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Recovery of long dicarboxylic acid chains from aqueous media with solvent-based membrane systems.

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Table of Contents

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Abbreviations

(ADH) alcohol dehydrogenase

(CAs) carboxylic acids

(CYP) cytochrome P450 monooxygenase

(DCAs) dicarboxylic acids

(DC12) Dodecanedioic acid

(DC16) Hexadecanedioic acid

(DC18) Octadecanedioic acid

(DSP) downstream processes

(EPA) Environmental Protection Agency

(FAs) unsaturated fatty acids

(FAldDH) fatty aldehyde dehydrogenase

(FAO) fatty alcohol oxidase

(ISPR) In situ product removal

(LDCAs) long-chain α,ω-dicarboxylic acids

(LLE) liquid–liquid extraction

(MC) membrane contactor

(ME) membrane-based extraction

(MBRE) membrane-based reactive extraction

(MSBE) membrane-based solvent extraction

(NADPH) Nicotinamide-adenine dinucleotide phosphate

(NF) Nanofiltration

(OBCR) Office of Business and Community Relations

(PE) Polyethylene

(PP) Polypropylene

(PTFE) Polytetrafluorethylene

(REACH) Regulation for Registration, Evaluation, Authorisation and Restriction of Chemicals

(SCCAs) short chains of carboxylic acids

(TOA) Tri-octylamine

(USD) United States dollar

(WODCA) waste oil to dicarboxylic acids

Abstract

The growing desire to produce organic acids through fermentative routes, as a starting point for bio-based plastics, has revived the scientific attention on organic acid removal from aqueous streams. In fact, biological fermentation is a promising method to produce organic acids from renewable biomass. [4, 10] The efficient separation of organic acids from a mix of multiple diluted components it is still a challenge and most of the production costs in microbial processes are higher than that in chemical processes, among which over 60% are generated by downstream processes (DSP). [5] Therefore, research on separation and purification processes is important for the future biorefinery industry: there is a need to develop a process that should ideally be simple to carry out and allow the purification of LDCAs directly from the fermentation broths. The WODCA (Waste Oil to Dicarboxylic Acids) project, carried out by several R&D partners including VITO, the Flemish Institute for Technological Research, is focusing on this framework. The overall goal of WODCA is in fact to create new, sustainable, fair-cost LDCAs molecules from lipid waste (used cooking oil). Thanks to my internship experience at VITO, I have been part of the WODCA project, working on DSP steps. Especially, the case study presented has been focused on the extraction of LDCAs (DC12, DC16, DC18) from a synthetic aqueous feed which simulate the real fermentation broth. For the recovery of the LDCAs, LLE and membrane extraction experiments, both physical and reactive (MBSE and MBRE), with microporous membranes as well as with tight dense membrane, have been performed. MBSE has showed low extraction efficiency (EE%), especially when ceramic membranes have been employed (7% DC12, 1% DC16 and DC18, pH 6, 30˚C, decanol), while polymeric ones have been able to reach higher values (47% DC12, 41% DC16 and 16% for DC18, pH 6, 30˚C, decanol). MBRE has instead achieved higher EE% thanks to the role of the reactive extractant. The best results in term of membrane extraction, have been achieved by the MBRE set-up constituted by polymeric membrane (PTFE 0.1) with Aliquat 336+ Octanol (96% DC12, 56% DC16 and 11% for DC18, pH 6, 30˚C), while the best results in absolute term have been achieved by reactive LLE with Aliquat+decanol. Unfortunately, even if LLE shows the best results, the problem faced up by this technology is that it could not be combined with bioproduction, since the solvents are toxic for the microorganisms, which need a separation layer that can be provided by the membrane. Further studies are thus required to enhance the EE% of DC16 and DC18 with MBRE, which are still far from the final goal (99-100%).

1. Introduction: the process sustainability

The growing concern over environmental sustainability and the urgent need to reduce our dependency on fossil fuels have spurred a remarkable paradigm shift in the field of bioprocessing and industrial fermentation. For decades, fossil fuels served as the primary substrate for several biotechnological processes, including the production of plastics, fuels, and other value-added chemical products. Fossil sources have been exploited as raw material to produce huge amount of polymeric chemicals, such as polyamide[1], but also as precursors for the manufacturing of powder coatings, lubricants, adhesives, and corrosion inhibitors. All these materials can be as well produced via bioprocesses, implying the substitution of the fossil-based substrate with a biobased one, or the recycle of a waste product. [5, 17] Thus, in an era characterized by environmental crises, resource scarcity, and the imperative to combat climate change, there is an escalating global effort to transition towards more eco-friendly and sustainable alternatives. One such transformative approach involves the employment of renewable sources, such as biobased fresh oil or waste cooking oils, potential sources of fatty acids (Fig. (1)), as substrates for fermentation processes effectively displacing the reliance on fossil fuels. [14, 23]

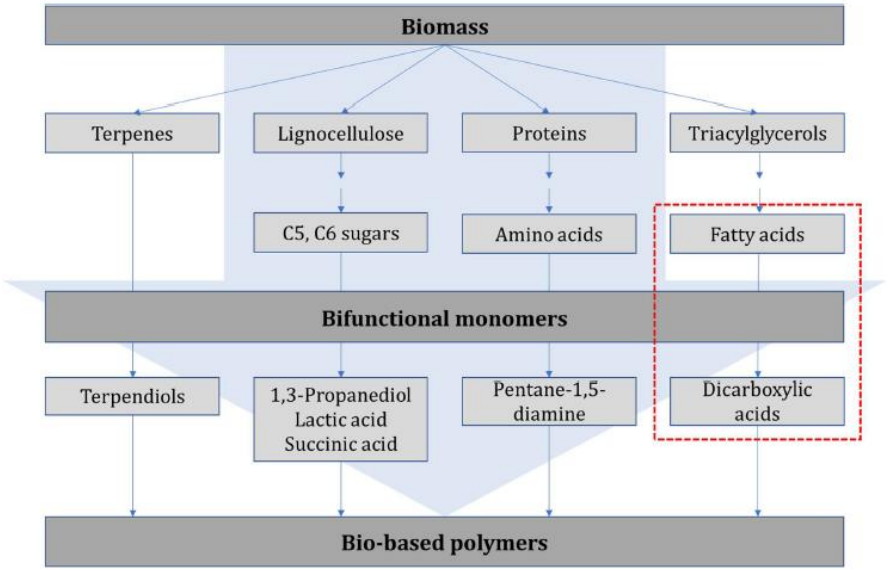


Figure 1: Production of biobased polymers from biomass [23]

[1] Such as nylon, polyesters, and polyurethanes

The shift towards the use of renewable sources as substrates stands for a pivotal departure from conventional practices. It aligns with the principles of green chemistry and sustainable industrial processes, aiming to mitigate the environmental impact while simultaneously addressing the ever-increasing energy demands of a burgeoning global population. [23] The importance of this shift cannot be overstated, as it holds the potential to reduce greenhouse gas emissions, conserve non-renewable resources, and promote a circular bioeconomy, where waste materials and by-products can be repurposed and valorised. Furthermore, in the quest for sustainable and environmentally responsible bioprocessing, the efficient recovery and separation of valuable compounds produced towards fermentation routes have become a pressing challenge. Among these valuable products, organic acids, especially carboxylic acids (CAs) and dicarboxylic acids (DCAs), have gained significant attention due to their applications in chemicals, pharmaceuticals, and biobased industries. [14] DCAs which include compounds like succinic acid, malic acid and adipic acid, are essential precursors for the production of various bio-based materials. [5] In addition to their biodegradability and environmental sustainability which makes biobased DCAs suitable for compliance with various regulatory norms laid by REACH and EPA in Europe and North America (Grand View Research 2017), they result in products with, e.g., higher flexibility, strength, durability, and anti-corrosion properties. This opens versatile fields of applications, e.g., in the textile, or automotive industry how has been pointed out by the 2016 OBCR Report. [5]

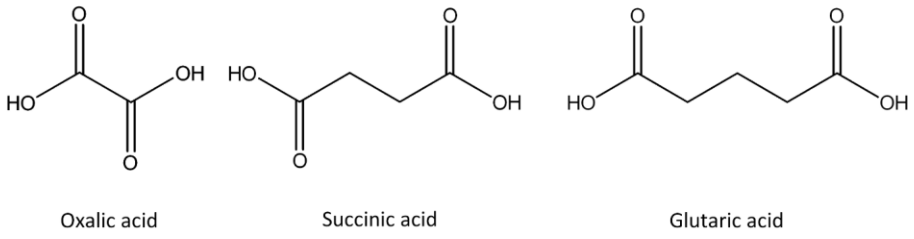


Figure 2: Dicarboxylic acids [18]

Among DCAs, especially the long-chain α,ω-dicarboxylic acids (LDCAs) (carbon atoms>12 [11]) are considered versatile chemical intermediates of industrial importance and can be employed as building blocks for the production of polymers, lubricants, or adhesives. [5] Although most of the industrial LDCAs are produced from petro-chemical resources, biotechnological production from renewable materials, like plant oil fatty acids by microbial

fermentation using oleogenious yeasts, could be an alternative.[2] The exploitation of downstream processes (DSP) such as membrane technologies to recover them from bioprocesses has the potential to significantly enhance the overall sustainability of the bio-based industry and leads to an increasing request in the last decay. The global market of LDCAs is in fact expected to reach USD 300 million by 2030, but the prohibitive costs and limited availability of them is hampering the market growth (Fig. 3) [5, 37]. As the world strives to transition towards more sustainable and environmentally responsible industrial processes, the innovative approach of membrane-based extraction of LDCAs, offers a promising future, stimulating the transition to a more sustainable bioeconomy inducing a recycle prospective and replacing chemical synthesis from fossil fuel and derived starting materials.

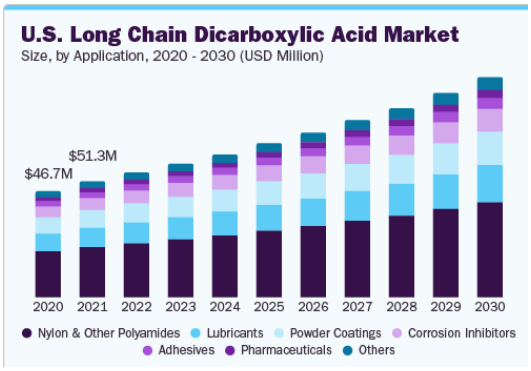


Figure 3: LDCAs Global market [37]

Although, some commercialized fermentation processes using engineered yeasts are reported, biobased LDCAs are still far from being a mass product. Further progress in bioprocess engineering and rational strain design is necessary to advance their further commercialization. [5] Their recovery from the fermentation broth is essential for the development of a sustainable bioprocess industry. Several studies indicate that separation processes account for over 30%-40% of the total processing costs in such processes. [5, 13] Traditional DSP, including solvent extraction and crystallization, have limitations in terms of sustainability and energy efficiency. Membrane-based extraction (ME), which comprehend membrane-based solvent extraction (MBSE) and membrane-based reactive extraction (MBRE), is a novel approach, offers instead a promising solution to this challenge by simultaneously transfer the DCAs from the fermentation broth to the solvent, which needs to be recovered afterwards. These method

[2] Oleogenious yeasts are natural long-chain DCAs producers, which must be genetically engineered for high-yield DCAs production.

leverages the unique properties of membranes and selective solvents, allowing for the efficient and sustainable separation of organic acids from complex mixtures while minimizing the need of energy-intensive processes. [5] By exploiting the affinity of a solvent with the target product, such as the DCAs, a selective pathway is created. The main advantage proposed by MBSE/ MBRE is that the fermentation broth and the extraction solvent are separated from each other thanks to the membrane layer, which presence is of utmost importance in the case of toxic solvent for the microorganisms. The presence of membrane itself is aimed to create a separation layer between the fermentation broth[3] and the organic phase, for this reason it is known as membrane contactor (MC). [10] In addition to that, this technology allows also to work with solvent with a similar feed density and to arrange their flow rate separately[4]. [7] In cases where it is not possible to extract purely on affinity or wherein selectivity is not high enough, the application of MBRE could lead to enhance the extraction process's yields since in reactive extraction (with or without a membrane) the solute generates a complex with the extractant. [3] Several studies have been proposed for the extraction of CAs and for short DCAs, but the state of arts for LDCAs is still at the beginning, although their wide kind of applications. In the following paragraphs the DCAs chemical and physical characteristics will be presented, along with their novel bio-production, extraction and possible exploitations routes.

1.1 The dicarboxylic acids: general introduction

DCAs are organic compounds that contain two functional carboxylic acid (–COOH) groups. Their molecular formula is $HOOC\ (CH_2)_n\ COOH$, where n can vary according to the acid: n is 0 for oxalic acid, 1 for malonic acid, 2 for succinic acid, 3 for glutaric acid, 4 for adipic acid, etc. According to the number of carbons they can be divided into short chain (5 or fewer atoms of carbon), medium chain (between 6 and 11) or long chain (12 or more atoms of carbon). [11] In the past the majority of DCAs have been derived from fossil feedstock using synthetic routes which often have suffered from low yield, complicated work-up, or toxic reagents employment[5]. [14] A large variety of substrates containing organic acids, such as oils[6], can be converted by biological fermentation into CAs or DCAs. [5] Until now several studies have

[3] An aqueous phase also containing the microorganism and the solvent.

[4] Extremely useful in the cases in which the amount of solvent is limited.

[5] Additionally it is not possible and surely not cost-effective to make LDCAs by chemical synthesis from fossil resources.

[6] Oils are rich in triglycerides. Each triglyceride is an ester containing one glycerol and three fatty acids (organic acids).

been conducted on the production of short chain DCAs as laboratory or industrial product, from fermentation routes, while the state of art for LDCAs is still at the beginning.

DCAs have a wide range of industrial applications directly or indirectly: succinic acid is used in the food and beverage industry, as an acidity regulator; in industrial applications, they are used as a precursor to active pharmaceutical ingredients, as additives, polymers, polyamides, and solvents. Adipic acid instead is used in production of nylon, but it is also used as a gelling aid and as a leavening and buffering agent. Glutaric acid is used in production of polyester, polyols, polyamides, ester plasticizers and corrosion inhibitors and in the synthesis of pharmaceuticals and surfactant compounds. [5, 17] Their functionalities make DCAs extremely useful as platform materials, and they are less toxic to the host cells than corresponding alkanes, alcohols, or aldehydes. [5]

Due to their functional group, CAs and the DCAs can be found in two different forms: undissociated or dissociated. Their chemical structure changes according to the environment, when it is undissociated the carboxylic group (-COOH) is intact, while in the dissociated form one proton (H^+) is released. DCAs are typically weak acids, meaning that they only partially dissociate into H_3O^+ cations and $RCOO^-$ anions in neutral aqueous solution (Fig. (4)). [11]

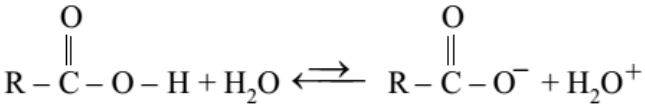


Figure 4: Dissociation of a carboxylic group [11]

Under acidic conditions, the carboxylic groups are almost completely undissociated and exist primarily in their protonated form (RCOOH). Under, basic conditions, it becomes nearly completely dissociated into the deprotonated form (RCOO−). If the radical group (R) of the Fig. (4) was a carboxylic group, the molecule would be a dicarboxylic acid. Since DCAs contain two carboxylic groups in the molecule they can dissociate twice. Their length can vary according to the number of atoms of carbon carried, as showed in Table (1):

Table 1: List of DCAs [11]

C length (straight)	*Product*	*CAS #*
C 2	Oxalic acid (ethanedioic acid)	144-62-7
C 3	Malonic acid (propanedioc acid)	141-82-2
C 4	Succinic acid (butanedioic acid)	110-15-6
C 5	Glutaric acid (pentanedioic acid)	110-94-1
C 6	Adipic acid (hexanedioic acid)	124-04-9
C 7	Pimelic acid (heptanedioic acid)	111-16-0
C 8	Suberic acid (octanedioic acid)	505-48-6
C 9	Azelaic acid (nonanedioic acid)	123-99-9
C 10	Sebacic acid (decanedioic acid)	111-20-6
C 11	Undecanedioic acid	1852-04-6
C 12	Dodecanedioic acid	693-23-2
C 13	Brassylic acid (tridecanedioic acid)	505-52-2
C 14	Tetradecanedioic acid	821-38-5
C 15	Pentadecanedioic acid	1460-18-0
C 16	Thapsic acid (hexadecanedioic acid)	505-54-4
C 18	Octadecanedioic acid	871-70-5

Among them, the most studied until now have been the oxalic, malonic, and succinic acid, which are all under the category of short DCAs. Nowadays several studies are concerning the LDCAs, since they seem to be promising from the industrial application point of view, and at the same time they allow to follow a sustainable production process. An example of this green process could be the bio-based production of long α,ω-dicarboxylic acids from renewable resources. [11]

1.2 Bio-based production of LDCAs

Bio-based long-chain α,ω-dicarboxylic acids (DCAs, ≥ C12 [11]) derived from renewable resources like plant oil fatty acids are important intermediates in the production of biobased polymeric chemicals such as polyamides, e.g. nylon, polyesters, and polyurethanes, but also as precursors for the manufacturing of powder coatings, lubricants, adhesives, and corrosion inhibitors. [14, 17] In addition to their biodegradability and environmental sustainability which makes biobased LDCAs suitable for compliance with various regulatory norms laid by REACH and EPA in Europe and North America, as pointed out by the Grand View Research 2017, they result in products with high flexibility, strength, durability, and anti-corrosion properties. [14] This opens versatile fields of applications in the textile, or automotive industry. (OBCR Report

2016). The global market of LDCAs is expected to reach USD 300 million by 2025, but the high costs and limited availability of them is hampering the market growth. [14] Unfortunately, nowadays most of the long-chain DCAs on the market are produced via chemical synthesis from petrol-chemical resources, with the largest volume manufactured via trimerization of butadiene, followed by hydrogenation and oxidation with nitric acid. [14] The chemical synthesis of long-chain DCAs can also start from unsaturated fatty acids (FAs)/esters via olefin metathesis in the presence of ruthenium-based catalysts. [14] The main drawback of this chemical approach so far has been the low conversion rates since the reaction is thermodynamically controlled. An alternative to the chemical synthesis is the biotechnological production of long-chain DCAs: microorganisms, such as yeasts of the genus *Candida*, are able to convert alkanes or plant oil FAs via the unique ω-oxidation pathway to the corresponding DCAs, which is further metabolized and degraded to acetyl-CoA via β-oxidation. [14, 23] Therefore, genetic engineering is required to generate production strains accumulating DCAs in the cultural medium. Although some fermentation processes using engineered yeast strains are already commercialized to produce specialized LDCAs, further progress in strain design and bioprocess development is necessary for their massive production. [14]

1.2.1 Microbial production of dicarboxylic acids

Fatty acids have been worldwide recognized as potential building blocks for the gradual replacement of the petrochemical platform: their subsequent chemical transformation up to DCAs in integrated biorefineries throughout fermentation can lead to the synthesis of attractive derivatives used in the production of solvents, polymers and fibres. Microbial production of DCAs has been considered a valid research field since the last century. In early 1970s the Japanese researchers Shiio and Uchio published the first extensive studies on the production of DCAs. [23, 33]

Specific microbe's strain or yeasts are able to exploit specific substrates and to start aerobic/anaerobic fermentation processes releasing as final product a DCAs mixture. An example of modified microbial strains that has been used for this purpose is the *Corynebacterium glutamicum*: it can produce succinic, fumaric, malic, and itaconic acids exploiting biomass as substrate. Unfortunately, since the fermentations should be carried out at neutral pH conditions, fully dissociated acids will be obtained instead of desired neutral

forms. If a conventional DSP is applied, the final broth should be acidified requiring the employment of a lot of chemicals and leads to salt waste generation. [14] For these reasons, innovative solutions that avoid these issues are required: the yeast *Saccharomyces cerevisiae* is regarded as an ideal organism for bio-based production of DCAs with high tolerance to acidic and hyperosmotic environments. It shows a robust growth for a wide range of substrates, great convenience for genetic manipulation, stable inheritance via sub-cultivation, and food compatibility. [21] Another yeast, *Wickerhamiella sorbophila*, has been developed as a promising microbial platform for the production of DCAs from biomass[7] [22]. Oleaginous yeasts like *C. tropicalis* or *Yarrowia lipolytica* not only have the natural ability to accumulate high amounts of lipids, but also to produce long-chain DCAs from hydrophobic substrates like n-alkanes, FAs, fats and oils during ω-oxidation. [21] The ω-oxidation is known as subsidiary pathway of the fatty acid degrading β-oxidation: this unique pathway is not present in the common microbial workhorses *S. cerevisiae* or *Escherichia coli*. Until now only some examples of possible microorganisms have been cited, but as the Table (2) shows, several strains are capable to carry out bioconversion exploiting organic acids as substrate up to DCAs.

