The inevitable depletion of non-renewable resources for the production of chemicals requires continued research efforts to make platform chemicals from renewable resources. In particular, the development and establishment of processing routes based on biomass should be prioritized in the short to midterm and further to mitigate climate change effects. Although several of these routes are technically feasible, different challenges and pitfalls towards better utilization of raw materials, emissions and overall sustainability have been identified. Particularly, in the case of the pathway from sugars to derivatives and materials via bio-based dicarboxylates, better technologies on downstream processing and upgrading of these dicarboxylates are required to minimize waste salt emission if efficient neutral pH bio-based transformations are used.

In this thesis, a contribution to a technological solution for such challenges is described. This involves a new approach in downstream processing for dicarboxylates, particularly for succinate and 2,5-furandicarboxylate. Critical aspects concerning the feasibility of this new alternative were studied.

Initially, the literature reviewed in chapter 2 showed that different methods, and consequently processes, can be devised for the recovery and downstream processing of a fermentative carboxylic acid, depending on its physicochemical properties and fermentation conditions. The main processing stages, its operations and applicability were discussed, with focus on primary recovery. In cases where a carboxylate salt needs to be converted into its acid form, stoichiometric amounts of inorganic salt are produced, which should be re-purposed as a market for the salt may be lacking and it may be unacceptable as waste. Alternatives to this issue are salt reuse within the process via splitting methods, which are relatively difficult, or avoiding salt formation by performing low pH fermentation. The literature research also revealed that the impacts of individual operations in overall processing are not well described in most publications, impeding an adequate comparison of recovery routes.

As identified in chapter 2, primary separation operations determine to a large extent the structure of downstream processing of carboxylates. Therefore, chapter 3 compared extraction and adsorption equilibrium for itaconate, fumarate, malate and succinate at neutral pH conditions. Two strong anion exchangers were tested, Aliquat 336 as extractant and Dowex Marathon A as sorbent, in their Cl^- and OH^- forms. The equilibrium behavior of the studied dicarboxylates showed no major differences in any of recovery systems. Furthermore, for both operations, the use of OH^- as counterion showed capacities about four times higher than using Cl^-, with a maximum capacity for sorption of about 2.3 mmol dicarboxylate/g dry resin with a defined 2:1, counterion to dicarboxylate exchange stoichiometry.

Although high capture capacity can be achieved with auxiliary phases using strong anion exchange with the adequate counterion, its conventional regeneration after a capture cycle produces waste salt. The conceptualization of non-conventional alternatives for regeneration, which could prevent such issue, led to the exploration of dicarboxylate upgrading operations. Among them, production of carboxylate esters was identified to be of relevance in the pathway towards polyesters. Therefore, it was clear that integration of capture operations and chemical upgrading for dicarboxylates could lead to a new process route without net waste salt production, assuming a viable upgrading chemistry.

The production of carboxylate esters is commonly carried out via Fischer esterification; however, when the carboxylic acid is not available in the undissociated form, other synthetic pathways are available, such as alkylation. In chapter 4, it was proven that strong anion exchange resins can act as phase transfer catalysts and thus sorbed succinate can be alkylated using a weak alkylating agent yielding the respective alkyl carboxylate. The conversion yield and kinetics of sorbed succinate to diethyl succinate using chloroethane depended on resin characteristics such as basicity and porosity. The reaction yield at 4 h of reaction at 80 °C was 70%, which was maintained after five sorption-reaction cycles. Moreover, green chemistry metrics such as atom efficiency and E-factor were improved in the proposed direct downstream catalysis when compared with a conventional acidification and esterification process.

As direct downstream catalysis of sorbed succinate to its ester was demonstrated, a complete integrated route from fermentative succinate production to dimethyl succinate was developed in chapter 5. The use of dimethyl carbonate as alkylating agent enabled such integration as it led to a mobile bicarbonate anion, used as counterion during sorption and useable as inorganic carbon source and neutralizing agent in bacterial fermentation. Using bicarbonate as counterion had a more favorable sorption equilibrium than using Cl^-, thus leading to a better capacity utilization of the resin. A reaction yield of 96% to dimethyl succinate was achieved after 6 h at 100 °C and the resin was regenerated to the bicarbonate form. Although methanol is produced as by-product due to the presence of bound water in the resin, it can be separated and recycled to dimethyl carbonate synthesis, for instance.

Finally, the proposed concept was validated in chapter 6 using bio-based succinate from *Clostridium glutamicum* and extended to 2,5-furandicarboxylate (FDCA) produced by *Pseudomonas putida*. The presence of competing anions from fermentation media and by-products decreased the dicarboxylate sorption capacity in the succinate case, and to a lower extent in the FDCA case. Conversion yields were high for both compounds, and for succinate also other carboxylate impurities were converted to their respective ester. Reaction kinetics were slower for FDCA, indicating that reaction rate correlates with pK_a.

The research performed in this thesis leads to the conclusion that an integrated direct downstream catalysis approach, in which sorbed bio-based succinate and FDCA are upgraded to methyl esters via O-alkylation using dimethyl carbonate, provides a processing route without stoichiometric waste salt production. Furthermore, it has been established that such approach is likely to be applicable to other fermentative carboxylates.