Table 2:Examples for DCA production yields with different microbial systems [22]

Strain	Substrate	Produced DCA (g/L)
C. viswanathii[a]	Dodecane (C12)	140
	Methyl tetradecanoate (C14)	210
	Oleic acid (C18)	100
Y. lipolytica[a]	Sunflower oil	23
C. maltosa[a]	Tridecane (C13)	15
S. bombicola[a]	Octadecane (C18)	5.6
C. cloacae[a]	Coconut oil	11
	Lauric acid (C12)	10
C. neoformans[b]	Pentadecane (C15)	0.61
P. aeruginosa[b]	Pentadecane (C15)	0.48
E. coli[a]	C12-fatty acid	0.16
	C14-fatty acid	0.41
S. cerevisiae[a]	Lauric acid (C12)	n.d.[c]

Unfortunately, the strains yield of production showed in the Tab. (2) is quite low, thus more accurate solutions should be found. Especially, novel studies are concerning the employment of *C. tropicalis* and *Y. lipolytica*, that are known for their ability to efficiently exploit alkanes and fatty acids as a carbon source. [23] In the first step of the LCDA bioprocess, the

[7] This strain has produced 92.5 g/l of dodecanedioic acid from methyl laurate over 126 h in 5-l fed-batch fermentation, with a productivity of 0.83 g/l/h. [14]

microorganisms will be brought in ideal growth conditions to enable the production of high amounts of biocatalyst. They will use oxygen (added as air) for growth and produce CO_2 during respiration. The amounts are calculated by resolving the stoichiometric equation of the microbial growth:

$$a\ C_6H_{12}O_6 + b\ NH_3 + c\ O_2 \rightarrow CH_{1.8}O_{0.5}N_{0.2} + e\ H_2O + f\ CO_2 \quad (1)$$

Using biomass yield of 5 g cell dry weight from 28 g glucose in batch conditions the obtained stoichiometric coefficients result in the consumption of 4.93 kg O_2/kg glucose and production of 1.03 kg CO_2/kg glucose [14], using a 200 m^3 reactor and 34.5 ton of O_2. In the second step, the biotransformation, the extra carboxylic group in LCDA demands one oxygen molecule. When using the molecular weight of LCDA C18:2 (312.4 g/mol), this corresponds to 0.11 kg O2/kg LCDA. [14] The metabolic pathway involves the import of aliphatic substrates, followed by a β-oxidation or an ω-oxidation. For β-oxidation alkanes and fatty acids are transported to the peroxisomes, whereas ω-oxidation is performed in the endoplasmic reticulum (ER). β-oxidation (on the left in the Fig. (5)) represents the main degradation pathway and involves several enzymatic steps, finally resulting in acetyl-CoA, which can directly enter the citric-acid cycle to provide the cells with energy. Acyl-CoA is catalysed to generate acetyl-CoA, which is further converted into succinate and can enter the citric acid cycle, while succinate represents a precursor for amino acid or carbohydrate synthesis. [23]

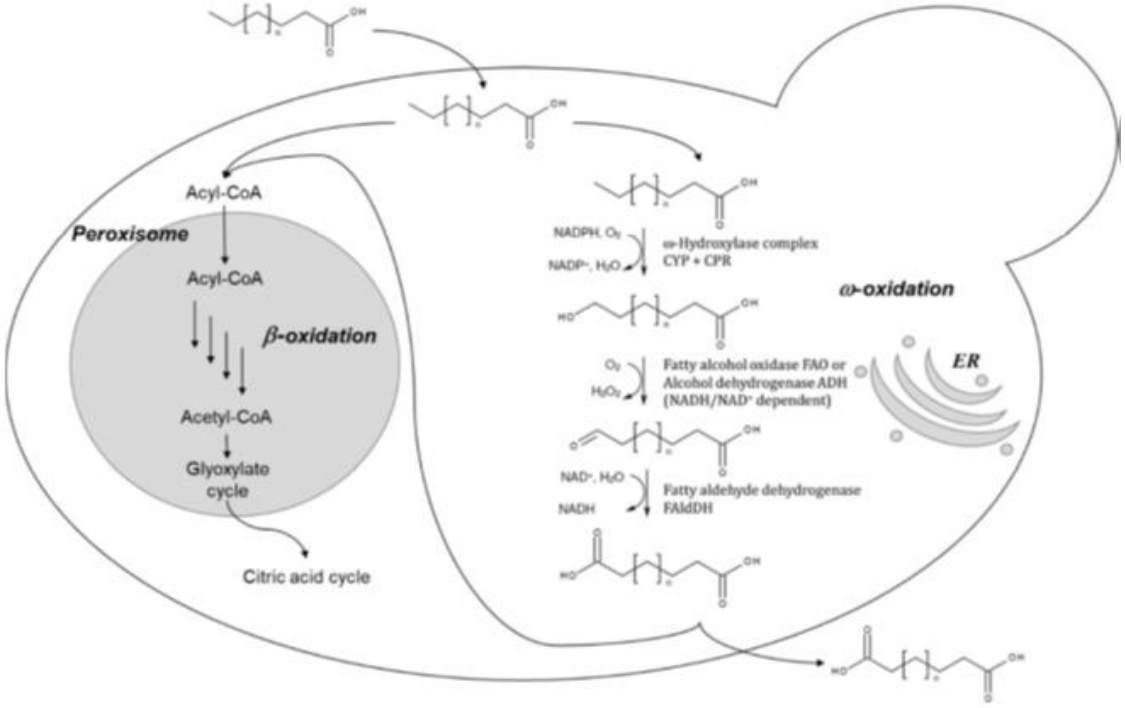


Figure 5:Aliphatic substrates involved in β-oxidation or w-oxidation. [23]

As an alternative pathway for the hydrophobic substrate degradation and for the production of DCAs, the ω-oxidation (on the left in the Fig. 6) can be followed. Its first and rate-limiting step is catalysed by a ω-hydroxylase complex, comprised of a cytochrome P450 monooxygenase (CYP) and an associated NADPH reductase (CPR). [23] The resulting ω-hydroxyl molecule is further converted to the corresponding aldehyde by a fatty alcohol oxidase (FAO) or an alcohol dehydrogenase (ADH). Finally, the aldehyde group is oxidized to a carboxyl group by a fatty aldehyde dehydrogenase (FAldDH). Then, the DCA is further degraded through the β-oxidation pathway in the peroxisome, which leads to extensive shortening of long-chain DCAs and finally to their total breakdown into acetyl-CoA units. Consequently, to enable the conversion of hydrophobic substrates into DCAs it is necessary to block β-oxidation via genetic modifications. [23, 14]

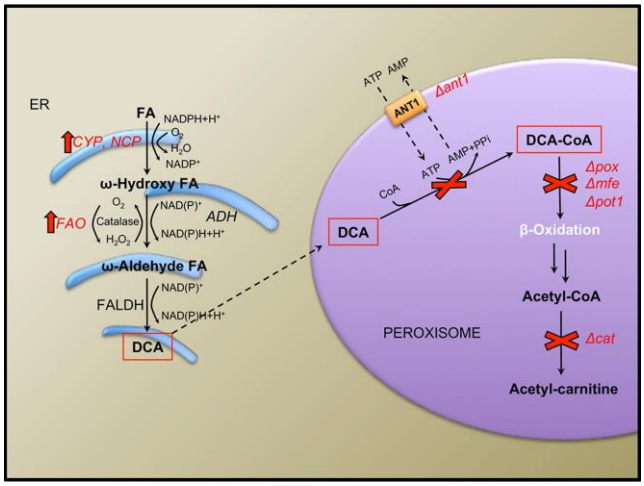


Figure 6: ω- and β-oxidation of dicarboxylic acids, and genetic targets for the genetic engineering. [14]

The bio-oxidation of fatty acids represents a highly complex pathway involving several enzymatic conversion steps and various co-factors. Therefore, *C. tropicalis* has been applied as a whole-cell biocatalyst for the biotransformation of fatty acids to corresponding DCAs based on its enzymatic machinery. [14] The industrial production of DCAs via biotransformation uses this unique ω-oxidation pathway to enable highly selective functionalization of fatty acids and alkanes towards selected DCAs of higher value. The application of microorganisms for the conversion of substrates can come along with several drawbacks, such as unwanted side-pathways and therefore occurrence of by-products. *C. tropicalis* for instance not only owns the desired ability of fatty acid oxidation to DCAs, but also supplies enzymes, which enable disadvantageous degradation of fatty acids. The undesired β-oxidation pathway could be eliminated resulting in increased production rates of DCAs. [23, 14, 20]

1.2.2 The issue of downstream processes

Specific separation processes are required in a biorefinery at different stages, primarily to separate and purify the product or an intermediate for the next stage of processing and to remove biomass components that are inhibitory at a particular stage of processing. As remarked until now, among the numerous chemicals, organic acids are more attractive targets for process development efforts in the renewable-based biorefinery industry, however, most of the production costs in microbial processes are higher than that in chemical processes, of which the 60% are generated by separation processes. [5] Therefore, the optimization research of separation and purification processes is fundamental for a promising biorefinery industry. In the specific case of the industrial production of DCAs, the downstream recovery process accounts for 30-40% of the production cost [5]. The major issue of this process is represented by the separation and recovery of DCAs from the matrix: mixed culture fermentation generates a mixture of carboxylates rather than a single product in high concentration. Furthermore, mixed culture conversions can be plagued by limited product titers and volumetric productivities due to end product inhibition. These limitations can be overcome by selectively removing carboxylates during fermentation. Once the DCAs are generated, they are released by the microbes in the fermentation broth as extracellular products. Supposing that this process occurs inside a bioreactor, the fermentation products need to be separated from the living organisms, avoiding to damaging them. The most common techniques of separation include membrane separation (microfiltration and ultrafiltration), precipitation, chromatography, extraction, and distillation [5, 8]. At industrial scale, the key requirements of a good recovery process are:

- Purity of the single product > 99.5%, especially for DCAs that need to be used for polymerization [5]
- High extent of recovery: 90–100% yield in the DSP. [5]
- Low chemicals and energy consumption, low waste production during product recovery. [5]
- Modest investment costs, due to efficient mass and heat transfer in the recovery equipment. [5]

To achieve the required purity of DCAs, the DSP typically must fulfil the following steps (Fig.7) [8]:

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 DCAs (if required).
4. Concentration/purification. Removal of the bulk solvent or capture of the DCAs, achieving concentration. Removal of remaining impurities.
5. Upgrading. Transformation to chemical derivatives (if required).
6. Formulation. Adaptation of product to storage and customer needs.

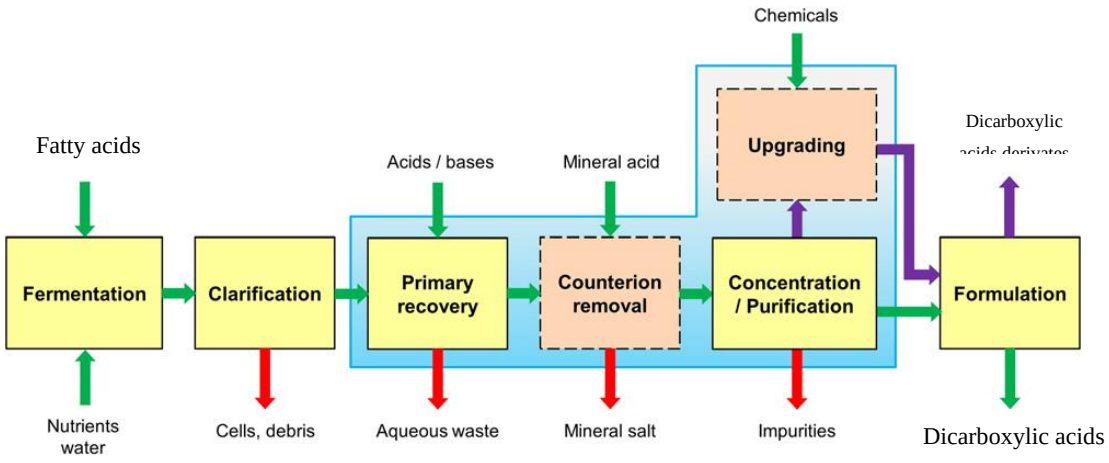


Figure 7: General downstream processing sequence for the recovery, purification and upgrading of fermentative DCAs. Dashed boxes are optional steps. [8]

Cell removal is usually the first downstream step required, executed by filtration or centrifugation. Cell retention could be favourable but results complicated if the fermentation leads to poorly soluble products. Since the physical properties of the target DCAs are widely different, only one recovery process would not be enough [5]. More in detail, the microbial product's recovery process can be divided into two main steps: the extraction and the refining. The extraction concerns primarily the pretreatment of the fermentation broth from which the target product can be extracted. If the target product is intracellular there will be the need to separate the cell from the broth and consequently disrupt the cell to get it, while if it is extracellular the last step is avoided. [5, 8] Then, the second step of refining can start: it involves different procedures of purification and polishing. The process can be schematized as follows:

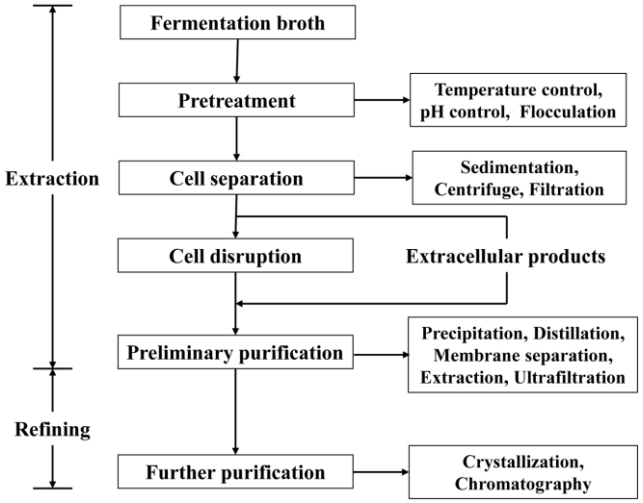


Figure 8: General flow of separation from fermentation broth. [5]

As shown in the Fig. (8), several techniques are used to recover products from the broth. The pretreatment step requires to manage the control of working parameters, such as temperature, pressure pH, conductivity. These parameters can be optimized and changed according to the aim of the process: for example, if the goal is to recover particles from the broth, it is feasible to achieve flocculation and precipitation by adding salts managing the pH. Then, to separate the cells from the broth, physical methods such as sedimentation, centrifugation and filtration can be employed. [6]

Table 3: DSP technologies to recover DCAs from fermentation broths. [6]

DSP step	Technology	Basic principle
Recovery and concentration	Precipitation	Solubility
	Solvent extraction	Chemical interaction
	Reactive extraction	Chemical interaction
	Membrane filtration (NF, UF)	Molecular size
Purification	Crystallization	Solubility
	Distillation	Volatility, difference in boiling point
	Chromatography	Affinity
	Adsorption	Affinity
	Electrodialysis	Ionic charge
	Evaporation	Physical interaction

If the product is intracellular an additional step for cell disruption is required, known as cell lysis. Cell lysis can involve physical (High Shear Homogenisers), chemical (surfactants), or mechanical methods (Bead Milling).[6] In case of extracellular products different methods can be employed to achieve separation, according to the size and chemical property of it. Especially, the organic acids are mainly extracellular products. [5, 6] Precipitation, membrane separation, and extraction are mainly used in preliminary recovery, whereas chromatography and crystallization are mainly applied in refining of organic acids as listed in the Tab.(3) [6, 8].

1.2.3 Separation Technologies for the target product

After removing cells from fermentation broth, the aqueous mixture will hold impurities and fermentation by-products such as proteins, debris derived from the cell lysis and/or decay and undesired DCAs. 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. [2] Some seldom used techniques in the field DSP of DCAs are briefly explained in the Tab.(4), while the most widely used, LLE and solvent extraction (MBSE/ MBRE), will be deeply analysed in the next sections.

Table 4: Separation methods that will not be analyzed in detail.

Method	Explanation	Advantage	Disadvantage	Examples	Source
Precipitation	Molecules can aggregate themselves according to the chemical conditions of the solution, and the aggregates, due of differences in densities, drop down creating a precipitate.	Highly selective method, no phase transition, high product purity. It can be applied as a preliminary separation step.	Difficult to find the right precipitants for the products and their constitutive consumption rises the cost. Further processes are required to get the purified product after.	$Ca(OH)_2$ or $CaCO_3$ are used to get calcium salt, that is then treated with a high concentration of sulfuric acid to free the desired acid.	[5, 8]
Chromatography	The mixture which holds the target product is dissolved in a fluid solvent (gas or liquid) called 'mobile phase', which carries it through a system (column) on which the 'stationary phase' is fixed (resin). The different constituents of the mixture tend to have different affinities for the stationary phase leading to be kept for different lengths of time depending on their interactions with its surface sites.	The resin shows good selectivity for DCAs, the separation process involves low energy consumption with no phase transition. Any co-products as calcium sulfate in precipitation are generated, the yields of products are high.	This process generates large amount of waste liquor, requiring high consumption of salt in the elution process and the exchange capacity of the resin will be weakened with increase of time.	DCAs separations operations are usually accomplished in a batch mode cantering on simulated moving bed (SMB) chromatography, in which acid is separated from a concentrated broth by resin adsorption and solvent elution.	[6]
Distillation	Allows to extract one or more products from a mixture based on differences in components volatility, reaching a high final purity.	Distillation is effective even at a low concentration of organic acid. It is a cheap (operational costs can vary according to the	It may be not the best option to recover DCAs: since their weight is very high[8] with respect to the solvents used, they will be recovered as a bottom product, together with	Reactive and extractive distillation exploit the separation of the target product (DCAs) using solvents. Since the carbonyl group of DCAs	[5, 6]

[8] The average molecular weight for a DC12= 230.3 g/mol, while a suitable solvent like octanol has a molecular weight of 130.2 g/mol. [29]

		energy requirement for the processes) and effective method (high recovery and purity values can be achieved)	the impurities, requiring additional DSP (rising the OPEX).	has a strong adsorb-electron effect, since most organic acids have a higher boiling point than water.	
Pressure driven Membrane Separation	Pressure-driven membrane processes are commonly divided into four overlapping categories of increasing selectivity based on pore size: microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and hyperfiltration or reverse osmosis (RO).A membrane is a thin artificial or natural selective barrier, which allows mass transport of molecules, ions or other small particles across the it to achieve the physical separation and enrichment purposes, with the aim to reach high purity and yield. The driving force for the molecules is the pressure applied (hydrostatic pressure gradient).	These processes aim to enhance the efficiency and versatility, since they can be utilized in many exciting applications. They generally require lower energy consumption than distillation or LLE. They are relatively cheap and require few manual actions.	Fouling: a solution or a particle is deposited on a membrane surface or in its pores so that its performance is degraded. It can cause severe flux decline and affect the quality of the product and severe fouling may require intense chemical cleaning or membrane replacement. This leads to an increasing of the operating costs of a treatment plant. Membrane pollution is considered another obstacle, leading to by-product salt formation during the ion-exchange process. Furthermore, the membrane itself sometimes is quite expensive since it needs to be compatible with the solution filtered.	MF is characterized by a membrane pore size between 0.05 and 2 μm and operating pressures below 2 bars, it is used to separate particles and bacteria from other smaller solutes. UF → between 2 nm and 0.05 μm and operating pressures between 1 and 10 bars, it is used to separate viruses, colloids like proteins from smaller molecules. NF → between 0.5 and 2 nm, pressure between 5 and 40 bars, it is used to achieve a separation between sugars, other organic molecules,	[25, 26, 27, 35]

1.3 Liquid-liquid extraction for organic acids

Liquid–liquid extraction (LLE), also known as solvent extraction and partitioning, is a common method used to separate compounds by their relative solubility. It is among the most common initial separation techniques, though some difficulties result in extracting out closely related functional groups. [20] In LLE a net transfer of one or more species from one liquid into another liquid phase, generally from aqueous to organic, occurs.[9] The transfer is driven by chemical potential, once the transfer is complete, the overall system of chemical components that make up the solutes and the solvents are in a more stable configuration (lower free energy). This technology is widely used in the production of fine organic compounds, the processing of perfumes, the production of vegetable oils and biodiesel, and other industries.[24] LLE can be divided into physical and reactive extraction. While for the former the solubility/affinity

[9] Such as water (polar) and an organic solvent (non-polar).

determines distribution of the solute over the two phases, for the latter the ability of reacting with the extractant is the key.

1.3.1 LLE Physical extraction

For the physical extraction the affinity between the target product and the solvent is the only mass transfer force. [10] The solvent that is enriched in solute(s) is called extract, while the feed solution that is depleted in solute(s) is called the raffinate. (Fig. (9)) The most known organic solvents, for the physical extraction, used to separate organic acids from broth until now have been ethyl acetate, diethyl ether, and diethyl ether-hexane, but especially for DCAs have been 1-octanol, 1-decanol and butanol [8].

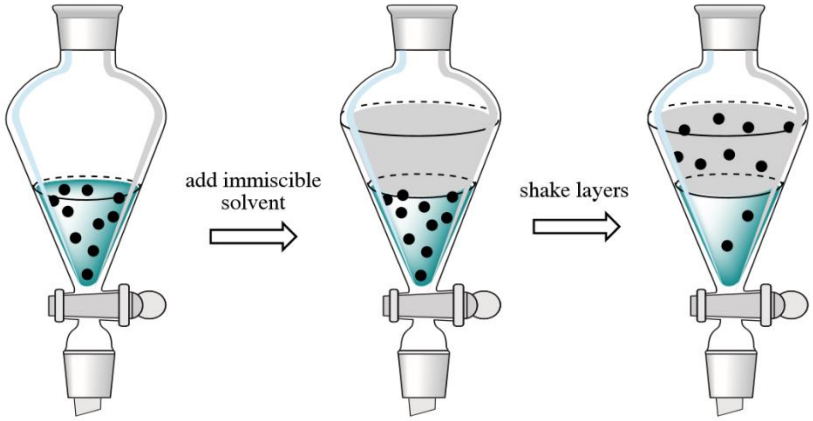


Figure 9: LLE scheme. [34]

1.3.2 LLE Reactive extraction

A more efficient method is the reactive LLE: 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. To make the desired product extract more easily, it is sometimes converted into another compound, or it is coupled with a reactive extractant to create a complex with. [8]. 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. [8] Thus, in

reactive extraction a diluent is often present: it can be active or inert based on its polarity. (Fig. (10))

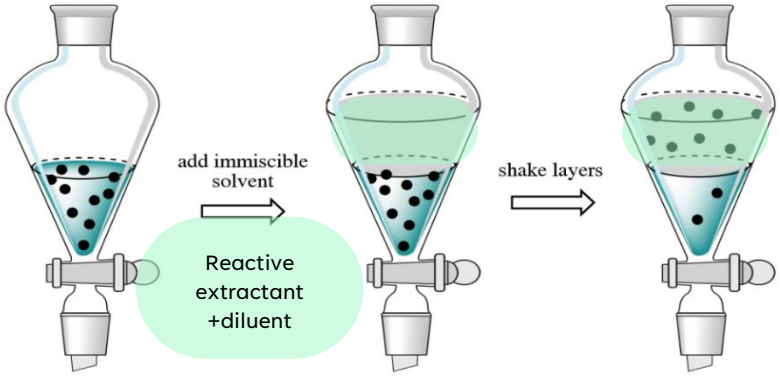


Figure 10: Reactive LLE scheme. [34]

The extent of complex formation is governed by the solubility of the extractant in the diluent and the strength of the interaction between the extractant and the solute. Hence, a diluent is used to control and improve physical properties like viscosity, density and surface tension which will affect the mass transfer and phase separation, and to stabilize the complex structure. Once the complex is formed it needs to be solvated in the extraction solvent. If the diluent does not have the required solvation power, 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. [5] Modifiers in general are less economical than diluents and do not provide enough good transport properties to be solely used with the extractant; long chain alcohols are the most frequently used modifiers. [5] They also influence the basicity of the solution and improve phase separation. [18]

1.3.3 Organic solvents for LLE

In bioprocesses coupled to LLE systems the pure extractants are often diluted with other organic solvents as a mean to enhance the biocompatibility, and to decrease the viscosity of the organic phase. As their high volatility would result in co-distillation with the target acid from the heavy extractant instead, solvents with low volatility are required to enable the acid to be recovered as a distillate; solvents that exhibit a high partition coefficient[10] (K_D) facilitate organic loading. The reactive extractant is used to transfer a solute from one liquid phase (usually aqueous) to another liquid phase, usually an organic solvent mixture (reactive

[10] The partition coefficient is introduced in the section "1.4.1 Parameters that influence acid loading in the organic phase".

extractant+diluent). [7] Organic solvents can be divided into two categories, diluents and reactive extractant, as shown in Fig. (11). Diluents can be classified as dissociating and non-dissociating, and as active (functional solvents) and inactive (hydrocarbon solvents). Dissociating solvents reduce the interactions between the oppositely charged ions through their high relative permittivity and facilitate acid dissociation upon complexation, while solvents with a low relative permittivity suppress dissociation, resulting in complexation between the amine and non-dissociated acids. Active diluents can participate in complex formation, e.g. through hydrogen bonding, while inactive diluents cannot. [3, 7] For the inactive diluents in fact, acids partition is based on hydrophobic interactions. [3] However, complexes in active diluents are seldom 1:1 in stoichiometry, and when the diluent is not stabilizing the extractant-acid complex, typically a second acid is involved that stabilizes the complex through hydrogen bonding (or even more acid molecules) [7]. Reactive extractant are divided into carbon bonded oxygen bearing extractants, phosphorus bonded oxygen bearing extractants and high molecular weight aliphatic amines.

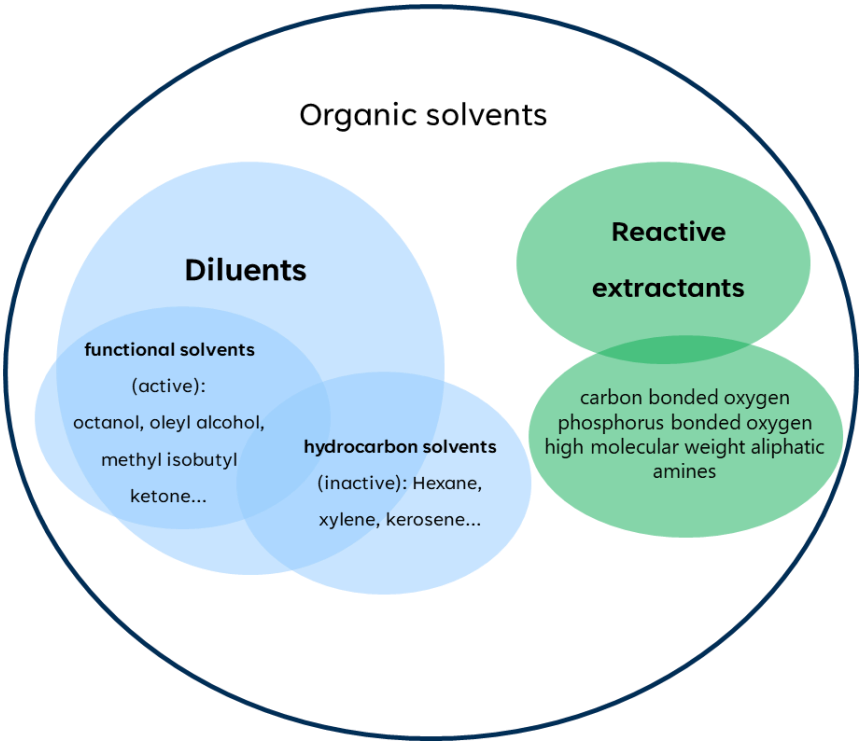


Figure 11: Schematization of organic solvents.

Hexane, xylene, and kerosene are common hydrocarbon solvents (inactive) used to dilute phosphine-oxide and amine based extractants, but these solvents are unsuitable for recovery of DCAs as overhead distillates due to their high volatility and their weak complex. [3] The functional solvents instead, which contain alcohol and/or ketone functional groups (e.g. 2-

octanol, oleyl alcohol, methyl isobutyl ketone), can form a hydrogen bond with DCAs and have been used successfully in the literature as extractant diluents. [3] Phosphorus bonded oxygen bearing extractants involve the solvation of the acid by donor bonds which are to be distinguished from strong covalent bonds and from ionic interactions while high molecular weight aliphatic amines involve a reaction. [10] Amine and phosphine-oxide based extractants are commonly employed to achieve good extraction performances and operate by forming either an ionic bond or a non-ionic hydrogen bond with DCAs present in the aqueous phase. [18] The choice of the reactive extractant that will be mixed with the diluent depends on the specific application, on the nature of the components to be separated, and on factors such as selectivity, efficiency, and cost-effectiveness. [18] DCAs 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 processes associated include hydrolysis, complexation, dissociation, and ion association in two phases, and finally phase equilibrium. (see Fig. (12)) [10]

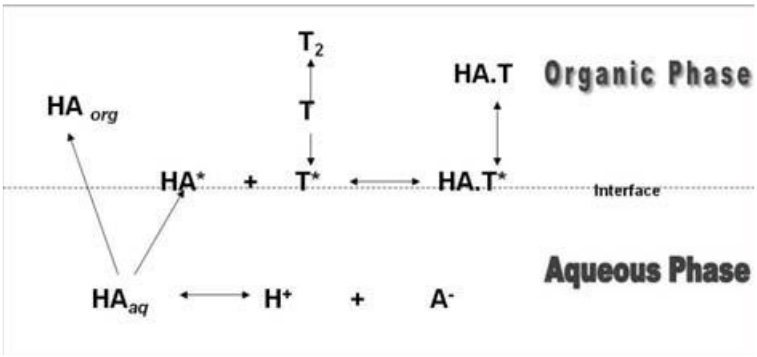


Figure 12: Mechanism of chemical equilibrium HA: acid, T: extractant [10]

The degree of ion-pair formation depends on the acid pK_a and the basicity of the amine, being important only if $pK_{a,amine} > pK_{a,acid}$. [18] In the case of reactive LLE for DCAs, the solvents that can form hydrogen bonds stabilize the dicarboxylic-acidtrioctylamine complex are preferred. [7] The state-of-the-art LLE solvent technology for the DCAs recovery is based on complexation with TOA, or the commercially available equivalent Alamine 336 (a mixture with C8-C10 alkyl chains), as complexing agent. Polarity and functional groups of the solvent and stability of the amine-acid complex are important factors to affect the yield. [18] Thus, alcohols are the most widely used species of solvents for this kind of reactive extraction and are expected to result in high extraction efficiencies. [7] The mechanism of chemical extraction

showed in Fig. (12) and described by the equations (2, 3) governed by the equilibrium constant (K_c). [10]

$$Solute + n.Extractant \rightleftharpoons Complex \quad (2)$$

$$K_c = \frac{[complex]}{[solute][extractant]^n} \quad (3)$$

The general steps for reactive extraction processes proceed as follows:

1. Contact with the Aqueous Solution
2. Formation of a Complex: In the extraction process, the reactive extractant is typically dissolved in an organic solvent, such as an aliphatic hydrocarbon or an organic phase and it generates a complex with the dissociated or undissociated form of DCAs.
3. Partitioning: The DCA molecules preferentially partition into the organic phase due to the formation of complexes. This partitioning is driven by the differences in solubility and chemical affinity between the organic and aqueous phases.
4. Phase Separation: After mixing and allowing the system to reach equilibrium, the two immiscible phases (organic and aqueous) are allowed to separate. The organic phase now contains the extracted DCAs.
5. Recovery: The organic phase enriched with the DCAs can be separated from the aqueous phase. Further processing, such as solvent evaporation, can be employed to recover the product in a concentrated form.

N-octanol is the most widely used solvent diluent for the reactive extraction of DCAs, although, the octanol-trioctylamine-water-carboxylic acid system is known to form stable emulsions. [5, 7] Unfortunately, the 1-octanol molecular level toxicity is too high to allow the direct coupling between LLE and a fermentation unit, thus, a separation between the microorganisms and the extraction process would be required to avoid molecular level toxicity. [7] Therefore, often decanol is used since it offers a good compromise between extraction efficiency and toxicity. [18] The Tab.(5) shows a selection of reactive extractants used in primary recovery of DCAs along with their solvents and modifiers (if required).[19]

Table 5: Selection of extractants used in primary recovery of CA and DCAs [19]

System	Extractant name	Functional group	Structure characteristics	Solvent (modifier)
Amine-based	N1923	Primary amine	Methyloctadecyl amine	1-Octanol Butyl acetate Hexane
	Primene® JM-T	Primary amine	Branched alkyl chains C16–C22	Kerosene
	Amberlite® LA-2	Secondary amine	Asymmetric alkyl chains C12–C15	Diethyl carbonate Methyl isobutyl ketone 1-Hexanol Kerosene (1-octanol)
	Tris(2-ethylhexyl)amine	Tertiary amine	2-Ethylhexyl alkyl chains	Kerosene
	Trihexylamine	Tertiary amine	Hexyl alkyl chains	1-Octanol
	Tri-n-octylamine (Alamine® 300)	Tertiary amine	Straight octyl chains	Kerosene Dodecane (1-decanol) n-Paraffins (isodecanol) Heptane (1-octanol, tripropylamine) Methyl isobutyl ketone Methyl isobutyl ketone (1-octanol) 1-Octanol
	Triisooctylamine	Tertiary amine	Isooctyl alkyl chains	Chloroform Heptane 1-Octanol
	Alamine® 336	Tertiary amine	Straight alkyl chains C8–C10	2-Octanol Kerosene 1-Octanol Decanol Cyclohexanone
Ionic liquids	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

In the past decade a new solvent class has appeared in the field of acid extractions: ionic liquids (ILs). [5] Ionic liquids are a group of organic salts, have drawn considerable attention and been developed rapidly. Most of them are imidazolium, quaternary phosphate, or quaternary ammonium salts, which are non-volatile, nonflammable, and liquid in a wide range of temperatures. [5, 18] Considering the (strong) intermolecular interactions between the ions of the ILs and the DCAs, they should be considered as a new promising class of reactive solvents, even if their cost is still an obstacle. [18] Their exploitation leads to extremely high distribution ratios[11] at very low acid concentrations, which is beneficial for recovery from dilute streams. They can be used in pure form as well as diluted with a co-solvent or diluent. [18] Thus, several extractants can be employed to enhance the solubility of the DCAs into an organic solution. The extractants that have been used for the case study presented are mainly the Aliquat 336 and TOA, that will be presented as follows. [18]

[11] The ratio of the acid concentration in the extract phase over the raffinate phase.

1.3.3.1 TOA

The reactive extractant TOA refers to Tri-octylamine, which is a commonly used chemical compound in reactive extraction processes. It is an organic compound with the chemical formula $(CH_3CH_2CH_2)_3N$, and it belongs to a class of chemicals known as tertiary amines. [29] It has melting point of −34 °C, boiling point of 365-367 °C at standard conditions; density of 0.810 g/mL and viscosity of 7.862 mPa.s. [29] It is known for its ability to extract various metal ions from aqueous solutions into organic solvents. It is often used in hydrometallurgical processes for the separation and purification of metals.

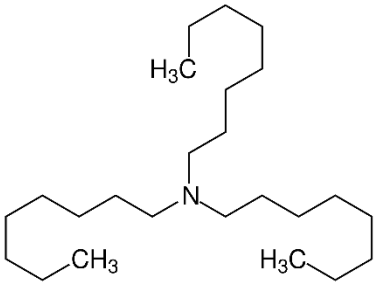


Figure 13: TOA [29]

In solvent extraction processes, TOA can form complexes with metal ions in the aqueous phase and transfer them into an organic phase. [30] This property makes TOA extractant valuable in industries such as mining, metallurgy, and chemical processing, where the extraction and separation of metals are crucial steps in production. It is often employed in solvent extraction processes for the separation and purification of various organic compounds, including DCAs. [29, 30] It can indeed be used for the recovery of LDCAs like dodecanedioic acid (DC12), hexadecanedioic acid (DC16), or octadecanedioic acid (DC18) in their undissociated form. The efficiency of this extraction process depends on various factors, including the choice of organic solvent, the concentration of the extractant, the pH of the aqueous solution, and the specific DCAs involved. [29] Optimization of these parameters may be necessary for a particular application. Furthermore, the recovered DCAs may need further purification steps, depending on the desired level of purity and the presence of impurities in the organic phase. [29,30]

1.3.3.2 Aliquat 336

Aliquat 336, also known as tricaprylmethylammonium chloride, is a quaternary ammonium salt used as a phase transfer catalyst and metal extraction reagent. [31] Aliquat 336 is well known for its versatility and is used in a wide range of chemical and industrial processes that

involve the transfer of ions or compounds between different phases. Its specific applications can vary depending on the process and the chemicals involved. [16] Aliquat 336 has a long hydrophobic tail (composed of three octyl groups) and a positively charged quaternary ammonium head (Fig. (14)). It is a colorless viscous liquid with a density of 0.884 g/cm^3, a boiling point of 225 °C (437 °F) and a flash point of 113 °C. [31] This compound is employed for solvent extraction of metals, acting as a liquid anion exchanger. It is often used diluted in hydrocarbon solvents since its high viscosity (1500 mPa·s at 30 °C). [31]

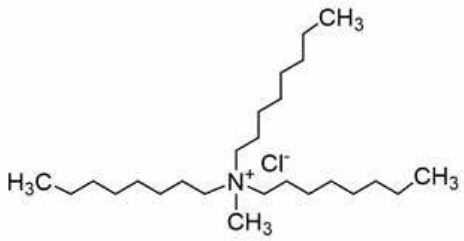


Figure 14: Aliquat 366 [16]

In solvent extraction is used to extract various chemical species from aqueous solutions into organic solvents. Furthermore, it is used in the ion-exchange chromatography where it can serve as an ion-exchange resin for the separation and purification of ions in aqueous solutions. Due to its amphiphilic nature (having both hydrophilic and hydrophobic parts), Aliquat 336 can also function as a surfactant or wetting agent in various applications. While tertiary amine extractants are very effective in capturing undissociated form of DCAs, ionic extractants, such as Aliquat 336, forms an ionic bond with the different acid's structure (see Fig. (15)).

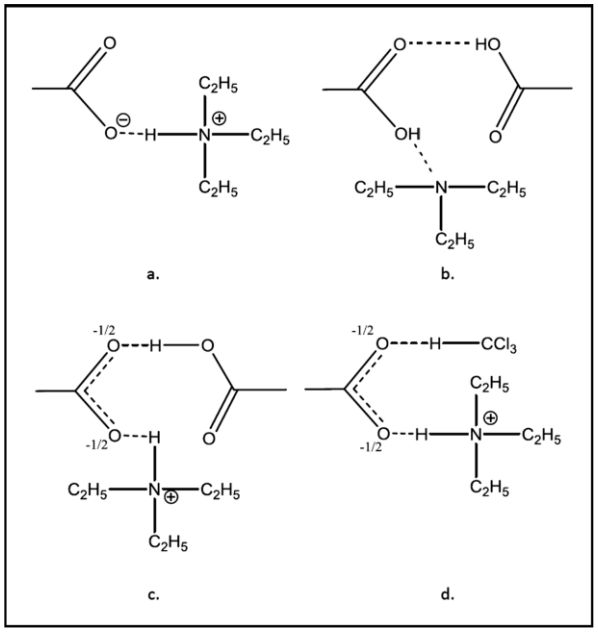


Figure 15: Mechanisms for interaction between acetic acid and triethylamine (similar compound to Aliquat 336), (a) in dissociating solvents, (b) in completely inactive diluent, (c) in non-dissociating solvent and (d) in chloroform. [18]

When it is employed in the solvent extraction of DCAs the complex formation will occur both with the dissociated and undissociated form of the acid. [31] For this reason, it requires a salt-breaking step to be employed before free acids can be recovered from them. [18] Unlike ionic extractants, the bond formed between a DCA and a non-ionic extractant can be thermally broken during distillation and the acid recovered directly in the column overhead. [18] When studying the effect of pH on the distribution coefficient of the DCAs it is verified that Aliquat can extract both the undissociated and dissociated forms of the acid. Even though a quaternary ammonium group can 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's counter ion) impeding hydrochloric acid formation as ion exchange product. Therefore, at low pH conditions extraction of uncharged DCAs by ammonium groups occurs by other mechanisms such as hydrogen bonding. [31, 16]

1.3.4 Operational parameter incidence on LLE

For DCAs, self-stabilization by interaction of both acid groups may occur but depends on the molecular structure. [10] Effect of temperature affects several factors in liquid-liquid equilibria of acids with amines, i.e., a small effect on the acid pK_a, increasing acid solubility in organic solvents, and even more in water with increasing temperature. [18] The most important

dependency is the decreasing acid-base complexation strength with increasing temperature; for this reason, reactive extraction with tertiary amines is much stronger temperature dependent than physical extractions. [18] Water co-extraction also increases with increasing temperature, while acid extraction decreases. [10] In case of phosphorous based extractant (tri-n-octyl phosphoric acid) and high molecular weight tertiary amines (tri-n-octyl amine, Alamine 336), extraction decreases with increase in pH and in case of quaternary amine it has certain optimum pH. [10]

1.3.5 Liquid-liquid extraction disadvantages

LLE, both physical and reactive, are valid methods to extract products from a solution. Nevertheless, in the chemical industry the challenging liquid separations at molecular scale, more specifically with the separation of different molecules with similar physical properties, is a concern. [4] LLE can offer a solution, but it also figures out limitations in chemical production:

- the separation is only possible if solute-solvent affinity is different for the components which have to be separated;
- a relative high solvent volume is required;
- an extra step is required to recover the product from the extractant phase;
- solvent choice is limited by amongst other density and surely in biological processes toxicity.

Even if this technique could be considered competitive in the extraction field in chemical processes, it could not be the same for food and pharmaceutical industries. The reason why this method could not be applied in the latter fields is that with LLE, the purity of the product extracted will always be less than the 100% (due to small percentage of extractant present in the recovered product). [5] In these industries high recovery and purity percentage of the products are required since they will enter in contact with the human body, and they could affect the entire population health. Especially the use of solvent for recovery is not allowed in these fields since some small percentage of it could remain in the final product. [5] When solvents are used, further separation processes are required, such as distillation or membrane technology. [13] A typical example of separation system in which a more advance method based on LLE should be employed is the DSP of bioproduction.

In the bioproduction processes the bioreactors with inoculum inside are connected to a separation system. The aim of the latter is to collect the extracellular products of the microbes that are released in the broth. [12] So, the first liquid to be recovered is the broth, that is in contact with the living organisms. To recover it, we need to control certain operative parameters such as operative conditions of pH, temperature, pressure, transmembrane pressure and so on. The role of the chemical engineer is to choose the best method to recover the products satisfying all these conditions. These conditions could not be satisfied in the case of the use of LLE, since the broth, which contains the living organisms, would be in contact with the solvent, which could damage them because of its toxicity. [15] At the same time the use of a solvent is required, since its potential recovery of extracellular products, such as DCAs, is high. Thus, the methods which allows to recover products in a proper way could be distillation processes, where the products contained in the broth could be separated in an efficient way from the solvent, but further processes would be required to extract the target product from the solvent used in distillation. An alternative method could be membrane extraction (ME), which implies the use of a physical separation (MBSE), or membrane reactive extraction (MBRE). [10]

1.4 Membrane Extraction

ME is a particular type of MC: MC can be in fact adapted to several applications according to the phases involved, to the profiles of the driving forces, and the main kind of polarization that may occur [40]. Several MC configurations such as flat sheets, capillary membranes, or hollow fiber are available but usually, as commercial contactors, hollow fiber are preferred because of their large surface area. [36] The Fig. (16) shows a summary of the different MC applications.

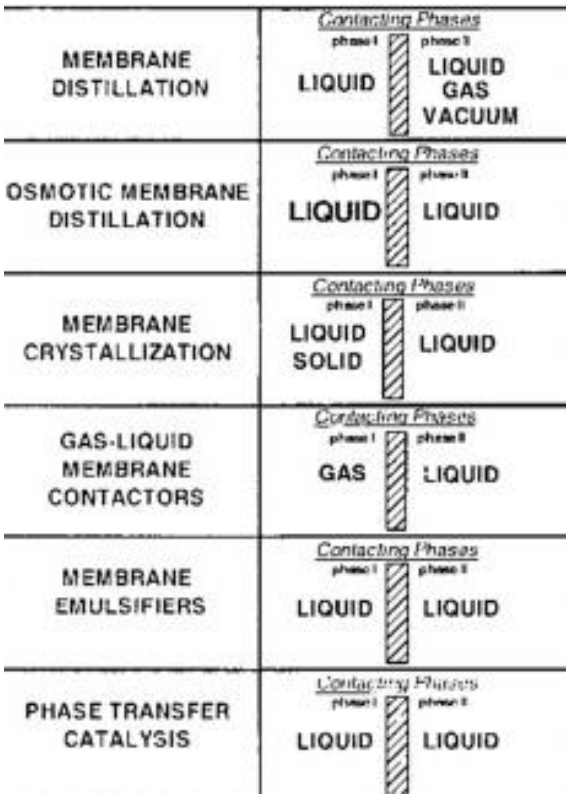


Figure 16: Overview of membrane contactors [40]

While in the pressure-driven membrane processes, the driving force for the separation is the pressure difference across the membrane and it acts as a selective barrier allowing certain molecules or ions to pass, in MC, if a microporous membrane is used as in the case of DCAs extraction, the membrane is not able control the transport between the two adjacent phases. [39] A microporous membrane is defined as a thin-walled structure having an open spongy morphology of precisely controlled pore size, typically ranging from 0.03 μm to 10 μm in diameter.[41] Therefore, when microporous membranes are employed they only covers the function to keep the two phases separated and in contact at the same time [37, 39] On the other hand, if the membrane pores are small enough (tight membrane), interactions with the membrane will play a role and in extreme cases, the membrane can take over the role of the

solvent.[41] ME for DCAs extraction process can be realized through the simultaneous application of MC and of LLE, as showed in the Fig.(17), providing in such way a contact area between the two phases with interesting properties. [36] Indeed, MC act as a barrier which prevents phase dispersion and emulsion formation near the contact areas, offering interesting opportunities for process intensification through its flexibility, compactness and modularity. [36]

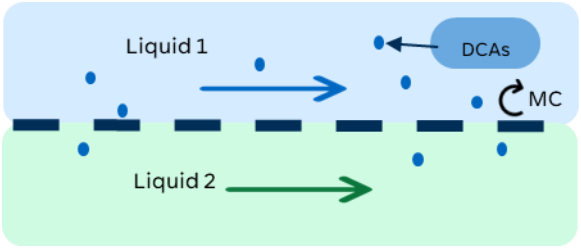


Figure 17: Scheme of a membrane contactor (MC) employed in ME of DCAs.

As showed in the Fig.(17) the liquid 1, which contains the target molecules (DCAs), circulates on one side of the membrane while the liquid 2 on the other. As time passes, DCAs are transferred from one phase (liquid 1) to the other (liquid 2). [36]

1.4.1 Membrane-based solvent extraction (MBSE)

Membrane physical extraction system exploits the presence of a solvent in contact with the aqueous solution through the MC. The aim of the solvent is to create a selective mass transfer of the target product from one side (aqueous phase) of the membrane to the other, where the solvent is present (Fig. 18). [36]

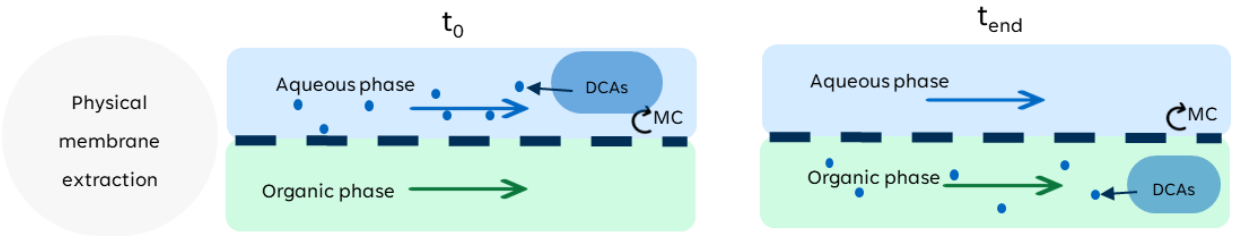


Figure 18: Scheme of physical membrane extraction process with MC.

This method is substantially the application of the LLE with a separation layer between the two phases, which is realised thanks to the membrane; especially for the recovery of organic acids this procedure shows high efficiency. [36] In particular, the physical extraction of DCAs

consists of two immiscible phases, namely the stationary phase (aqueous phase) and the transport phase (organic solvent or supercritical fluid) as is reported in the Fig. (19). [15]

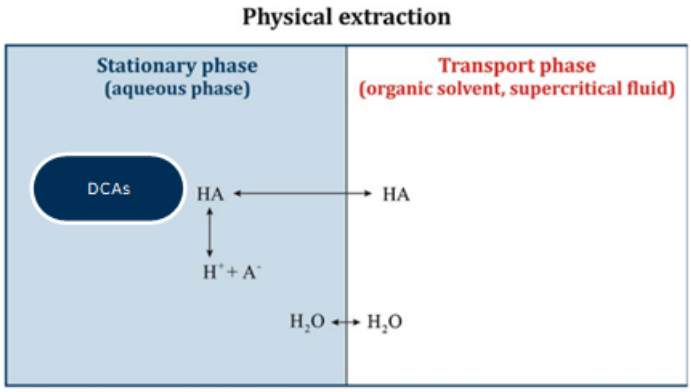


Figure 19: Schemes of physical extraction process of acids from aqueous solution through a membrane [15].

The acid (HA) is transferred from the aqueous phase into a transport phase until thermodynamic equilibrium in the multiphase system is reached. [15] The solvents that can be used to perform this method can be several: polar-nonpolar, protic-aprotic, inorganic-organic, natural solvents. [10] In particular, for the recovery of DCAs, 1-octanol, 1-butanol and 1-decanol are the most used[12]. [10, 13, 15] MBSE can be also used for various tasks such as separation, selective extraction of products, retention of the catalyst, distribution/dosing of a reactant, and catalyst support. It is suitable for performing equilibrium-limited endothermic reactions due to their ability to enhance conversions compared to the equilibrium value. [15] Reversible reactions are usually limited by thermodynamics: when direct and reverse reactions, whose rate depends on reactants and product concentrations, are balanced, a chemical equilibrium state is achieved. If temperature and pressure are fixed, the equilibrium state is a constraint for the ratio of products versus reactants concentrations, obstructing the possibility to reach higher conversions. [5] A solution offered by MBSE could be to remove a product of the reaction: in this way, the system cannot reach equilibrium and the reaction continues, reaching higher conversions.[15]

1.4.2 Membrane-based reactive extraction (MBRE)

MBRE system has shown efficient results in the field of organic acids recovery since it allows a selective transport of the target product thanks to the complexing agent presence (Fig. (20)).