Samenvatting

De onvermijdelijke uitputting van niet-duurzame grondstoffen voor de productie van chemicaliën vraagt om onderzoek naar nieuwe routes om platform chemicaliën te maken vanuit duurzame grondstoffen. Daarnaast kan het gebruik van biomassa als grondstof een bijdrage leveren aan het beperken van klimaatveranderingseffecten. Ondanks dat er een aantal van deze routes al technisch mogelijk is, zijn er nog steeds uitdagingen op het gebied van grondstoffen, emissies en globale duurzaamheid van deze processen. Bijvoorbeeld voor de productie van *bio-based* dicarboxylaten zijn betere scheidings- en opwerkingstechnieken nodig om nevenproductie te minimaliseren van zouten die in stoechiometrische hoeveelheden ontstaan wanneer de dicarboxylaten bij een neutrale pH geproduceerd worden.

Dit proefschrift beschrijft een technologische oplossing voor deze uitdagingen. Er wordt een nieuwe scheidings- en opwaarderingsmethode voor dicarboxylaten gepresenteerd, de kritieke aspecten van deze methoden worden bestudeerd en deze methode wordt gedemonstreerd voor succinaat en 2,5-furandicarboxylaat.

De literatuurstudie in hoofdstuk 2 beschrijft de verschillende methodes en processen voor de scheiding en opwaardering van carbonzuren geproduceerd door fermentatie met een focus op de primaire scheiding. De keuzes voor processen en procescondities zijn afhankelijk van de fermentatie condities en fysische eigenschappen van het product. In processen waar een carboxylaatzout omgezet moet worden in het carbonzuur worden stoechiometrische hoeveelheden anorganisch zout geproduceerd, wat resulteert in een grote hoeveelheid bijproduct wat als afval gezien kan worden. Mogelijkheden om zulk afval te voorkomen zijn methoden om de zouten te splitsen en fermentatieprocessen bij een lage pH. Hoofdstuk 2 laat ook zien dat de effecten van individuele processtappen op het gehele proces niet goed beschreven zijn in de literatuur, wat het maken van een goede vergelijking tussen verschillende routes lastig maakt.

Zoals in hoofdstuk 2 beschreven, bepalen de primaire scheidingsstappen voor een groot deel de configuratie van de scheidingsstappen van carboxylaten. In hoofdstuk 3 worden extractie en adsorptie evenwichten vergeleken voor itaconaat, fumaraat, malaat en succinaat onder neutrale condities. Twee sterke anionenwisselaars zijn getest: Aliquat 336 (extractant) en Dowex Marathon A (sorbent), beiden in hun Cl^- en OH^- vorm. Het evenwichtsgedrag van de dicarboxylaten toonde geen grote verschillen voor de verschillende scheidingssystemen. Zowel bij extractie als adsorptie zorgde het gebruik van OH^- als tegenion voor een vier keer hogere capaciteit vergeleken met Cl^-, met een maximum capaciteit van 2.3 mmol dicarboxylaat/g droge ionenwisselaar, voor een gedefinieerde tegenion:dicarboxylaat verhouding van 2:1.

Ondanks de hoge scheidingscapaciteit die behaald kan worden met hulpfasen en sterke anionenwisselaars wordt er nog steeds zout gevormd als bijproduct wanneer de ionenwisselaar op conventionele wijze wordt geregenereerd. Om de mogelijkheden voor het voorkomen van zoutvorming te bepalen werd het ook het proces na de primaire afscheiding van de carboxylaten geanalyseerd. Eén van de in de industrie gebruikte verwerkingsstappen is het veresteren van de carboxylaten, bijvoorbeeld voor de productie van polyesters. Wanneer de vorming van deze esters geïntegreerd kan worden met de primaire scheiding door ionenwisseling, kan er een nieuwe procesroute ontwikkeld worden waarin zoutvorming wordt voorkomen.

De conventionele productie van carboxylaatesters gaat via Fischer verestering. Wanneer het carbonzuur in gedissocieerde vorm aanwezig is dan zijn er alternatieve methodes beschikbaar, zoals alkylering. In hoofdstuk 4 wordt aangetoond dat sterke anionenwisselaars als fase-transfer-katalysator kunnen optreden voor deze reactie en het geadsorbeerde dicarboxylaat omgezet kan worden tot alkylcarboxylaat, wanneer er een zwak alkylerend reagens wordt gebruikt. De conversie en kinetiek van de omzetting van geadsorbeerd succinaat naar diethylsuccinaat met behulp van chloorethaan was afhankelijk van de eigenschappen van de ionenwisselaar zoals basiciteit en porositeit. De reactie opbrengst na 4 uur bij 80 °C was 70%, en dat percentage bleef gelijk gedurende vijf adsorptie-reactie cycli. Verder werden *Green Chemistry* parameters zoals *atom efficiency* en *E-factor* verbeterd ten opzichte van het conventionele proces waarin aanzuring en verestering gecombineerd worden.