[12] The solvents are the same that have been already analysed in the chapter "1.3.3 Organic solvents for LLE".

[15] Usually the complexation reactions with bond energy less than 50 kJ/mol are similar to ordinary associations by van der Waals forces in the condensed state and can readily be reversed. [10] This kind of process applied to the DCAs involves two immiscible phases: a stationary phase (aqueous phase that includes the DCAs) and a transport phase (organic solvent or supercritical fluid) containing a complexing reactant.

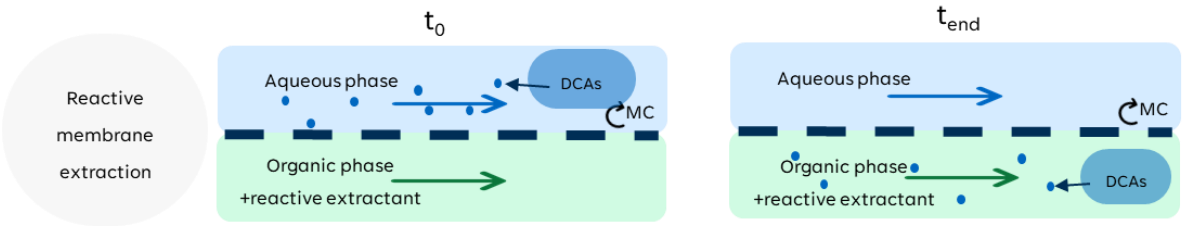


Figure 20: Reactive membrane extraction [36]

As previously mentioned, complexing reactants used in reactive extraction processes have high viscosity and corrosivity thus, they must be dissolved in solvents (diluent) beforehand. The type of applied diluent plays an important role in MBRE. Further, the solvent properties affect the structure of complexes formed by the reactant with the acid, as well as the formation of the third phase in the system. For example, proton-donating solvents can form hydrogen bonds with the complex and thus, stabilize its structure.

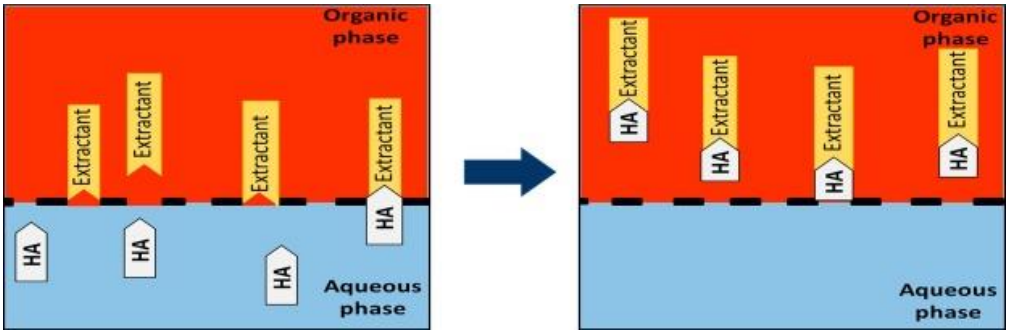


Figure 21: Scheme of reactive membrane extraction process with MC.

The complex formation occurs between the acid (HA) and the complexing reactant generating $(HA)_a(G)_b$, which is soluble in the transport phase (Figure 22). In the complex structure "a" denotes the number of molecules of the acid while "b" the number of molecules of the complexing reactant. The reaction of the complex formation is reversible.

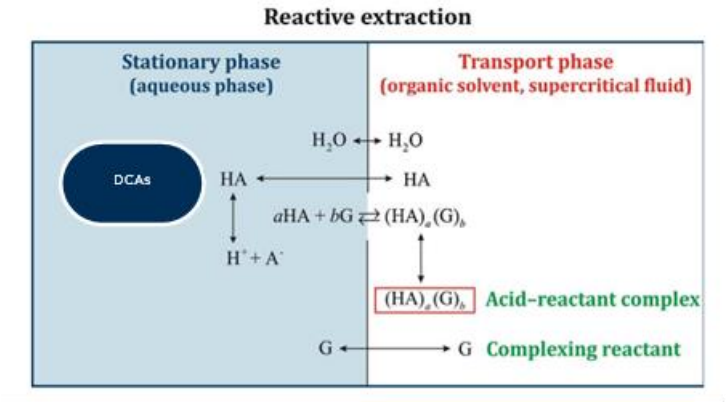


Figure 22: Schemes of reactive extraction process of acids from aqueous solution. [15]

As cited in the section regarding reactive LLE, the complexing reactants most employed for the recovery of DCAs from fermentation broths are, also for the MBRE, the high-molecular weight aliphatic amines and organophosphorus compounds. Among the high-molecular-weight aliphatic amines, tertiary amines are the most effective reactants widely applied in the separation of DCAs. [10, 15] The mechanism of complex formation between DCAs and the tertiary aliphatic amine is based on hydrogen bonding or ion-pair formation. Both forms of complexes may coexist simultaneously in the transport phase at equilibrium. The mechanism of complex formation strongly depends on the basicity of the amine and on the DCAs acidity, as well as the properties of the solvent and the pH of the aqueous phase. [15] Among the organophosphorus compounds, tri-n-butyl phosphate and tri-n-octyl phosphine oxide are most used as the complexing reactants. Unfortunately, organophosphorus compounds have been found to be more expensive and less effective than tertiary amines. [15]

Both MBSE and MBRE methods can be coupled directly with the bioproduction process of DCAs. The separation of inhibitory compounds as they are produced in biotransformation and fermentation systems is known as "*in situ product removal*" (ISPR). It can be realised by coupling the bioreactor (fermentor), where the microorganisms are producing the target product released in the fermentation broth, with the MC unit. Thanks to the membrane layer, microorganisms can be protected and separated from the solvent.

1.4.3 In situ product removal (ISPR)

ISPR exhibits improved sustainability characteristics in applications including continuous flow reactors, biofuel fermentation and waste utilization. It allows the fast removal of extracellular products, thereby preventing its subsequent interference with cellular or medium components. [5] This technology requires low energy consumption and minimal chemical addition, its Environmental (E)-factor (kg of waste/kg product) can be reduced by a factor of 96.4% with respect to conventional methods. [5] The coupling of the fermentation with separation, including extraction, resin, and membrane, leads to realize a continuous process. The continuous set up system includes a bioreactor, which can be fixed bed type, batch or continuous, coupled with nanofiltration, anion exchange chromatography, or membrane extraction (Fig. (23)).[5]

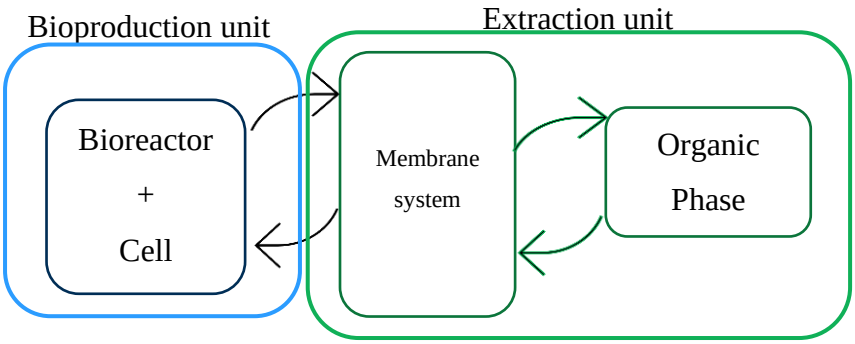


Figure 23: ISPR scheme applied to bioproduction.

As shown by the Fig. (23) in-situ *extractive* technology can be implemented, by introducing an organic phase able to selectively extract the target product from the fermentation broth. ISPR allows a selective separation of the product during its fermentation production stage, improving the volumetric productivity by alleviation of product inhibition and leading to a decrease in DSP costs. [5] According to different sources, the productivity of the extractive fermentation process is in fact 4.3 times higher than conventional DSP processes, thanks to the lack of inhibition. [28] For a successful implementation of it, the extractant should be not toxic to the cells and should even be efficiently extracting at relatively low product concentration. [28] The main advantage of this technology is that by removing acids from the broth promptly, the product inhibition is reduced, and pH is controlled, leading to higher utilization of feedstock, improvement of productivity, reduction of DSP load, and total cost reduction. By removing the target product from the fermentation broth, the probability of side reactions decreases while

the process flows required[13] is reduced and the yield is enhanced. [5] Moreover, the removal of products can minimize the toxicity of products to microorganisms. In the case of acids, the broth pH will decrease during fermentation but, thanks to ISPR, it can be controlled by balancing fermentation and extraction instead of using base addition. [8] Moreover, the phase separation characteristics of the extraction system (density difference, viscosity and interfacial tension) should not impact adversely the bioconversion itself. [28] The path to follow to detect the best method to recover the target product coupled with the presence of a bioreactor can be schematized as shown in the Figure (24):

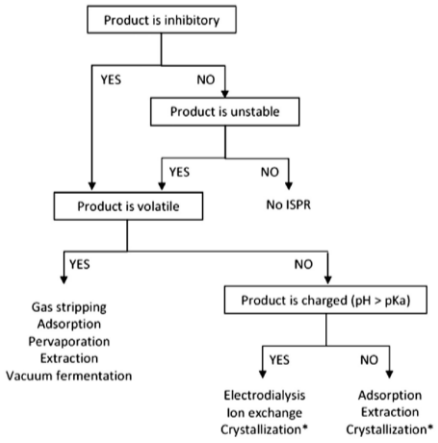


Figure 24: Heuristics for selection of an appropriate ISPR technique [2]

The classification proposed is based on the physical characteristics of the product. It may also be noted that although ISPR is predominantly used to remove the target product from the production medium, it may also be used in certain cases to separate inhibitory by-products whose high concentrations affect the titer of the target compound (e.g. a protein). [2] There is to notice that based on this selection procedure, extraction seems to not be the best option at all pH, but the in case in which the pH selected is higher than the pK_a, as in the case of DCAs, extraction is one of the viable ways. A possible set up of the ISPR could be as the one presented in the Figure (25):

[13] Meaning an amount decrease of wastewater per weight unit of product.

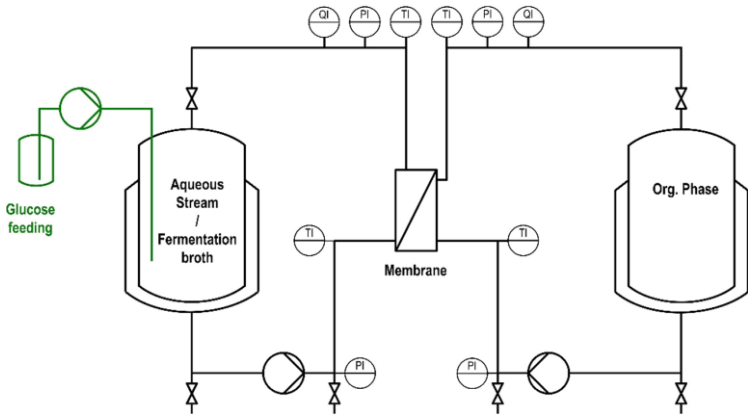


Figure 25: Set up of ISPR. [7]

Two gear pumps are used to pump the phases through the pressure driven membrane system and back into the container. The pressure of both phases can be adjusted by needle valves. Flow meters record the flow as well as the density of both phases. For continuous fermentation experiments, a glucose-feeding[14] pump can be connected to the aqueous phase vessel. The ME separation setup needs to be cleaned after every experiment using acetone, then dried using oil-free compressed air. When this system is used with microorganisms, the setup needs to be disinfected with 70 vol% ethanol for 20 min. Afterwards, the setup is purged with ethanol and dried using oil-free compressed air. [7]

1.4.4 ISPR for DCAs

In most cases, bioproduction of DCAs are carried out batch wise followed by separation of the product from the biomass and further purification of the target compounds.[2] In the last decades attention shifted more and more from DSP to ISPR since the products from a reactor can be concentrated while solvent loss can be minimized by using continuous separation techniques. Furthermore, it leads to increased productivity and yield due to a decrease in product inhibition and induces spontaneous regulation of the pH in the reactor without adding equimolar amounts of base [6]. Different configurations are possible depending on the location of the separation unit (internal or external). Main separation involving ISPR technologies for DCAs technique are extraction, adsorption, evaporation, precipitation/crystallization, electrodialysis, complexation and membrane-assisted separations. [6] Among them, the most

[14] Glucose could be the feed for the microbial strains inside the bioreactor, which need to be fed in order to perform fermentation.

frequently applied for acid recovery are extraction, electrodialysis and ion exchange when fermentation is operated at a pH above the dissociation constant of the organic acids. [6] The ISPR method realised coupling DCAs bioproduction and ME, is shown in the Fig. (26). DCAs are produced by living organisms during fermentation inside a bioreactor (1) as extracellular products. They need to be separated from the fermentation broth through the MC, in which a mass transfer occurs: the DCAs are extracted from the fermentation broth into a solvent stream, which flows in counter current (2) with respect to the broth stream. The bioreactor media and cells are recycled (3) while the organic phase is passing into a distillation column (4) where neat free acids are vaporized (5) recovered as a distillate from the heavy organic phase (6).[6]

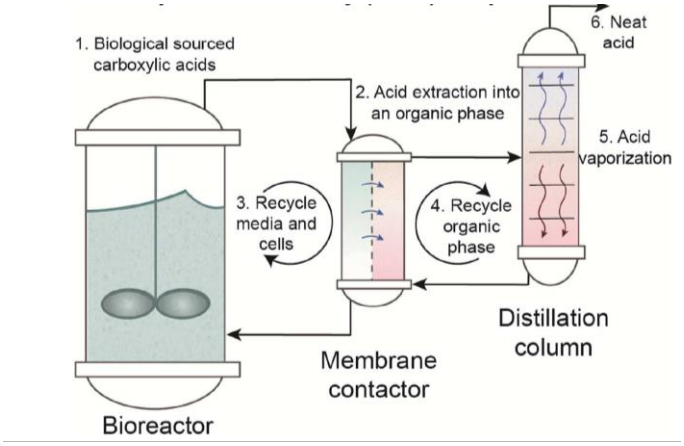


Figure 26: ISPR coupled with distillation [6]

This method shows how, a ME based ISPR system can be integrated with downstream distillation to selectively purify free DCAs. Thanks to multiple extraction-distillation cycles a complete recovery of acids from the extractant and recyclability of the organic phase can be reached. This system, applicated to the recovery of acetic acid, integrated with downstream distillation, has an estimated carbon footprint of less than 0.36 kg CO_2/kg, and provides a green approach to enable both new industrial bioprocesses. [2] By using a membrane as interface for the extraction, emulsion formation can be prevented and there is more flexibility in solvent/feed ratios lowering the total solvent consumption. [6] One of the disadvantages of this method is the difficulty of finding a common biocompatible solvent that has a high extraction coefficient for the product, as well as the ability of the solvents to function at the fermentation pH (ranging between 5.5 and 7), which is the interesting range for DCAs production. [2] Anyway, the application of ISPR can be proposed in several configurations according to the target product needed to be recovered. For instance, since LDCAs are difficult to extract by distillation

because of their high molecular weight, so the setup of the previous case (Fig. (26)), has been replaced by a back-extraction unit Fig. ((27)).[36]

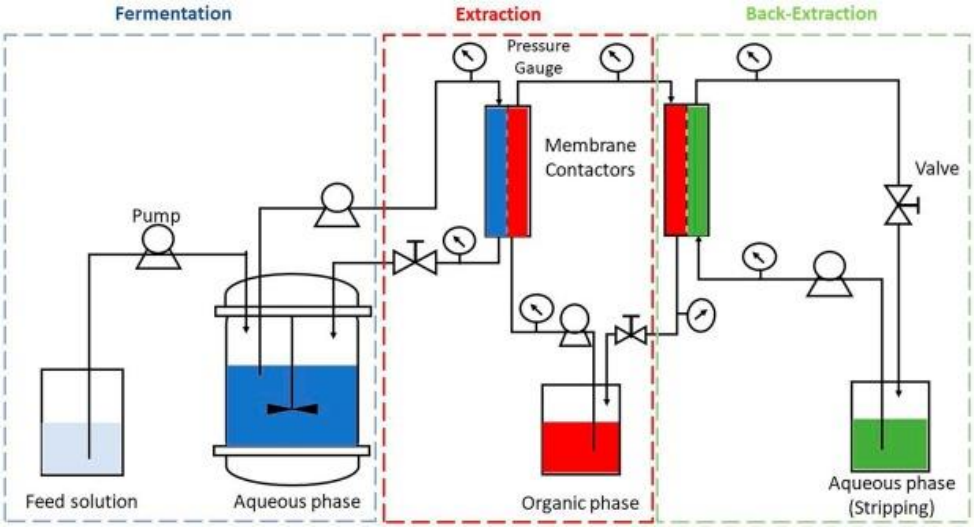


Figure 27: Implementation of LLE of organic acids assisted by MC in a relevant ISPR approach [36]

New technologies need to be investigated to back-extract LDCAs with the highest purity yield.

1.5 Solvent regeneration and acid recovery

Several regeneration methods for the recovery of the solvents and acids after acid extraction have been proposed in the past decades. In case of volatile acids, volatile solvents or both, the simplest option appears to be evaporation or distillation of the acid directly from the solvent, or vice versa [18]. Regeneration, in the case of acids that are reasonably volatile might be performed through direct evaporation of the acids from high boiling solvents. In such case, the theoretical heat duty required for it corresponds to the heat of evaporation of the acid and of the co-extracted water, and subsequent distillation of the aqueous acid stream. [18] But, when the acid is not volatile, such as the case of LDCAs, other regeneration strategies need to be followed, such as back-extraction or reactive back extraction. [18] In order to back-extract the bound acid into water, the acid complexes, if present, need to be split. When the complexes are present, they are usually stabilized by hydrogen bonds with the solvent (such as n-decanol and n-octanol). With the addition of another solvent which cannot donate or accept hydrogen bonds (like heptane) the nature of the complexes and the stoichiometry changes and the complexation constant can be reduced [7]. Additionally, an increase in temperature of the organic phase could reduce the complexation constant even more, increasing the acid recovery rate. However, the

added solvent needs to be removed after back-extraction. Ther Fig. (28) shows a typical set up of back-extraction, applied for CA, but suitable also for LDCAs.

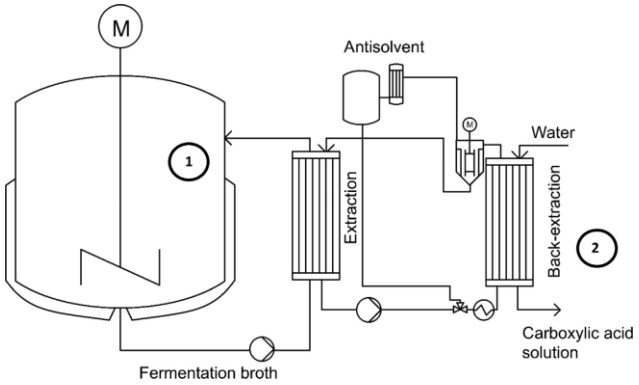


Figure 28: Setup for a fermentation with in-situ removal of CA coupled with back-extraction.[7]

Lactic acid is produced in a fermenter ①, extracted into 20 wt% TOA in n-decanol and then back-extracted in a temperature and solvent-swing process (using heptane as antisolvent) into a pure water stream. [7] Due to the significant boiling point difference of heptane and n-decanol (98 °C resp. 230 °C under ambient pressure), heptane can be removed out of the organic phase by evaporation after the back-extraction.

1.6 Extraction parameters: the equilibrium constant

When the DCAs create a complex with the solvent, a chemical equilibrium is achieved. The chemical equilibrium can be studied introducing the equilibrium constant (K_c). K_c is a measure of the ratio of the concentrations of the products to the reactants, each raised to the power of their stoichiometric coefficients in the balanced chemical equation. [10] The equilibrium constant for acids, including DCAs, is represented by K_a. The K_a values can vary depending on the specific acid in question, it requires experimental determination since is influenced by operational and physical factors such as temperature, pressure and organic phase (solvent). [6] The aim of the organic extractants is to achieve the highest K_a of the target molecule for the organic phase. [6] To calculate it in the solvent, the case of phosphine-oxide based organic phase will be followed[15]. From the following equations (4, 5) is possible to see how to determine the equilibrium constant for complexation of free acid in the aqueous phase with an extractant in the organic phase ($K_{EQ,EXT}$ equal to K_a) for an acid:

$$\mathrm{HA\ (AQ) + n \cdot EXT\ (ORG) \overset{K_{EQ,EXT}}{\longleftrightarrow} HA - n \cdot EXT\ (ORG)} \tag{4}$$

$$K_{EQ,EXT} = \frac{[HA{-}EXT_{ORG}]_{EQ}}{[HA_{AQ}]_{EQ}[EXT_{ORG}]_{EQ}^{\,n}} \tag{5}$$

Where $[HA_{AQ}]_{EQ}$ is the free acid concentration in the aqueous phase at equilibrium (mol/L); $[EXT_{ORG}]_{EQ}$ is the concentration of extractant in the organic phase at equilibrium (mol/L) which is determined from a mole balance. $[HA - EXT_{ORG}]_{EQ}$ is the concentration of the acid-extractant complex in the organic phase at equilibrium (mol/L), and n is the stoichiometric coefficient for the extractant [6]. Eq. (4) is used with an assumed n-value of 1 and with experimental equilibrium acid concentrations to determine $K_{EQ,EXT}$ of organic acids for the extractants. The goal of the extractant is to react with the HA and to build a complex (HA-Extractant) of which the molar production is governed by the equilibrium constant, as is schematized in the following Fig. (29):

[15] according to the font [6].

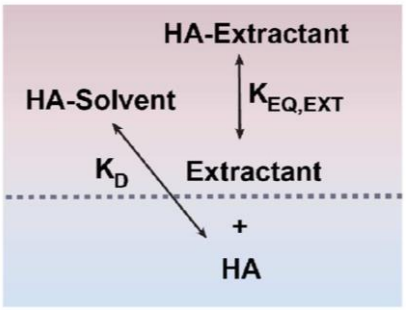


Figure 29: Free acids distribution between an organic and aqueous phase. [6]

The case of two widely employed non-ionic extractants, trioctylamine (TOA), and Cyanex 923[16], in equilibrium with different organic acids, is reported in the Fig. (30). They have been chosen as model extractants in different studies since they are commercially available, miscible with diluents, have relatively low freezing points and have lower toxicity than shorter chain derivatives.

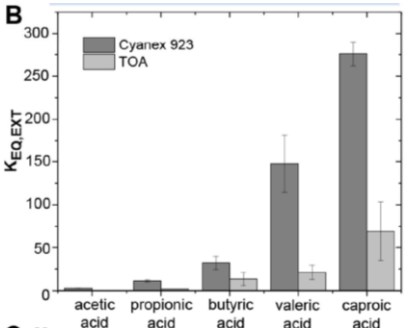


Figure 30: The equilibrium constant of different acids [6]

As shown, different short chains of carboxylic acids (SCCAs) have been tested: acetic, propionic, butyric, and valeric acid and caproic acid. From the results of the Fig. (30) reported above, the equilibrium constant of phosphine-oxide based extractant Cyanex 923 is higher than that of TOA. Thus, since the driving force for extraction is higher for Cyanex 923 over TOA, Cyanex 923 is more useful in ISPR systems to prevent product accumulation and growth inhibition in a bioreactor. [6] Instead, if tertiary amines, in the reactive extraction, are employed alone, only the undissociated form of the acids will be extracted. This means that, according to the pK_a of the CAs[17], the process is more efficient at low pH, since at low pH the percentage of undissociated form is higher. [7] However, when working with fermentation broths, the pH must be kept higher than 5 for optimal culture conditions. In the case of ME (in particular in

[16]Cyanex 923 is a mixture of phosphine oxides.

[17] The pK_a for the carboxylic and dicarboxylic acids changes according to the chain length, an average value of it is 4. [29]

the case of microporous membrane), the presence of the membrane does not influence the phase equilibrium in any way since it is non-selective, thus the equilibrium constant is still calculated through the Eq. (5).

1.6.1 Parameters that influence acid loading in the organic phase.

The ratio of the concentration of acid in the organic solvent to the concentration of undissociated acid in the aqueous phase, at equilibrium, can be expressed through K_D as is showed in the Eq. (6). [3]

$$K_D = \left(\frac{[HA{-}S_{org}]_{EQ}}{[HA_{AQ}]_{EQ}}\right) \quad (6)$$

K_D is called the partition coefficient of free acids into the solvent, $[HA_{AQ}]_{EQ}$ is the free acid concentration in the aqueous phase at equilibrium (mol/L), and $[HA{-}S_{ORG}]_{EQ}$ is the concentration of the acid solvated by the organic solvent at equilibrium (mol/L). [6] The extraction efficiency (EE%) can be expressed as follows:

$$EE_{DCA}\,(\%) = \left(\frac{C_{DCA,org}}{C_{DCA,acq}+C_{DCA,org}}\right) \cdot 100 \quad (7)$$

Where C_{org} is the concentration of the component in the organic phase after extraction and C_{aq} is the concentration of the component in the aqueous phase after extraction. [3] Accordingly, there are five key drivers that determine the EE% at equilibrium:

1. the total initial acid concentration in the bioreactor (Fig. 31A),
2. the initial pH (Fig. 31B),
3. the volume ratio of the organic to aqueous phase (V_R) (Fig. 31C),
4. the extractant concentration in the organic phase
5. the solvent selected for extractant dilution

As can be seen in the Fig. 31A, the concentration of the acid-extractant complex in the organic phase at equilibrium (mol/L) ($[HA - EXT_{ORG}]_{EQ}$) has a straight linear trend for the long chain of CA, correspondent to a linear increasing of their initial concentration. From the experimental studies in fact, $K_{EQ,EXT}$ increases with increasing acid chain length which results in a higher

equilibrium acid concentration in the organic phase. Along with decreasing the volume ratio of the organic to aqueous phase, the acid's concentration increases in the organic phase (Fig. 31C). As the V_R approaches zero, the equilibrium aqueous acid concentration approaches the initial total acid concentration and the acid concentration in the organic phase increases exponentially. [6] The Fig. 31B shows the influence of pH on the equilibrium acid concentration in Cyanex 923 at a constant $V_R = 0.2$, where the equilibrium acid concentrations were normalized to a pH of 3. [6]

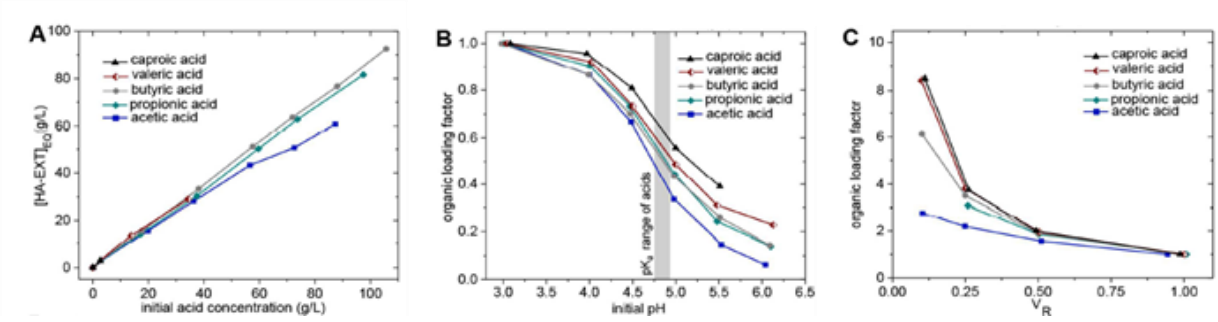


Figure 31: The equilibrium concentration of acid in the organic phase ($[HA\text{-}EXT_{ORG}]_{EQ}$). [6]

The free acid concentration in the aqueous phase rapidly decreases as the pH drops below the pK_a, and as a result, the acid concentration in the organic phase also drops off rapidly near the pK_a of the acid. The pK_a values of CAs and DCAs generally fall in a range of approximately 2 to 5, although there can be variation depending on the specific compound and experimental conditions. [6] The pK_a value represents the acidity of the acid, specifically the negative logarithm (base 10) of the equilibrium constant for the dissociation of the acidic proton (H^+) from the carboxyl group (COOH) in water. [29] LDCAs, such as DC12, DC16 and DC18, have two carboxylic groups and the pK_a values for each of them can vary slightly depending on the specific isomer and experimental conditions. The approximate pK_a values can be found in literature and it is for all of them the same ($pK_a \approx 4{,}48$) [29] When these values are compared with those of alcohols, such as ethanol ($pK_a = 16$) and 2-methyl-2-propanol ($pK_a = 19$), DCAs result stronger acids.[29]

Furthermore, electronegative substituents near the carboxyl group act to increase the acidity. In a water solution each extremity of a DCA composed by a carboxyl group, reacts with the hydrogen from H_2O, releasing H_3O (Fig. (32))

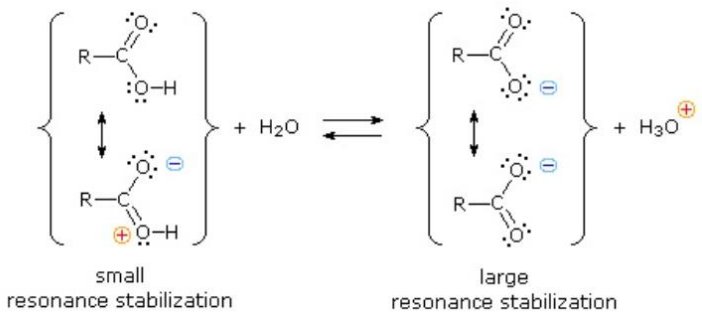


Figure 32: Carboxyl group in water solution [17]

Water is the standard base used for pK_a measurements; consequently, anything that stabilizes the conjugate base ($A:^{(-)}$) of an acid will necessarily make that acid (H–A) stronger and shift the equilibrium to the right. The equilibrium favours the thermodynamically more stable side; thus, the magnitude of the equilibrium constant reflects the energy difference between the components. In an acid-base equilibrium, the equilibrium always favours the weaker acid and base since these are the more stable components. [17] Both the carboxyl group and the carboxylate anion are stabilized by resonance, but the stabilization of the anion is much greater than that of the neutral function. In the carboxylate anion the two contributing structures have equal weight in the hybrid, and the C–O bonds are of equal length (between a double and a single bond). [17] The equation which describes the reaction between the water molecules present in the solution and the acid are the following ones:

$$H\text{–}A + H_2O \rightleftharpoons H_3O^{\oplus} + A:^{\ominus} \qquad K_{eq} = \frac{[H_3O^{\oplus}]\,[A:^{\ominus}]}{[HA]\,[H_2O]}$$

$$K_a = \frac{[H_3O^{\oplus}]\,[A:^{\ominus}]}{[HA]} \qquad pK_a = -\log K_a = \log\left(\frac{1}{K_a}\right)$$

Figure 33: Acid dissociation in water [17]

From which the value of pK_a for a specific DCA can be calculated, knowing the K_a.

1.7 Aim of the thesis

The growing desire to produce organic acids through fermentative routes, as a starting point for bio-based plastics, has revived the scientific attention on organic acid removal from aqueous streams. In fact, biological fermentation is a promising method for the production of organic acids from renewable biomass. [4, 10] In the past decades, a significant number of research papers appeared, especially for the extraction of CAs from the fermentation broth, while for the DCAs and especially for LDCAs, have been a few. The efficient separation of organic acids from a mix of multiple diluted components it is still a challenge and most of the production costs in microbial processes are higher than that in chemical processes, among which over 60% are generated by DSP. [5] Current methodologies used in recovery processes all have their limitations, and their improvements are especially needed regarding yield, purity, and energy consumption. Therefore, research on separation and purification processes is important for the future biorefinery industry: there is a need to develop a process that should ideally be simple to carry out and allow the purification of DCAs directly from the fermentation broths. Besides this, the emergence of new materials and the development of technologies would boost the recovery processes, which would make the biological process more competitive than the chemical routes and promote the development of green chemistry. One Flemish project that is focusing on this framework is the WODCA (waste oil to dicarboxylic acids) project, carried out by several R&D partners, including VITO, the Flemish Institute for Technological Research. The following scheme (Fig. 34) summarizes the rationale of WODCA.

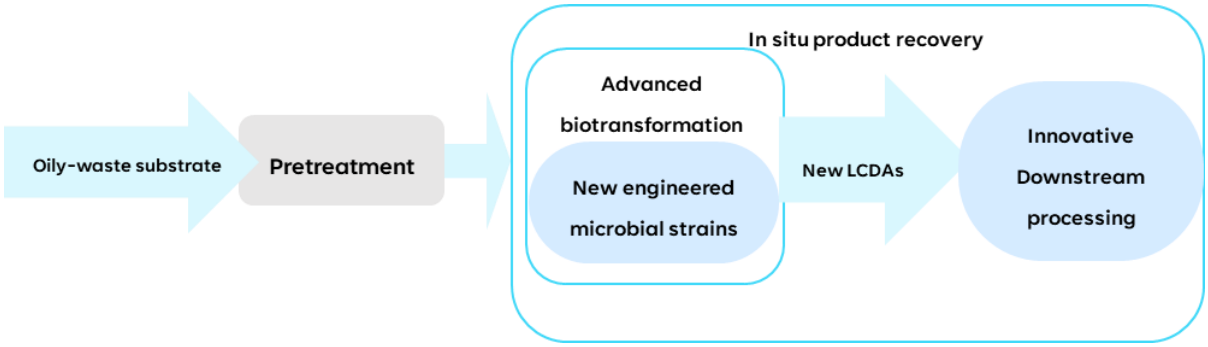


Figure 34: WODCA project.

The project, funded by VLAIO–Catalisti, follows the value chain approach, starting from lipid waste from households, restaurants and food industry collected by oil logistics, over the biotechnological production of DCAs as building blocks that can serve a variety of end-users. The overall goal of WODCA is in fact to create new, sustainable, fair-cost LCDA molecules

from lipid waste (used cooking oil) relevant for specific industrial applications. For this purpose, some of the project partners are developing a novel and adapted non-pathogenic microbial biocatalyst and an optimized biotransformation process, while VITO is responsible for the DSP consisting out of innovative membrane processes, to deliver LDCAs of high purity appropriate for several applications of interest to Flemish companies. DSP will include several steps. Two steps are investigated to start with: the recovery of the LDCAs from the aqueous fermentation broth and the separation/purification of the different compounds. For the recovery of the LDCAs, 'standard' membrane extraction with microporous membranes have been employed, while the last step of DSP consisted of ME with tight membranes or nanofiltration (NF) (replacing chromatography, distillation or crystallization described in the state-of-the-art). The goal is to achieve at least 90% LCDA recovery and relevant purity (ranging between 90 and 99.5%) for the final applications selected by the advisory board.

Thanks to my internship experience at VITO, I have been part of the WODCA project. Thus, my thesis's work aims to provide a comprehensive exploration of the extraction of LDCAs from aqueous model solutions, mimicking fermentation broths using membrane-based techniques. The stage for the ensuing research has been already proposed in the previous chapter, by discussing the significance of DCAs in modern industrial applications as well as the challenges associated with their extraction from fermentation broths. The potentiality of membrane-based separation systems and its capability to revolutionize the bioprocessing industry will be showed through to the performed experiments. A wide range of polymeric and ceramic membranes, diluents and reactive extractants have been tested to reveal the potential of MBSE and MBRE for the recovery of this type of compounds.

2 Material and methods

2.1 Calculations and analytics

In order to calculate the extraction efficiency (EE%) of each DCA from the feed mixture, the following eq. (8) has been employed:

$$EE_{DCA}\ (\%) = \left(\frac{C_{DCA,org}}{C_{DCA,theoretical}}\right) \cdot 100 \quad (8)$$

Where $C_{DCA,org}$ is the concentration of the component in the organic phase after extraction (mol/L) and $C_{DCA,theoretical}$ is the concentration (mol/L) based on the amount of DCAs added, in the preparation phase, to the system. The theoretical feed concentration has been used to calculate the EE%, instead of the sum $C_{DCA,acq} + C_{DCA,org}$, because the mass balance in ME tests was not closed and precipitation was observed, on the other hand in LLE the mass balance was satisfied and in that case the formula with the sum can be used.

The ratio of the concentration of acid in the organic solvent to the concentration of undissociated acid in the aqueous phase, at equilibrium, is K_D:

$$K_D = \left(\frac{C_{DCA,org,eq}}{C_{DCA,acq,eq}}\right) \cdot 100 \quad (9)$$

K_D is called the partition coefficient of free acids into the solvent, where $C_{DCA,acq,eq}$ is the free acid concentration in the aqueous phase at equilibrium (mol/L) and $C_{DCA,org,eq}$ is the concentration of the acid solvated by the organic solvent at equilibrium (mol/L). The mass extracted from the feed to the extractant has been calculated as:

$$mass_{DCA,extracted} = mass_{DCA,feed}(t_0) - mass_{DCA,feed}(t_{end}) \quad (10)$$

$$mass_{DCA,feed}\ (t_0) = C_{DCA,acq,eq}(t_0)\ \cdot V_{feed} \quad (11)$$

$$mass_{DCA,feed}\ (t_{end}) = C_{DCA,acq,eq}(t_{end})\ \cdot V_{feed} \quad (12)$$

Theoretically, the mass extracted calculated as Eq. (10), is also equal to:

$$mass_{DCA,extracted} = mass_{DCA,extractant}(t_{end}) - mass_{DCA,extractant}(t_0) \quad (13)$$

And:

$$mass_{DCA,extracted,feed} = mass_{DCA,extracted,extractant} \quad (14)$$

unless accumulation in the system occurs, case in which the equation (14) could not be satisfied. This problem could show up during MBSE and MBRE when the DCAs can accumulate over the membrane surface. Therefore, it is always challenging to analytically determine the initial (theoretical) feed concentration of DCAs. Since a lot of mass could not be detected in the feed, there is the need to calculate the mass balance based on actual doses of each DCA. However, all the DCAs in the extractant phase are remaining in the soluble form and thus the measured (analytical) DCAs concentrations have been considered to calculate the mass balance:

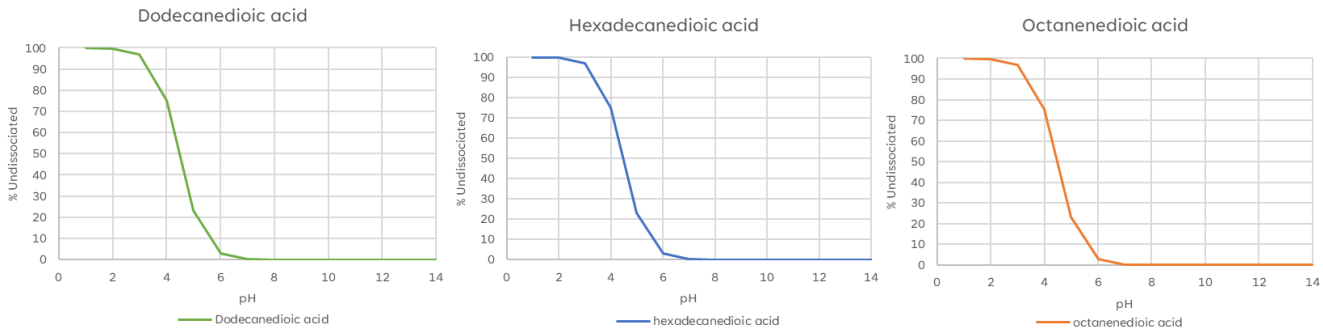
$$\boldsymbol{Mass}\ (\%) = \frac{(\boldsymbol{DCA}\ (\mathbf{t})_{\boldsymbol{feed}} \cdot \mathbf{V}(\mathbf{t})_{\boldsymbol{feed}} + \boldsymbol{DCA}(\mathbf{t})_{\boldsymbol{extr}} \mathbf{V}(\mathbf{t})_{\boldsymbol{extr}})}{\boldsymbol{DCA}_{\boldsymbol{actual}}\ (\boldsymbol{t_0}) \cdot \mathbf{V}_{\boldsymbol{feed}}(\boldsymbol{t_0})} \quad (15)$$

Where, $\boldsymbol{DCA}_{\boldsymbol{actual}}$ is the actual concentration of DC12, 16 and 18 weighted during the feed preparation (dosed concentration) and $\mathbf{V}_{\boldsymbol{feed}}(\boldsymbol{t_0})$ is the volume of the feed at the start of the experiment, $\boldsymbol{DCA}_{\boldsymbol{feed}}$ is the measured concentration of DC12, 16 and 18 in the feed in a specific time, $\boldsymbol{DCA}_{\boldsymbol{extr}}$ is the measured concentration of DC12, 16 and 18 in the extractant in a specific time. In case of liquid loss, as happens for leaking, the mass balance is unsatisfied. At the same time if precipitation occurs, the right DCAs concentration cannot be detected, resulting again to an unsatisfied mass balance. So, at the end of the experiment the mass (%) will be expressed as mass DCA at t_{end} (in both feed and extractant phase) divided by the mass DCA at t_0 (in both feed and extractant phase, with the consideration that DCA at t_0 in the extractant phase will be zero).

To determine the percentage of dissociated and undissociated form of the acids at a fixed pH present in an aqueous solution, the Henderson-Hasselbalch equation can be implemented. Once the experimental pK_a of DC12, 16 and 18 has been retrieved, the pH is fixed and the concentration can be obtained through calculation.

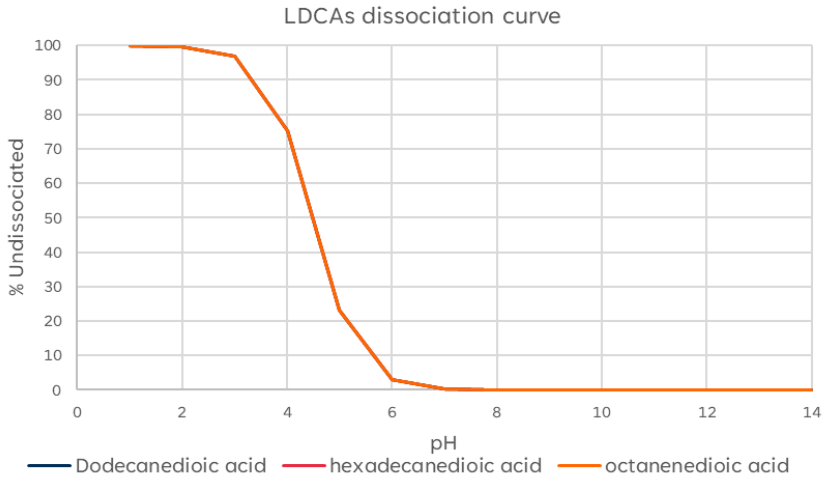
$$pH = pk_a + \frac{log_{10}[conjugate\ base]}{[weak\ acid]} \quad (16)$$

The Eq. (16) relates the pH of an aqueous solution, in which the acid is dissolved, to the acid dissociation constant (pK_a). [29] For DC12, DC16 and DC18 the dissociation curves can be plotted for the whole pH range, fixing pK_a= 4,48 [29]



Plot 1,2, 3: DC12, DC16, DC18 undissociated percentage curves versus pH.

Plotting all the curves in a single graph:



Plot 4: LDCAs undissociated percentage curves versus pH.

The dissociation curves for the LDCAs are crucial for the application of LLE, MBSE and MBRE. Since the chosen type of solvent and, especially, of extractant for the separation, create a different complex according to the acid's form, it is necessary to know the percentage of undissociated acid (and consequently of the dissociated).

2.2 Aqueous feed phase

To simulate the fermentation broth, synthetic aqueous feeds of LDCAs have been prepared. Synthetic mixtures of dicarboxylates with equal molarity of DC12 (mass fraction of 99%, Merck, Germany), DC16 (mass fraction of 99%, Merck, Germany), DC18 (mass fraction of 99%, Merck, Germany) were prepared in distilled water (RO). Solutions with total molarities ranging from 0.0109 mol/L to 0.0217 mol/L were investigated. The pH of the solution was adjusted to 4, 6 and 8 by adding NaOH 25% to increase it, or HCl to decrease it. [3]

Figure 35: Aqueous feed phase at the set-up phase of ME experiment, pH 6

The preparation for feed solutions involved a first phase of mechanical magnetic stirring lasting 24-48h, during which the temperature has been raised and kept to 30˚C, followed by alternating cycles of ultra sonification and mechanical stirring (350 rpm). The pH has been adjusted to the required value (usually 6, which is the closest to the real fermentation broth) using NaOH 25%. After 48h the feed has become homogeneously hazy and ready to be used.

2.3 Solvent screening (LLE)

Once the feed phase has been prepared, it can be tested in combination with a set of solvents to find the most promising in terms of DCA extraction from the mixture. To test different operative conditions, three feeds with 5 g/L of equimolar DCAs (DC12, DC16, DC18) in RO water have been created with pH: 4, 6, 8. The test involved physical extraction (S1-S5) and reactive extraction (S6-S11). For the reactive extraction the extractant molar ratio has been considered doubled with respect to the DCAs mixture, as it was composed only by DC12 (with concentration 5 g/L), so that solvent in excess is present for extraction of total mixture of DCAs. The aim of this test is to achieve an extraction of 90% LCDA, from the aqueous feed phase into the organic solution.

Table 6: Extractant solution for the shaking experiment.

Number	Extractant solution tested:
S1	BuOH
S2	Ethyl acetate
S3	1-butylacetate
S4	1 octanol
S5	1 decanol
S6	TOA+1 octanol
S7	TOA+1 decanol
S8	TOA+butanol
S9	Aliquat 336+1 octanol
S10	Aliquat 336+1 decanol
S11	Aliquat 336+ butanol

For the set-up a multireax (Heidolph), a shaker (Ivaki), a centrifuge (5804R Eppendorf) and vial recipients of 50 ml have been required.

The work procedure has been the following one:

- Take 10 mL of the feed phase and transfer it in a 50 mL vial.
- Indicate the liquid level.
- Add 10 mL of the extractant phase to the vial.
- Vortex for 10s.
- Put the recipients in the shaker for 2 hours (at 30°C).
- Remove the recipients from the shaker and put them in the centrifuge (10 min, 5000 g) (at 20°C)
- Check liquid level.
- Take a sample (2 mL) of the extractant phase (top) and feed phase (bottom).

2.4 Membrane screening

Suitable membranes (hydrophobic and solvent-compatible) to extract LDCAs have been chosen according to their physical properties and to the dropping test. For this sake, different polymeric membranes (PTFE, PDMS) have been subjected to hydrophobicity and wettability tests. When a membrane is hydrophobic it cannot get wet with water, this results in a high contact angle between the droplet and the membrane surface (>90°). In the case of hydrophilic membrane, it can instead easily get wet with water, which will penetrate in the pores. [36] This

phenomenon is related to the surface tension of the liquid and the surface energy of the membrane. In the case of hydrophobic membranes, the water surface tension is much higher than the surface energy of the membrane. [36] If the membranes are tested with aqueous feed droplets instead of water, as occurs with the dropping test, the surface tension of the liquid (feed) is affected by the presence of compounds in the water (DCAs). Wetting can occur although not expected based on water contact angle. DCAs can in fact act as surfactant when they are in contact with the membrane surface.