Na verwezenlijking van deze nieuwe technologie voor omzetting van geadsorbeerd succinaat tot de bijbehorende ester, *direct downstream catalysis*, kon een compleet geïntegreerde route voor de productie van dimethyl succinaat vanuit fermentatieve succinaat ontwikkeld worden, zoals beschreven in hoofdstuk 5. Het gebruik van dimethylcarbonaat als reagens voor alkylering maakte deze integratie mogelijk omdat er een bicarbonaat-anion gevormd wordt, dat gebruikt werd als tegenion bij de ionenwisseling, en kan dienen als anorganische koolstofbron en voor neutralisatie tijdens de fermentatie. Bicarbonaat zorgde ook voor een verbeterd adsorptie-evenwicht vergeleken met Cl^-, waardoor de capaciteit van de ionenwisselaar beter benut werd. Een dimethylsuccinaat opbrengst van 96% werd gerealiseerd na 6 uur bij 100 °C, waarbij de ionenwisselaar geregenereerd werd in de bicarbonaat vorm. Tijdens deze reactie werd methanol gevorm als bijproduct door de aanwezigheid van water dat gebonden was aan de ionenwisselaar, maar als dit methanol teruggewonnen wordt kan het bijvoorbeeld gebruikt worden voor de productie van de dimethylcarbonaat.

In hoofdstuk 6 werd het voorgestelde concept gevalideerd met behulp van bio-succinaat, geproduceerd met *Clostridium glutamicum*, en 2,5-furandicarboxylaat (FDCA), geproduceerd met *Pseudomonas putida*. The aanwezigheid van anionen en bijproducten in het fermentatiebeslag resulteerde in een lagere dicarboxylaat adsorptiecapaciteit van de ionenwisselaar voor succinaat, en in mindere mate ook voor FDCA. De conversie van beide producten was hoog en in het geval van de succinaat werden ook andere carboxylaat bijproducten omgezet tot hun bijbehorende methylesters. De reactiesnelheid was lager voor FDCA, wat een correlatie tot de pK_a kan suggereren.

Het onderzoek beschreven in dit proefschrift laat zien dat *direct downstream catalysis*, waarbij *bio-based* succinaat en FDCA opgewaardeerd worden tot hun methylesters via O-alkylering met dimethylcarbonaat, een alternatieve procesroute kan bieden waarbij stoechiometrische zoutproductie wordt voorkomen. Daarnaast laten de resultaten ook zien dat dezelfde aanpak waarschijnlijk ook toepasbaar is op andere door fermentatie geproduceerde carboxylaten.

Curriculum vitae



Camilo Sixto López Garzón was born on the 22nd of October 1983 in Bogotá, Colombia. He finished his Higher Secondary Education in 1999 and started his Diploma degree in Chemical Engineering at National University of Colombia, Campus Bogotá, in 2000. During his degree, he focused on transfer phenomena and bioprocess engineering, and graduated in 2005 with his Diploma Thesis "Design and assessment of an extractive fermentation system for ethanol production with immobilized yeast" under supervision of Prof. Pedro Bejarano Jimenez.

After a research internship at Biotechnology Institute at National University of Colombia and a short professional experience in the natural gas industry he moved in February 2007 to Campinas, São Paulo, Brazil, to pursue his Master of Science education in (Bio)Chemical Process Development at University of Campinas (Unicamp). During his Master degree, he conducted research on production of bio-based lipids from C5 sugars leading to his Master Thesis "Microbial Production of Lipids", under supervision of Prof. Dr. Telma Teixeira Franco.

In autumn 2009, he joined the Bioseparation Technology group (currently Bioprocess Engineering) at Delft University of Technology, Delft, The Netherlands, and started his PhD training under supervision of Prof. dr. ir. Luuk van der Wielen and Dr. ir. Adrie Straathof as co-promotor. During his PhD research, he developed a new process alternative for recovery and upgrading of bio-based dicarboxylates which is presented in this Thesis.

In November 2013, he moved to Copenhagen, Denmark and joined the Recovery Development department at Novozymes A/S headquarters in Bagsværd, where he currently holds a position as Project Leader and Recovery Scientist.

List of publications

Part of the research conducted in this thesis led to the following publications:

1. **López-Garzón, C.S.**, Ottens, M., van der Wielen, L.A.M. and Straathof, A.J.J., **2012**. Direct downstream catalysis: From succinate to its diethyl ester without intermediate acidification. *Chemical engineering journal*, *200*, 637-644.

2. **López-Garzón, C.S.**, van der Wielen, L.A.M. and Straathof, A.J.J., **2014**. Green upgrading of succinate using dimethyl carbonate for a better integration with fermentative production. *Chemical Engineering Journal*, *235*, 52-60.

3. **López-Garzón, C.S.** and Straathof, A.J.J., **2014**. Recovery of carboxylic acids produced by fermentation. *Biotechnology advances*, *32*(5), 873-904.

4. **López-Garzón, C.S.**, van der Wielen, L.A.M. and Straathof, A.J.J., **2016**. Ester production from bio-based dicarboxylates via direct downstream catalysis: succinate and 2,5-furandicarboxylate dimethyl esters. *RSC Advances*, *6*(5), 3823-3829.

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