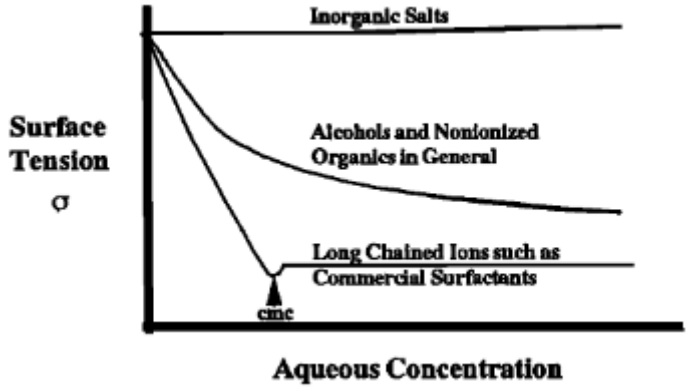


Figure 36: Surface tension trend with respect to aqueous solution concentration [42]

As showed in the Fig. (36), the surface tension of the solution decreases with increasing solute concentration and the wetting of the membrane occurs as soon as the surface tension of the solution is lower than the surface energy of the membrane. Wetting is therefore depending on the type of solute, on its concentration, and on the membrane material. PTFE, for instance, has a lower surface tension than PP, PE and PES. Ceramic membranes have been also considered for this test, since they will be employed for MBSE and MBRE, but the dropping test could not be performed since all of them show the hydrophobic layer in their internal cylindric surface.

2.5 Membrane based physical extraction (MBSE)

Once the solvents have been selected on the results of the shaking experiment, they can be tested with suitable membranes. Thus, the best extracting solvents will be employed in the ME experiments, with solvent-compatible and hydrophobic membranes approved by the dropping test. With this aim, a membrane screening has been performed, consisting of a test of two kinds of membrane, polymeric and ceramic, in combination with solvents as extractant. The objective

of ME is to extract at least 90% LCDAs building blocks from the model mixture as well as complex composition of the fermentation broth.

Table 7: Membrane screening specifications.

Membrane screening			
Membranes	**Solvent**	**Feed pH**	**Feed concentration**
PDMS (1)	Decanol	6	2.5 g/L
Ceramic (4)	Decanol	6	2.5 g/L
Ceramic (5)	Decanol	6	2.5 g/L
Ceramic (7)	Decanol	6	2.5 g/L

The selected ceramic membranes listed in the Table (7) are commercial dense membranes (tight) with in-house developed specific surface modifications. For the separation of the LDCAs or to avoid liquid transfer during recovery research is moving from the microporous membrane toward tight membranes. With tight ME the membrane provides interface but, differently from the microporous case, affinity for membrane will also play a role (combination). In addition to them, a set of polymeric membranes has been tested:

Table 8: Polymeric membrane test specifications.

Polymeric membrane screening			
Membranes	**Membranes**	**Feed pH**	**Feed concentration**
PTFE (A) 0.02 µm	Decanol	6	2.5 g/L
PTFE (B) 0.02 µm	Decanol	6	2.5 g/L
PTFE (C) 0.2 µm	Decanol	6	2.5 g/L
PTFE 0.1 µm	Decanol	6	2.5 g/L

The selected polymeric hydrophobic membranes are flat-sheet membranes and are available in large rolls, they need to be cropped from it with a specific size, glued (the glue used is LOCTITE UK5400+UK8101) and dried overnight before the experiment.

Figure 37: Roll of polymeric membrane PTFE 0.1 µm.

Figure 38: Membrane PTFE 0.1 µm cropping.

The glue layer (Fig. 39) is required for supported membranes since the sealing is not good enough without glue, especially if the support has a rough structure, thus, the free-glue layer will be in contact with feed, while the support (glued side) with extractant.

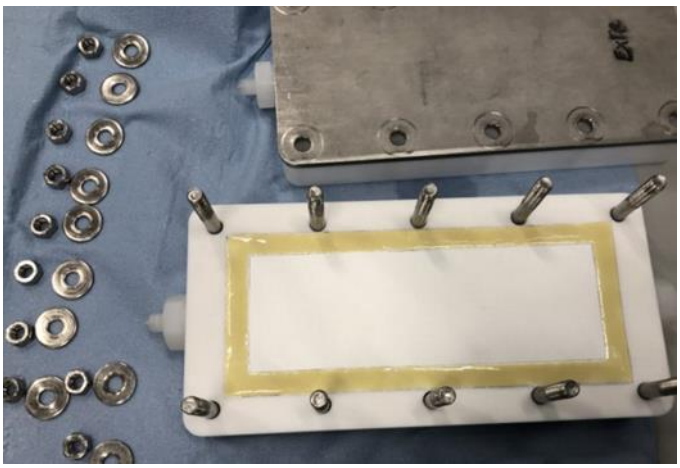


Figure 39: Membrane installation inside the stuck.

The membrane is then fixed in a specific module, consisting of two support pieces including gasket O-rings, which aim is to maintain the membrane in a fixed position, and polymeric nets to create homogeneous flows over the whole membrane surface. (Fig. (40)).

Figure 40: Internals of the module.

Then the module is closed tightly. To start the experiment the feed and extractant (400 mL of each) are poured in two bottles, containing a magnetic stirrer (which speed is 350 rpm). The mixtures start to circulate at a flow rate of 20 L/h and 30°C, maintaining a slight overpressure of 400 to 600 mbar for the feed mixture. The flow rates are measured by two manual flow meters, while the temperature is stabilized at the set point by a temperature control system linked to two heat exchangers, while the pressure is controlled by two pumps.

Figure 41: Set up with polymeric membrane.

When ceramic membranes are used, a metallic cylindric membrane housing needs to be installed.

Figure 42: Ceramic membranes in their protective box.

The set up with the ceramic membranes is analogous to the previous:

Figure 43: Set up with ceramic membranes.

When the experiment starts, the volume in feed and extractant bottle, pressures and temperature after the first circulation are taken. Samples of 2 ml from the feed and extractant bottle are taken at 1, 3, 6, 24 hours. At the end of the experiment the feed pH, feed and extractant volume are taken to calculate the liquid transport and losses. Then, the membrane is removed, and the system is cleaned with Ethanol 96% (VWR, Leuven, Belgium) and twice with RO water for 2 cycles.

2.6 Membrane based reactive extraction (MBRE)

To develop an efficient reactive extraction system for the recovery of DCAs from fermentation broths, reactive extraction experiments with different extractants (Table 9) mixed in a diluent

have been performed. The diluent selection has been based on the chemical resistance of the membranes. For all MBRE experiments the organic phase consisted of Aliquat 336 dissolved in alcohols, the extractant molar mass ratio has been chosen 1:2 to provide an excess of extractant. In presented cases, 1-octanol (Alfa Aesar, Germany) was used as diluent for Aliquat 366 at pH 6 experiments.

Table 9: MBRE specifications.

MBRE			
Membranes	**Solvent**	**Feed pH**	**Feed concentration**
PTFE (A) 0.02 µm	Aliquat 366+ Octanol	6	2.5 g/L
PTFE (B) 0.02 µm	Aliquat 366+ Octanol	6	2.5 g/L
PTFE (C) 0.2 µm	Aliquat 366+ Octanol	6	2.5 g/L
PTFE 0.1 µm	Aliquat 366+ Octanol	6	2.5 g/L

Procedure

The set-up for the MBRE is equal to the one for ME, except for the extractant preparation steps. In this case the reactive extractant is poured first in a bottle, and then diluted with 400 ml of alcohol (1-octanol), stirred until it is well dissolved. The extractant molar ratio has been considered doubled with respect to DC12 (predicting the mixture was composed only by DC12 2.5 g/L), so that excess solvent will be present for extraction of total mixture of DCAs. Then the experiment proceeds as the one cited for MBSE. The extraction percentage expected would be higher with respect to MBSE, since the reactive extraction enhance the probability to bind DCAs.

2.7 Development of an improved sampling process

During experiments performed before the start of my internship, difficulties appeared in interpretation of the measured feed concentrations. There was a discrepancy between the amount of LDCAs used for the preparation of the feed solution and the concentration measured by the analytic method. For future experiments, an exact analytical method is thus required to determine the real broth concentration. Therefore, a new way of sampling was tested and compared with the method used so far. For this sake, an improved sampling process has been tried, involving the sampling in two different ways: one only keeping the feed in 2 ml sample and the other, by diluting the feed sample (100 µl) in absolute EtOH (900 µl) (WWR, Leuven, Belgium). A 300 ml feed with 3.35 g/L of equimolar DCAs (DC12, DC16, DC18) has been

prepared at different pH (4, 6, 8) and tested in a temperature range 30-80˚C. The feed has been kept for 1h at the selected temperature before taking the samples.

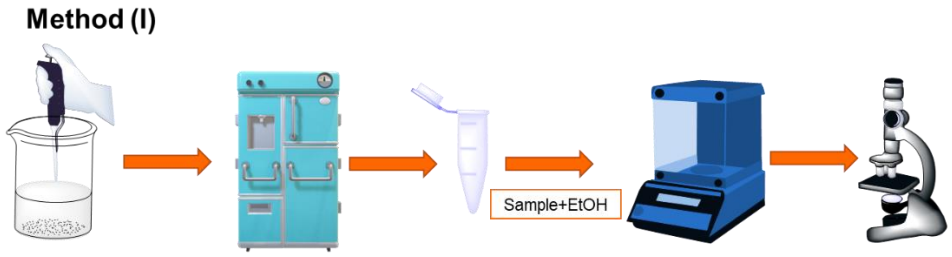


Figure 44: 1st sampling method.

As the Fig. (44) shows in the 1st method samples of pure feed (2 ml) are stored in the fridge. The room temperature feed samples are vortexed for homogenous mixing, pipetted and diluted with absolute EtOH by the analytic team, prior analysis. In this case precipitates are visible in the samples sent to analysis: this is a sign of low dissolution that can lead to a wrong concentration measurement.

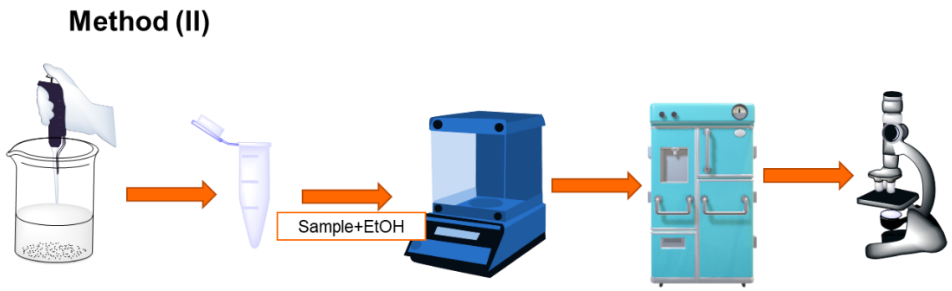


Figure 45: 2nd sampling method.

With the 2nd method the samples have been taken by directly diluting 100 µl of feed in 900 µl absolute EtOH. In this case the majority of DCAs are well solubilised before storing in the fridge and analysis.

3 Results and interpretations

The experiments have been performed and will be presented following the goals highlighted in the Fig. (46):

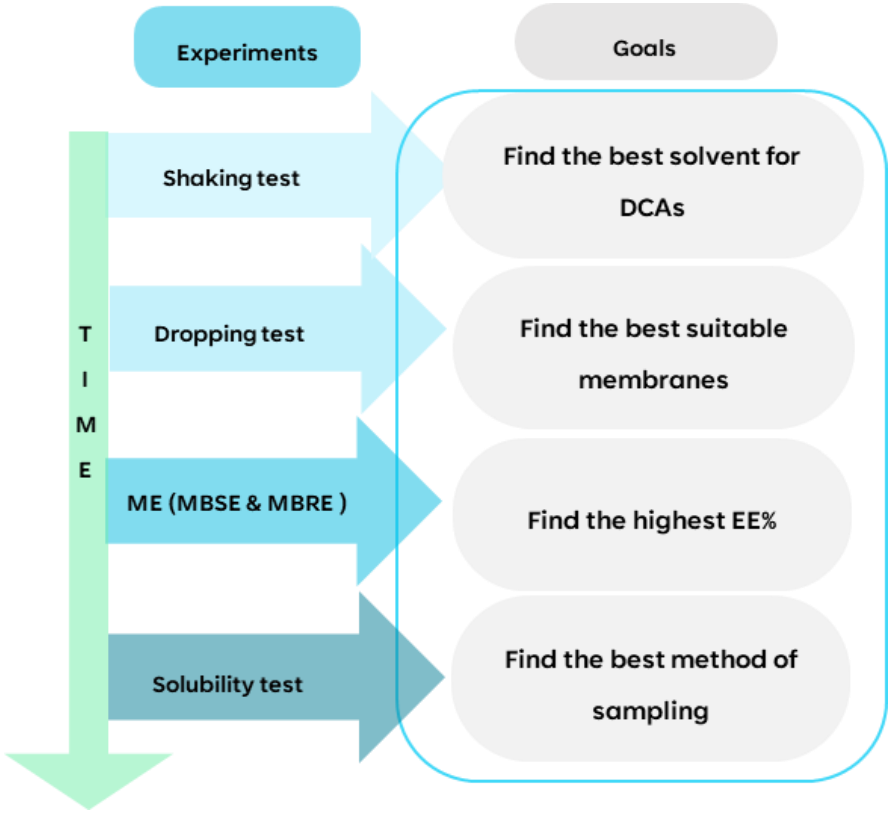


Figure 46: Experiments goals.

As first step a solvent screening has been developed, through a shaking experiment, with the aim to find the best solvent able to effectively extract the target products (DCAs). Once the best solvents have been selected, polymeric and ceramic membranes (hydrophobic and solvent-compatible) should be tested to find the suitable ones according to their physical properties and their feed compatibility (tested with the dropping test). After having selected a set of suitable membranes, the one able to extract DCAs the most in term of extraction efficiency needs to be found. For this sake, ME experiments (MBSE and MBRE) have been developed.

Since an exact analytical method able to detect the DCAs dissolved in the solvent needs to be developed, a more efficient sampling procedure (with respect to the one used in the past) has been tried. In fact, due to sampling issues, the measured concentration of the organic acids is not equal to the added (theoretical) one, therefore a new sampling method was performed.

3.1 Solvent screening

The feed has been tested in combination with different solvents to find the most promising one in term of DCAs extraction from the mixture. The extraction efficiency (EE%) has been calculated by measuring the DCAs (12, 16, 18) concentrations in the feed and in the extractant after the shaking, at different pH (4, 6, 8) as shown in the previous eq. (1). The LLE process allows to separate and concentrate the DCAs from an aqueous solution into the extractant phase. The EE% can be influenced by factors such as the type and concentration of the extractant, the feed pH, temperature, speed of agitation, extraction time and volume ratio.

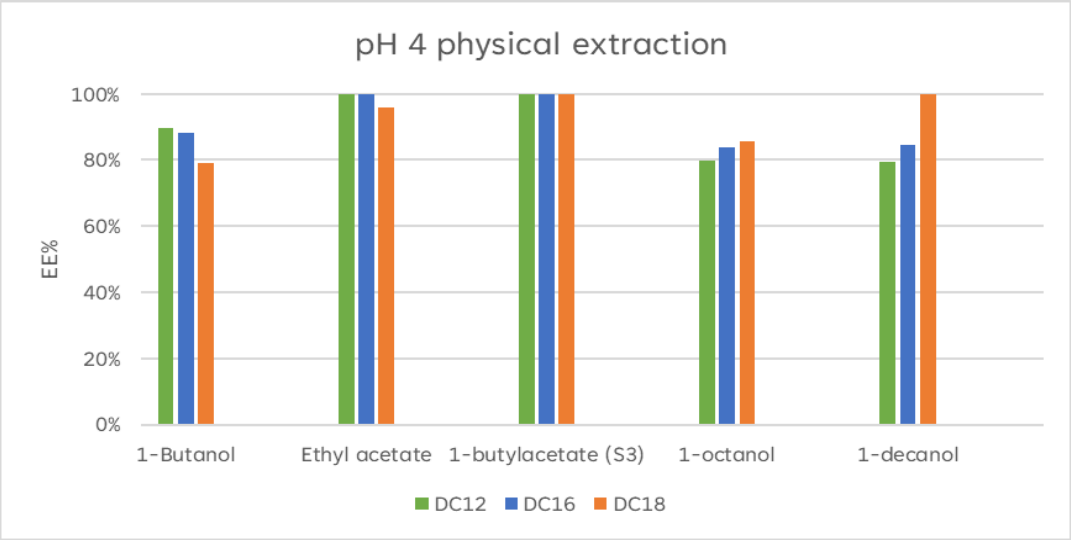
Figure 47: Vials of 50 ml for the shaking experiment, inside two phases can be recognised: the feed (bottom) and the extractant (up).

What could be expected from the solvent screening is that, according to the affinity between the solvent and the single DCA (12, 16, 18), the interactions are different. The dissolution of the DCAs in pure solvent, in fact, is not influenced only by the solvent polarity but also by intermolecular interactions and the ability of the solvent to form hydrogen bonds with them, which can be related to the acid form (dissociated or undissociated). Each DCA could act in a different way according to the pH, as shown in the plot (4) by the undissociated percentage curve versus pH. A parameter that could help to predict and understand the interactions between the components and the solvents is the log (P): more polar, hydrophilic compounds have lower values with respect to polar and hydrophobic compounds.

Table 10: Log(P) for different compounds. [43]

Substance	***Value***
log P (DC12)	3.06
log P (DC16)	4.62 - 5.05
log P (DC18)	5.39
log P (1-butanol)	0.78
log P (EtAc)	0.71
log P (BuAc)	1.82
log P (OctOH)	2.34
log P (DecOH)	3.12

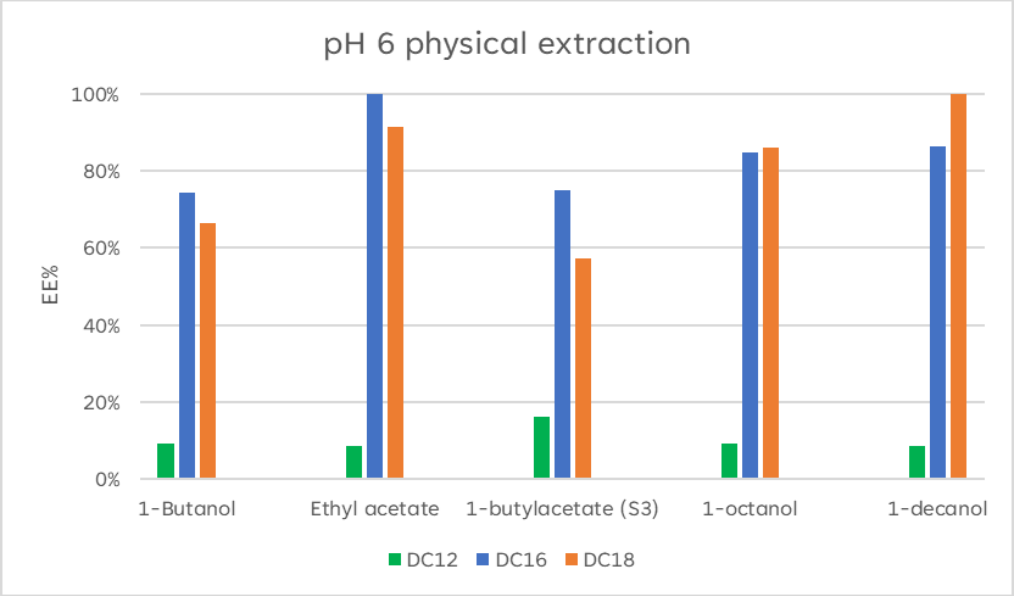
Since similar values of log(P) leads to a high level of interactions, high extraction efficiencies for DC12, DC16 and DC18 could be expected employing decanol and octanol.



Plot 5: Physical extraction at pH 4, feed: 5 g/L of equimolar DCAs.

As expected, each DCA shows its own behavior: their EE% depends on the solvent used, since the interactions between them are different. At pH 4 with physical extraction, all the presented solvents show high results (>80%): the highest ones are reached when using 1-butyl-acetate, which can extract all the DCAs with an efficiency of 100%, followed by ethyl-acetate, which instead has less power to extract DC18. When the pH <pKa, as in this case, all DCAs are undissociated and with a hydrophobic (non-polar) behavior and thus they prefer to be with hydrophobic (non-polar) solvents (following the like attracts like phenomenon), thus most of the solvents used show very good extraction for all the DCAs. The hydrophobicity of the

molecules is influenced by the pH. The carbon chain is hydrophobic at all pHs, but the carboxylic head is more hydrophilic in the deprotonated form (higher pH). The last effect is similar for all three DCAs, but the 'weight' of the carbon chain is depending on the chain length.



Plot 6: Physical extraction at pH 6, feed: 5 g/L of equimolar DCAs.

The most promising solvents at pH 6 from the Plot (6) seem to be 1-decanol and ethyl-acetate, but the EE%, especially of DC12, decreases with respect to pH 4. This could be linked to the dissociation curve (see Plot 4) which shows that at pH 6 most of the DC12 are dissociated. At pH 6, in fact, a mixture of dissociated (96%) and undissociated (4%) acids is present and the hydrophobicity of them decreases since shorter chain length leads to have lower hydrophobicity. Therefore, the extraction of DC12 (which is the shortest and so the one with lowest hydrophobicity) is lower than DC16-18. In fact, the DC16-18, that should be dissociated with the same percentage of DC12, show a higher physical extraction.

Additionally, molecular interactions could play a fundamental role in extraction: as cited in the introduction section, hydrogen bonds can be formed between solvent and DCAs [44]. DC12, as well as DC16-18, are able to bind their H atom of the carboxyl group with the O atom of the carbonyl group of the solvent. The Fig. (48) shows an example of this bond for DC12 of which interaction energy can be calculated and it is equal to 42.5 kJ/mol.[44]

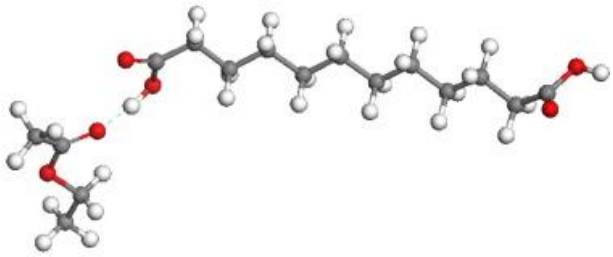
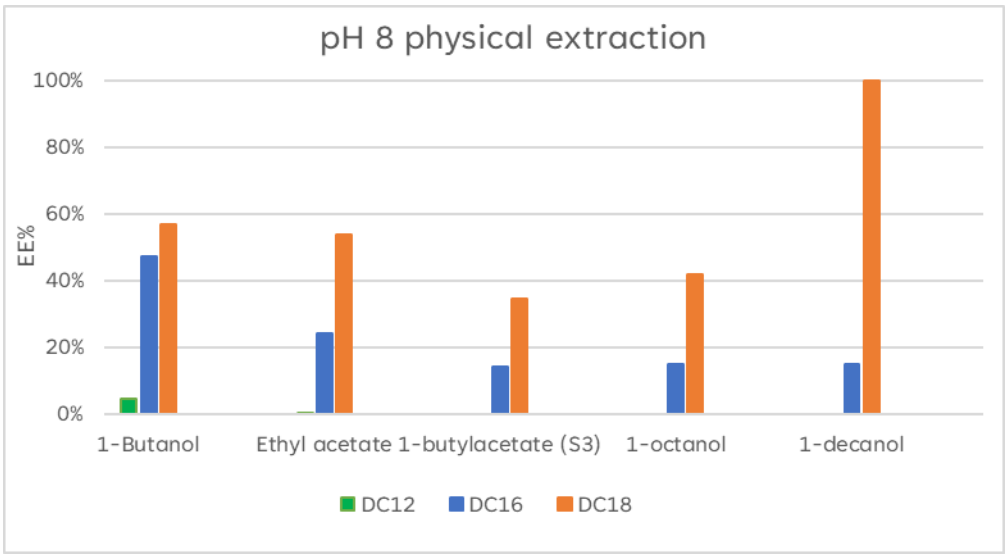


Figure 48: Hydrogen bonds between DC12 and ethyl-acetate. [44]

The larger the interaction energy, the stronger the interactions between DCA and the solvent molecules.[44]



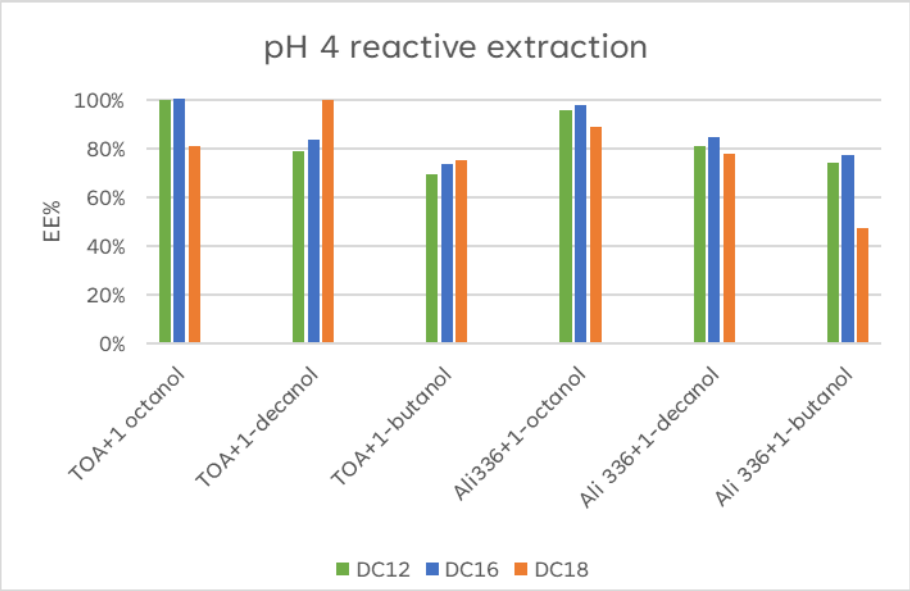
Plot 7: Physical extraction at pH 8, feed: 5 g/L of equimolar DCAs.

At pH 8, 100% of the acids are dissociated and their extraction drastically decreases. Nevertheless, DC16 and 18 are still being extracted while the extraction of DC12 is near to 0. The EE% of DC16 reaches 45% for 1-butanol, while DC18 has been entirely extracted by 1-decanol, as in the previous cases. Again, the dissociated molecules will be less hydrophobic than their undissociated form, but they will still be apolar enough to prefer the solvent phase above the aqueous one. So longer molecules such as DC16 and DC18 show extraction anyway.

It is possible to deduce that EE% is therefore affected by acidity (expressed by pK_a) and hydrophobicity (expressed by logP) of the solutes. In this case study, all three components have (almost) the same pK_a and they are for 96% dissociated. Nevertheless, there is a difference in extraction efficiency at pH 6 and 8 and, based on these results, the extraction is controlled rather by hydrophobicity of the acids than by their acidity. This effect is much more pronounciated using a mixture, in fact it seems that when the components are competing for

extraction, the role of hydrophobicity (which is increasing with chain length) becomes more important.

To enhance the EE% of the DCAs, two reactive extractants (TOA and Aliquat 336) have been tested diluted in alcohols. What could be expected from their employment is that the complex formed between the reactive extractant and the DCAs could be able to extract a higher percentage of the acids from the feed with respect to the physical extraction.

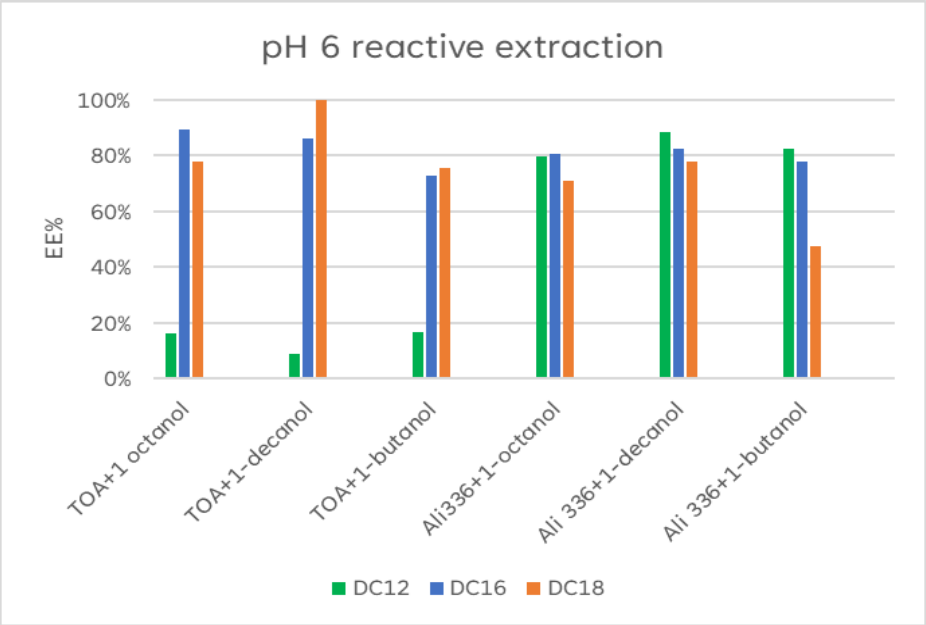


Plot 8: Reactive extraction at pH 4, feed: 5 g/L of equimolar DCAs.

As explained in the section 1.3.3.2, the extraction process with Aliquat 336 and TOA does not involve a real chemical reaction between the extractant and the acid. Instead, it relies on the difference in solubility of DCAs in the aqueous phase (water) and the organic phase. When the two phases (aqueous and organic) come into contact and are mixed, DCAs molecules in the aqueous phase tend to partition into the organic phase containing the extractant. This partitioning is driven by the preference of DCAs for the more lipophilic environment provided by an alcohol. Especially, the complex created has a higher solubility in the solvent with respect to the feed. Since Aliquat 336 has a hydrophobic long alkyl chain and a positively charged quaternary ammonium group, when is dissolved in octanol, it forms a lipophilic phase that is immiscible with the aqueous phase. While dicarboxylic acids alone are water-soluble due to their carboxylic acid functional groups and they can form hydrogen bonds with water molecules. [16] The case of reactive extraction at pH 4 shows high EE%, especially for the combination TOA+1-Octanol and Aliquat 366+1-Octanol (both in the range 80-100% of

extraction), but it is not exceeding the results from the physical extraction, as instead it was supposed since here a complexation is present.

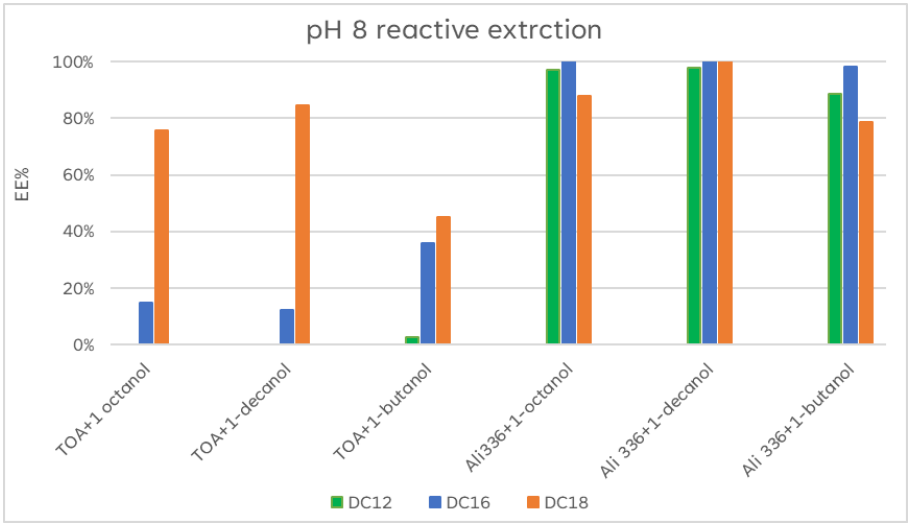
The reason why the EE% in reactive extraction are lower with respect to the physical one is that the diluent used (BuOH, OctOH and DecOH) are active diluents. They are not only used to adapt viscosity or density of the solvent phase (TOA or Aliquat), but they are adding an extraction power. Due to their hydrophobicity and to the hydrophobicity of the LDCAs, they have high extraction power for undissociated acids themselves, thus adding the reactive compound does not really have an added value at this pH (4), where both acidity and hydrophobicity of the components are in favor of physical extraction already.



Plot 9: Reactive extraction at pH 6, feed: 5 g/L of equimolar DCAs.

At pH 6 the extraction decreases, especially for DC12 in the case in which TOA is used. As pointed out in the introduction chapters, TOA can bind only with the undissociated form of DCAs, which are present in high percentage when the pH is lower than 6, while Aliquat can bind itself also to the dissociated ones. For the sake of the experiments, we need to simulate a feed around the pH 6 at which 96% of the acid is in dissociated form, thus it would be better to select Aliquat 336 as reactive extractant with respect to TOA to achieve maximum extraction efficiency. At pH 6 DC12 is less hydrophobic than DC16-18, especially because the hydrophilicity of the acid group is higher due to dissociation (carboxylate ion is more

hydrophilic than carboxylic acid functional groups) so it shows lower physical extraction power that could be enhanced by using Aliquat.



Plot 10: Reactive extraction at pH 8, feed: 5 g/L of equimolar DCAs.

At pH 8 the EE% of DC16 becomes low and TOA is not able to bind DC12 since it is less hydrophobic then the other DCAs; on the other hand, the EE% with Aliquat are high (near to 100%). As preannounced, at pH 8 physical extraction mechanism based on solubility shows its minimum and so only extraction based on hydrophobicity, which is enhanced by the complex formation, occurs. Anyway, the pH 8 is too far from the pH selected (6), so it would not be used for MBSE or MBRE to simulate the real feed.

3.2 Membrane selection based on solvent-compatibility and hydrophobicity

To select suitable membranes, the available polymeric ones have been tested as showed in the Fig. (49). Their wettability has been investigated with droplets of a feed of 2.5 g/L at different pH 4, 6 and 8.

Figure 49: Dropping test.

At zero time, all the contact angles between the droplets and membrane surface are high. Especially, the results obtained after 2 hours show a high contact angle for PTFE (C) at pH 4, 6 and 8, while in the other cases the droplets are spread on the surface, leading to a low contact angle (wetting), except for pH 4. The pH 6 instead shows wetting for PTFE (A, B), especially for (B). Anyway, a working pH 6 should be selected rather than 4 since it is the closest to the real feed pH value. These results are different with respect to what could have expected from previsions, in fact, since PTFE (A) and (B) have the same pore size, the expectation was that their behavior regarding wettability could have been the same. Furthermore, having PTFE (C) the largest pore size, the expectation was that it could be wetted more than the remaining two. The observed behavior could be justified by the presence of surface modifications that are characterizing the different membranes, which are leading to reduce (or enhance) their wettability. Another polymeric membrane (PTFE 0.1µm) has been tested, only at zero time. The droplet surfaces create a high contact angle with the membrane surface, which is a symbol of low wettability, as the Fig. (50) shows:

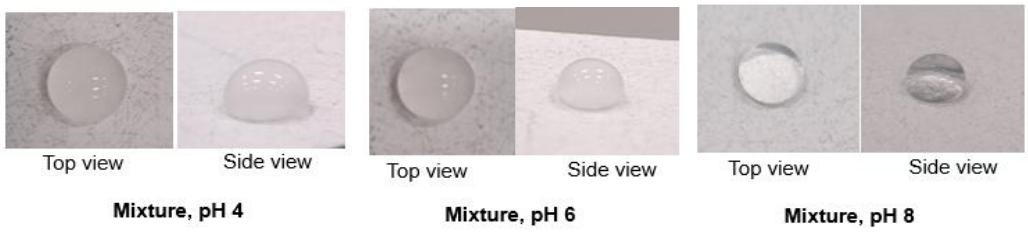


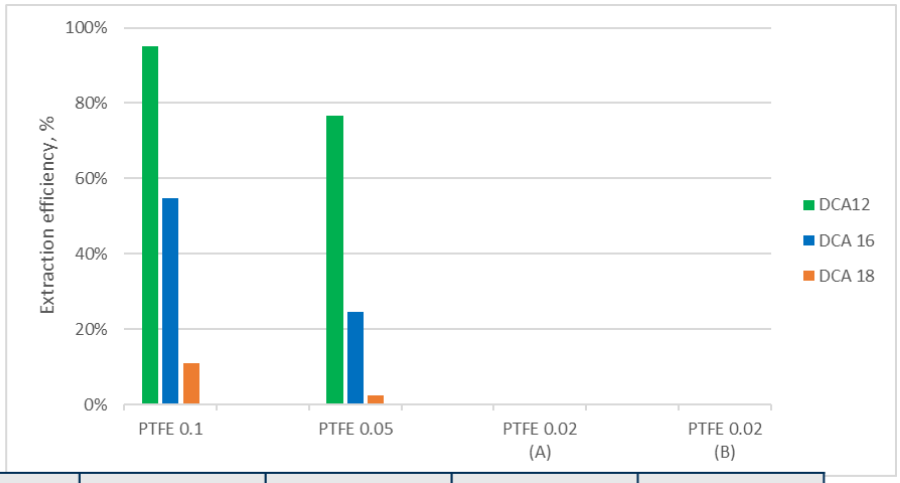
Figure 50: PTFE 0.1 µm membrane.

3.3.1 Membrane-based solvent physical reaction (MBSE)

Due to precipitation during the ME experiments, the mass balance was not fitting and thus, to calculate the EE% of each DCA the eq. (1) showed in the section results 2.1 and here reported, has been employed:

$$EE_{DCA}\ (\%) = \left(\frac{C_{DCA,org}}{C_{DCA,theoretical}}\right) \cdot 100 \quad (1)$$

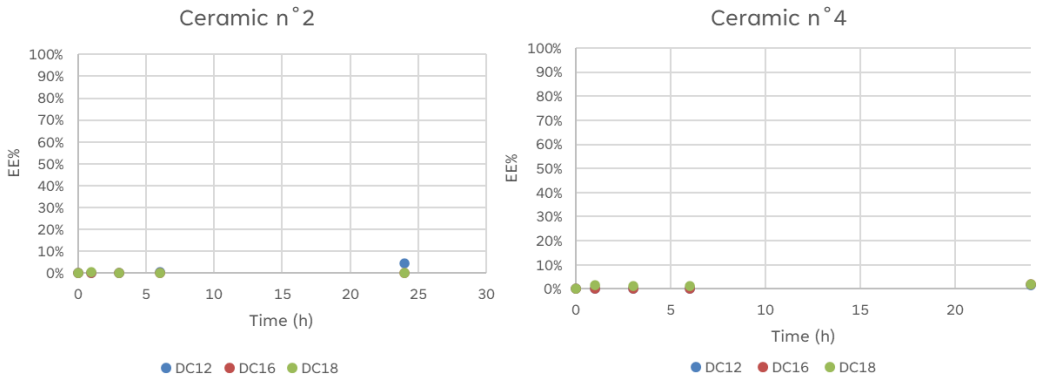
Where $C_{DCA,org}$ is the concentration of the component in the organic phase after extraction (mol/L) and $C_{DCA,theoretical}$ is the concentration (mol/L) based on the amount of DCAs added to the system. The theoretical feed concentration has been used instead of $C_{DCA,acq} + C_{DCA,org}$, because the mass balance in ME tests was not correct and precipitation was observed. An example of this issue can be seen from the mass balances reported in the Fig. (51):



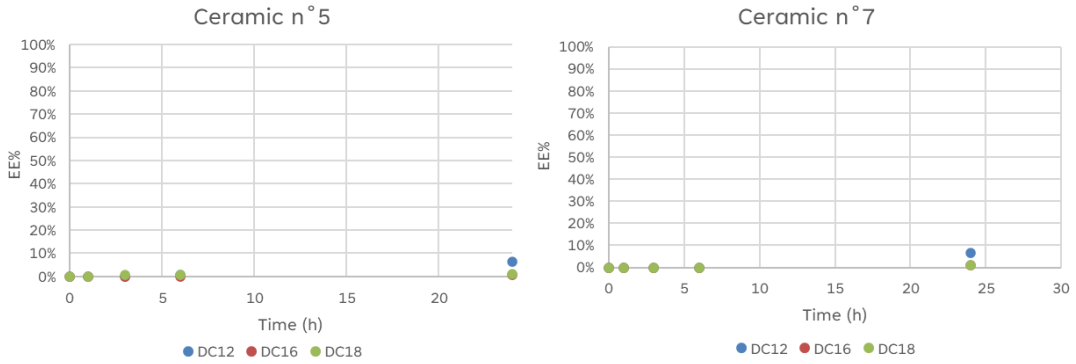
DC12	102	77	107	111
DC16	62	38	42	0
DC18	15	4	4	0
Mass balance after 24 hours (%)				

Figure 51: Mass balances of DCAs for different polymeric membranes after 24h.

Reactive extraction was not selected as the first experiment with ceramic membranes because it would have made it more difficult the observation of the membrane modification effect; at the same time, the stability of the membrane modification would have been checked for these compounds. Thus, the choice of decanol as solvent for the physical extraction comes from the good EE% results obtained from the shaking experiment. As reported in the chapter 1.3.3, decanol is often used since it offers a good compromise between extraction efficiency and toxicity. Although ethyl-acetate showed good EE% during shaking experiment, it was not selected for ME experiment due to its incompatibility with the available ME set-up. The EE% results of the ceramic membranes tested are reported as follows.

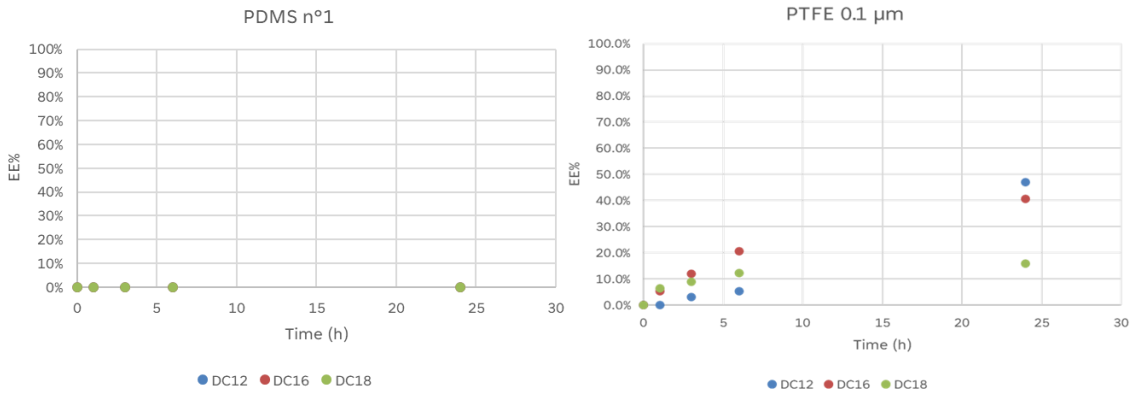


Plot 11, 12: Ceramic membranes (2, 4), Feed: 2.5 g/l DCA mixture, Extractant: decanol.



Plot 13, 14,: Ceramic membranes (5, 7), Feed: 2.5 g/l DCA mixture, Extractant: decanol.

All the tested ceramic membranes unfortunately have not shown good extraction results, reaching a maximum value of 5-7%. This could be due to the membrane surface modification, as well as the lack of hydrophobicity throughout the whole membrane thickness, which can lead to the absence of contact between the phases, or to the short experiment time selected. Polymeric membranes have also been tested for physical ME, such as PDMS (1) and PTFE (1) 0.1 µm:

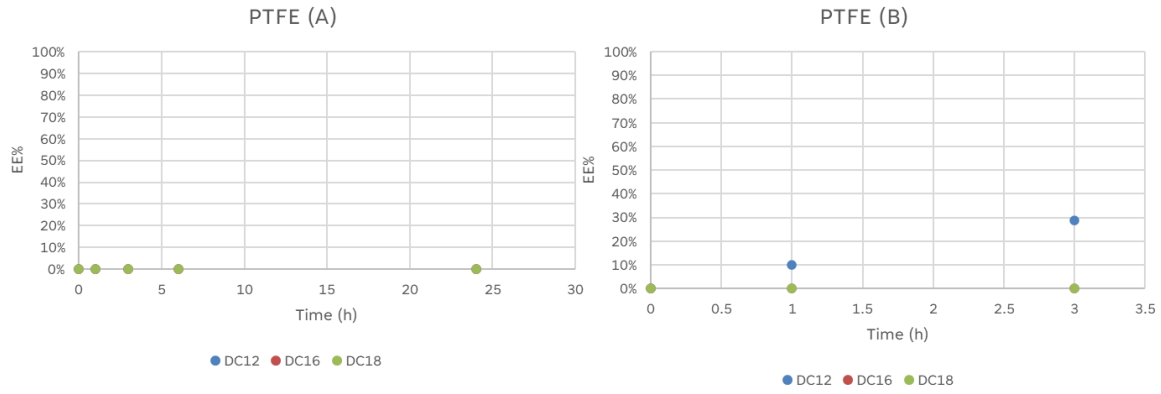


Plot 15, 16: PDMS (1), PTFE 0.1 µm, Feed: 2.5 g/l DCA mixture, Extractant: decanol.

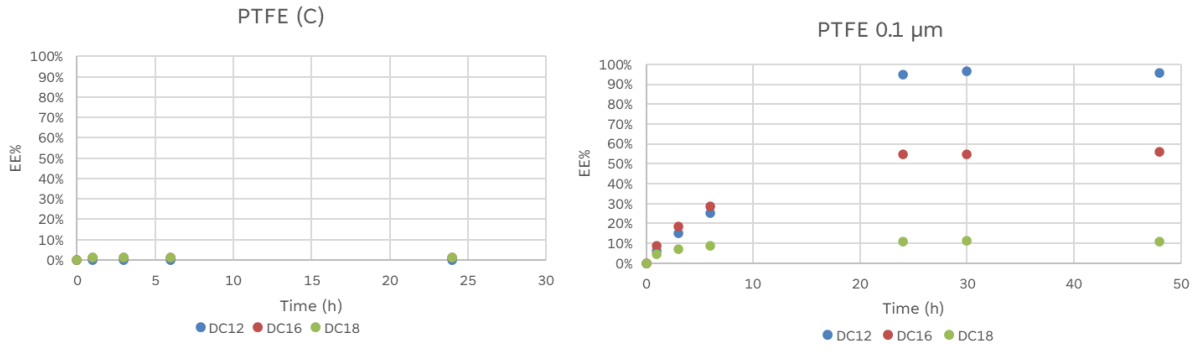
While the PDMS (1) shows no extraction, the PTFE 0.1 µm shows high results with respect to the ceramic ones, reaching 47% of extraction for DC12, 41% for DC16 and 16% for DC18. Unfortunately, these results are lower than the one obtained with LLE, maybe due to the membrane presence since its surface modifications may interfere with the DCAs. From the results it is possible to notice that somehow DC12 and 16 are more able to pass through the membrane than DC18. A hypothesis could be that, a percentage of DC16 and mostly DC18, which has the longest -CH_2 chain among them, interact with the membrane surface creating a hydrophilic layer, instead of passing through.

3.3.2 Membrane-based solvent reactive reaction (MBRE)

For the reactive extraction polymeric membranes (PTFE), have been tested in combination with Aliquat 366 + Octanol as extractant and a feed of 2.5 g/L of DCAs (12, 16, 18) at pH 6.



Plot 17, 18: PTFE A, B, Feed: 2.5 g/l DCA mixture, Extractant: Aliquat 366 + Octanol.



Plot 19, 20: PTFE C, PTFE 0.1 µm, Feed: 2.5 g/l DCA mixture, Extractant: Aliquat 366 + Octanol.

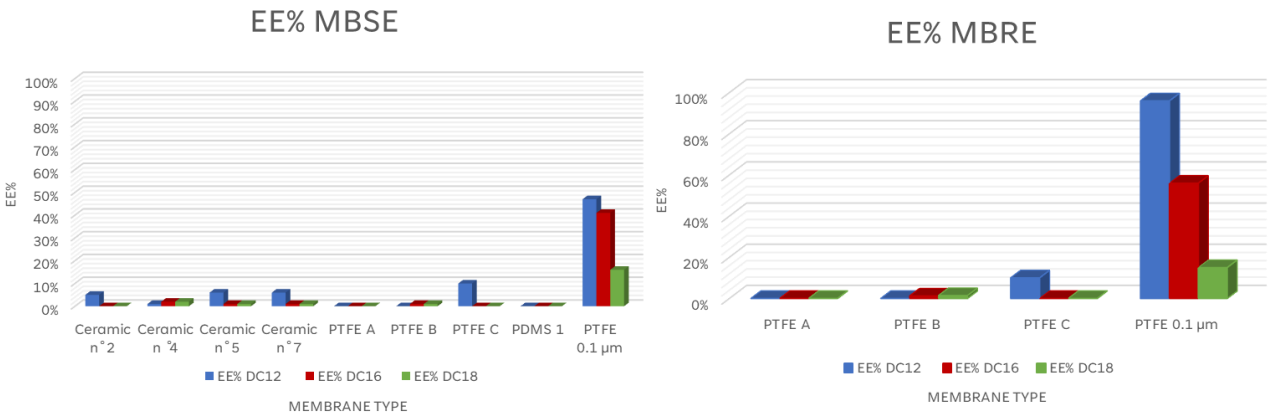
The best result has been obtained with the PTFE 0.1 µm membrane, using Aliquat 336 and octanol, that has been tested for 48 hours. The DC12 extraction using PTFE 0.1 µm has almost reached >90% (96%) achieving thesis goal, while for DC16 and for DC18 are 56% and 11%, respectively. The reason of succeeding for the DC12 could be linked to the surface modification done on the membrane in combination with the use of the most effective reactive extractant (Aliquat 366 + Octanol). From the results it is possible to see that while DC12 is well extracted, DC16-18 are less, even if, based on hydrophobicity as previous explained, we would have expected higher extraction of them. Nevertheless, DC16-18 are removed from feed phase and precipitates were seen in circulation loop and membrane module at feed side. Their low EE% could be due to their interaction between each other, interaction with the membrane surface, possible micelle formation or precipitation.

Results regarding Plot (18, PTFE B membrane) are not attainable at the 3rd hour since a volume shift was observed and thus LLE occurred, in fact the EE% rapidly increases. The volume shift may be caused by the interaction between DC18 and the membrane surface since it could be capable of generate a hydrophilic layer over it.

Further tests could be performed trying to extract the DCAs one by one (only DC12, DC16 or DC18), selecting the proper conditions.

3.3.3 Comparison between MBSE & MBRE

Making a comparison between all the tested membranes, the following graphs can be obtained:



Plot 21, 22: MBSE and MBRE efficiencies, pH 6.

As showed by the Plots (21, 22), among the 13 tested configurations the most promising method to obtain the highest EE% at pH 6 (fermentation pH) has been the MBRE with Aliquat 336 + Octanol, tested with the membrane PTFE 0.1µm. The reactive solvent in combination with PTFE 0.1µm, allows to the organic acids to efficiently pass through the membrane to create complexes. Aliquat, in fact, can bind itself both to the dissociated and undissociated form of DCAs leading to reach higher extraction efficiencies. Even here, the hydrophobicity plays a role in the extraction efficiency both for MBSE and MBRE: at pH 6 all the DCAs are at 96% dissociated and among them DC12 is the least hydrophobic, since it is the shortest. Because of DC12 scarce hydrophobicity, using MBSE is only possible to reach its EE% near to 50%. This value can be instead enhanced using MBRE, thanks to the complex formation with Aliquat. So, it seems that the scarce hydrophobicity can be compensated by the presence of an efficient reactive extractant. In fact, even if DC16-18 are more hydrophobic than DC12 (being longer than DC12) they are less extracted. Their low extraction could be caused by interactions with the membrane surface (on which they can bind), by micelle formation or by precipitation in the extraction system (in pipes, membrane surface, membrane support). Certainly, what can be observed is that the presence of the membrane influences the EE% for both MBSE and MBRE, unfortunately hindering the total extraction of DCAs.

3.4 Results of the improved sampling process

As cited before, due to sampling issues the measured DCAs concentration is not equal to the added (theoretical one). Therefore, there is a need to find a suitable method to detect the exact concentration of DCAs in the solutions. This problem must be solved especially to exactly determine the amount of each DCAs present in a real fermentation broth, which would be of unknown concentration. As showed in the Table (11) analytical measurement errors can occur, since a mass percentage could not be detected in the feed because of precipitation.

Table 11: DCAs concentration detected with respect to actual ones, data from ceramic-membrane n°2 MBSE.

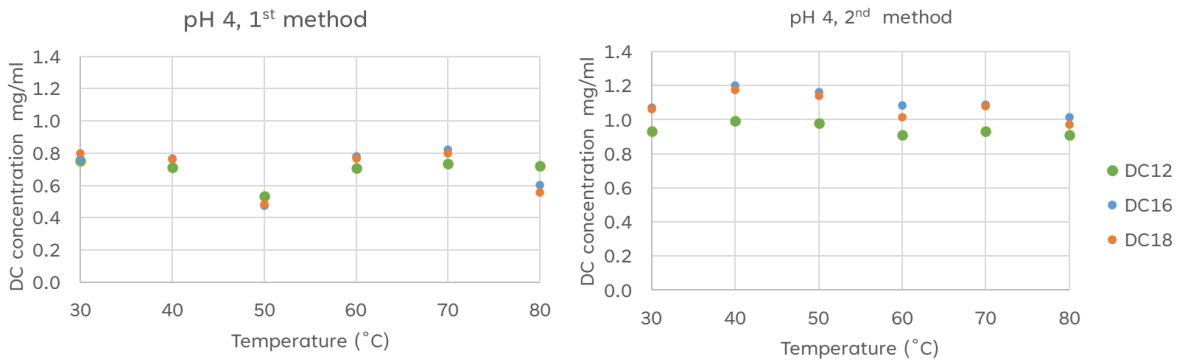
DCAs	***Amount detected at zero time [mg/ml]***	***Actual amount in feed preparation [mg/ml]***
DC12	0.807	0.825
DC16	0.790	0.825
DC18	0.787	0.825

Since an exact analytical method is required to determine the real broth concentration, a new way of sampling was tested and compared with the method used so far. For this sake, a solubility test has been tried, involving the sampling in two different ways: one only keeping the feed in 2 ml sample and the other, by diluting the feed sample (100 µl) in absolute EtOH(900 µl) (WWR, Leuven, Belgium). The discrepancy on the measurement between them can be evident in the Table (12):

Table 12: DCAs concentration analytically detected by two methods.

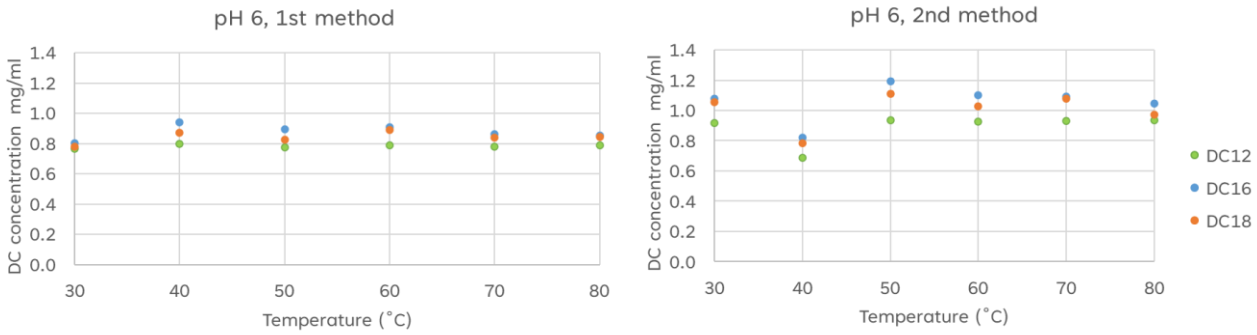
	Pure feed method (1st)			***Diluted method (2nd)***		
Temperature	***pH 4***	***pH 6***	***pH 8***	***pH 4***	***pH 6***	***pH 8***
˚C	***DCAs [mg/ml]***	***DCAs [mg/ml]***	***DCAs [mg/ml]***	***DCAs [mg/ml]***	***DCAs [mg/ml]***	***DCAs [mg/ml]***
30	2.3	2.4	2.4	3.1	3.0	2.9
40	2.2	2.6	2.5	3.4	2.3	3.0
50	1.5	2.5	2.5	3.3	3.2	3.0
60	2.3	2.6	2.6	3.0	3.1	3.0
70	2.4	2.5	2.5	3.1	3.1	3.0
80	1.9	2.5	2.5	2.9	3.0	3.1

The second method is considered more accurate since the solute (DCAs) is well dissolved in the alcoholic solution and in this way its quantification is simpler, for this reason it will be employed for future experiments to detect the exact concentration of DCAs. With the second method the detected concentrations are closer to the actual feed value of 3.35 g/L, thus this lead to consider it more accurate with respect to the first. In addition, to understand the correlation between the solubility, temperature and pH, the DCAs concentration have been plotted for both sampling methods at different temperatures at constant pH:



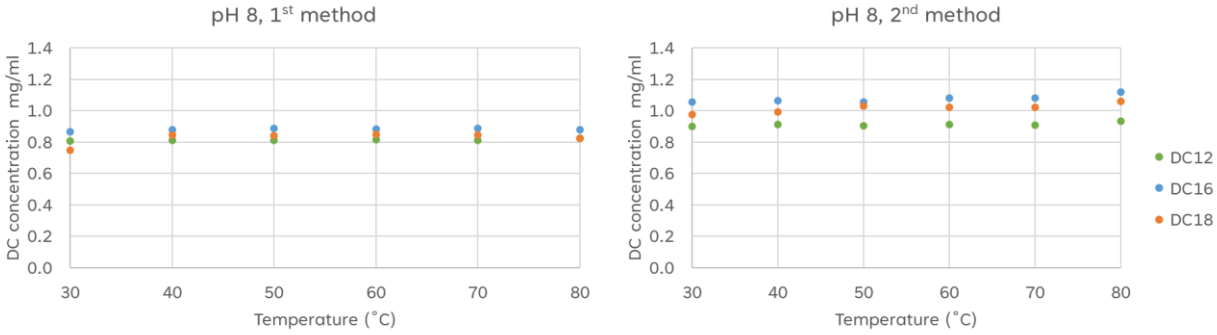
Plot 23, 24: Solubility test, pH 4.

The first difference that can be noticed from the results is that the DCAs solubilities values are higher for the diluted method and so closer to the weighted initial value. According to the 1st method at pH 4 the DCAs are well solubilized at temperatures lower than 40˚C and higher than 60˚C, while in the middle (50˚C) their solubility drops to a minimum. While, according to the 2nd method, the highest solubility value is reached at 40˚C for all the DCAs, and then a slow decreasing trend is detected. The solubility minimum in the 1st case could be caused by an analytical error, since in the 2nd is not detected, related to the precipitation of DCAs in the sample. In both cases the highest solubility values are reached by DC16, which achieves 1.2 mg/ml, followed by DC18 (1.18 mg/ml), while DC12 shows lower values (1 mg/ml). Most of the DCAs at pH 4 are under the undissociated form, as showed by the dissociation curve (Plot 4, section 1.5.2), thus their solubility should be lower than the one related to higher pH, since they do not bind with water molecules.



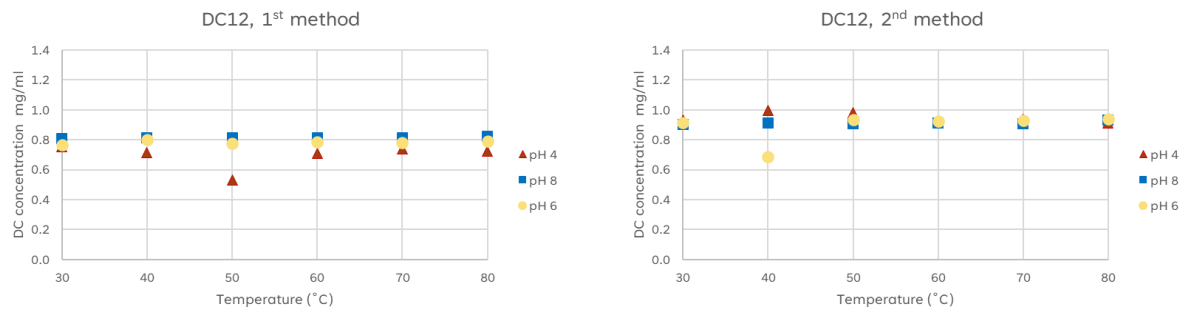
Plot 25, 26: Solubility test, pH 6.

As expected, increasing the pH, the solubility increases according to the 1st method. This could be caused by the increasing percentage of dissociated DCAs. The component most solubilized is the DC16, which arrives to 0.95 mg/ml at 40˚C for the 1st and 1.2 mg/ml at 50˚C for the 2nd. In the 1st case there is not a minimum of solubility related to the temperature and the trend is quite constant while it is different for the 2nd method, where a minimum of solubility at 40˚C (which could be an analytical error since is not detected by the other method) is showed and the maximum is instead reached at 50˚C.



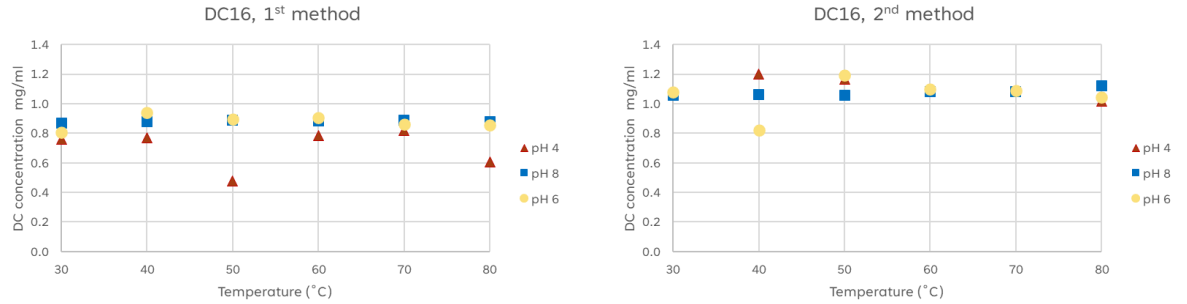
Plot 27, 28: Solubility test, pH 8.

At pH 8 the behavior is flatter with respect to the pH 6 for both methods, but the solubility values are lower. The DCAs should be all in the dissociated according to the pk_a curve (Plot (4)). From both methods the component most solubilized is the DC16, which achieves 0.87 mg/ml at 50˚C (1st) and 1.15 mg/ml at 80˚C. The lowest values are reached by DC12, which tends to be less solubilized. The same results can be also plotted in a different way, considering the single DCA.



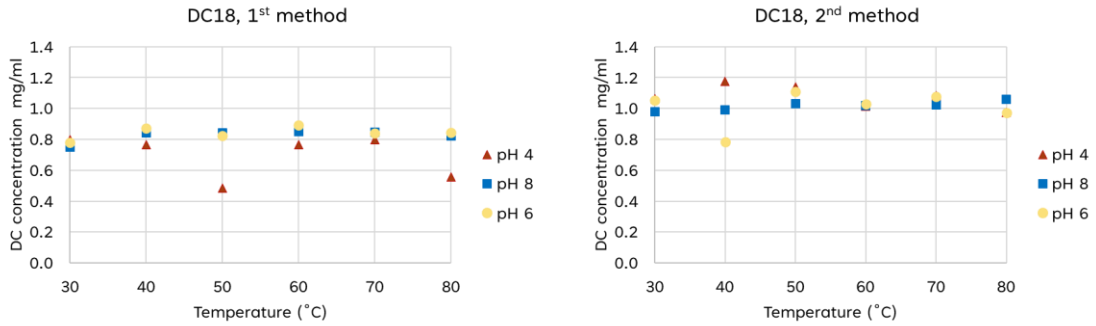
Plot 29, 30: Solubility test, DC12.

The solubility of DC12 drops around 50 ˚C pH 4 for the 1st and at 40 ˚C at pH 6 for the 2nd while the maximum is reached at pH 8 according to the 1st method and at pH 4 (40 ˚C) according to the 2nd.



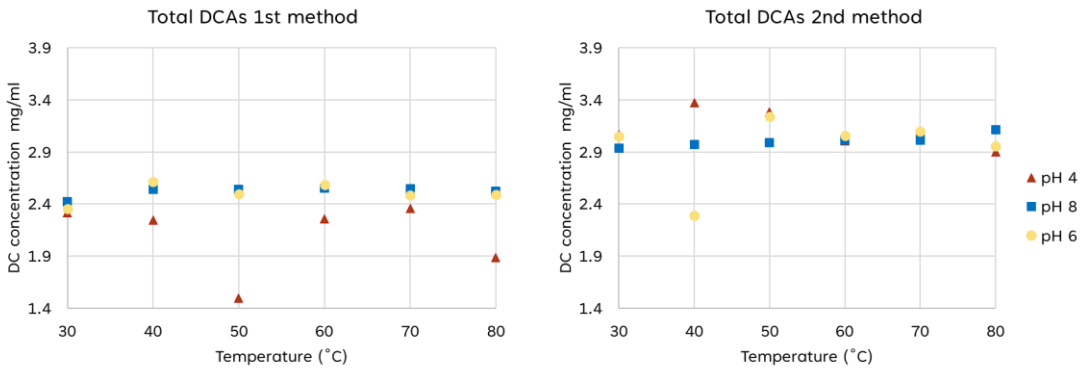
Plot 31, 32: Solubility test, DC16.

The solubility of DC16 drops around 50˚C at pH 4 and reaches its maximum at pH 6 at 40˚C according to the 1st method, while for the 2nd the highest value is reached at pH 4, 40˚C and the minimum at pH 6, 40˚C.



Plot 33, 34: Solubility test, DC18.

The solubility of DC18 drops around 50 and 80˚C at pH 4 and reaches its maximum at pH 6 at 60˚C according to the 1st method, while it shows higher results for the 2nd, reaching 1.2 mg/ml at pH 4. Calculating the total concentration of the DCAs for each single temperature data, the following plots are obtained:



Plot 35, 36: Solubility test, respectively 1st and 2nd method, total concentration.

They show that the highest solubility values of the DCAs mixture are reached at pH 6 and 8, especially at 40 and 60˚C. Since our goal is to work at pH 6, the selection of the temperature around 40˚C according to the 1st method could be a good choice. On the other hand, with the diluted method the Plot (36) the calculated concentrations are higher (closer to the actual value of 3.35 g/L) and while from the Plot (35) the best working temperature at pH 6 seemed to be 40˚C, from the Plot (36) it is not. For this reason, the selection of a lower or higher temperature is needed. The 30˚C could be a good option since the heat duty required to warm up the feed is low and in case of solvent employment (MBSE and MBRE) the evaporation is limited. Also, at 30˚C all the DCAs solubility values are similar, such that the extraction could proceed simultaneously. Even if the pK_a values has been considered equal for all DCAs, as showed in the Plot (4) presented in the section 1.5.2, the results from the solubility test can point out that the pK_a could instead be different for each of them, being the cause of different interactions according to the pH. The Fig. (52) shows the change in color of the feed solution related to the pH rise, and thus related to different interactions.

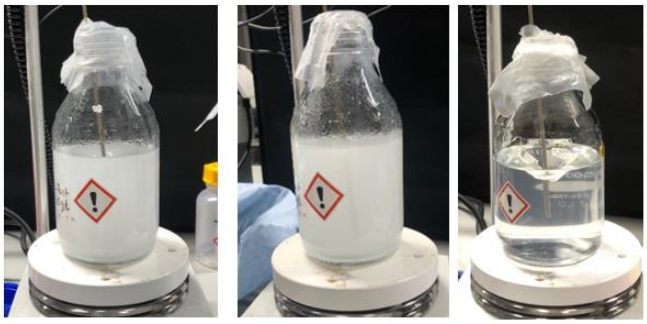


Figure 52: pH 6 (80˚C), pH 4 (80˚C), pH 8 (80˚C)

The solution is hazy at pH 4 and 6 while it becomes clear at pH 8, without the presence of precipitate. The change in sharpness could be caused by the highest degree of acid dissociation with the increasing of the pH, as pointed out by the plot 4.

4. Conclusions

The rapid industrialization and increasing competitiveness among various sectors of industries in the last few decade have initiated chemical engineers to select a process or unit which is smaller, safer and cheaper, creating less waste generation and requires low energy consumption. Downstream processes are one fundamental step in any chemical industry operation, required to recover chemical species from dilute solutions with high selectivity and capacity. Successfully employed methods in this fields have been LLE, MBSE and MBRE, especially for the recovery of (L)DCAs. DCAs can be obtained both by petroleum and fermentation route, they are widely used in food, chemical and pharmaceutical industries. Biological fermentation has been found to be a promising method for the production of LDCAs from renewable biomass. However, it is still a challenge to efficiently separate the organic acids from a mix of multiple diluted components. Current methodologies used in recovery processes all have their limitations, and their improvements are especially needed with regard to yield, purity, and energy consumption. Therefore, there is a need to develop a process that should ideally be simple to carry out and allow the purification of LDCAs directly from the fermentation broths. Thanks to the WODCA project, carried out by VITO and collaboration universities, several downstream configurations (LLE, ME, MBRE) have been designed and tried, among which the most promising in term of extraction efficiency (EE%) have been reactive LLE and MBRE.

Unfortunately, several tested configurations have not been successful: MBSE has showed low EE% especially when ceramic membranes have been employed (7% DC12, 1% DC16 and DC18, pH 6, 30˚C, decanol), while polymeric ones have been able to reach higher values (47% DC12, 41% DC16 and 16% for DC18, pH 6, 30˚C, decanol). MBRE has reached higher EE% thanks to the role of the reactive extractant. The best results, in term of highest extraction of DC12 have been achieved by the MBRE set-up constituted by polymeric membrane (PTFE 0.1 µm) with Aliquat 336+ Octanol (96% DC12, 56% DC16 and 11% for DC18, pH 6, 30˚C).

The results have pointed out that the EE% is affected by acidity (expressed by pK_a) and hydrophobicity (expressed by logP) of the solutes: in this case study, all three components have the same pK_a and they are for 96% dissociated at pH 6. Based on the results, is possible to affirm that the EE% is controlled rather by hydrophobicity of the acids than by their acidity. This effect is much more pronounciated using a mixture, in fact it seems that when the

components are competing for extraction, the role of hydrophobicity (which is increasing with chain length) becomes more important.

Further studies are required to enhance the EE% of DC16 and DC18, which are still far from the final goal (99-100%). One viable road could be the progressive extraction of a single DCAs from the mixture. The best overall results have been obtained with reactive extraction without membranes (LLE, pH 6) when using Aliquat 336+Decanol and Aliquat 336+Octanol, thanks to which also high EE% of DC16 and DC18 can be achieved. Thus, what is possible to conclude is that the membranes used so far are hindering extraction of DC16 and DC18. Further research is required to avoid this and understand the reason behind, for this sake other technologies (NF) will be tested. Unfortunately, even if LLE shows the best results, the problem faced up by this technology is that it could not be combined with bioproduction, since the solvents employed are toxic for the microorganism and thus, they need a separation layer provided by the membrane (such as in the case of ISPR). Besides this, the emergence of new materials and the development of technologies would boost the recovery processes, which would make the biological process more competitive than the chemical routes and promote the development of green chemistry.

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Annex

Issues

Non successful experiments have been also tried as shown in the Table (13): the most frequent problem that has been faced up is the volume shift from the feed to the extractant side. This phenomenon could be caused by several reasons, such as wetting or swelling of the membrane, the use of a damaged membrane, or a membrane with too large pore size with respect to DCAs. The swelling of a membrane occurs when a solvent or chemical is absorbed by the membrane, from the support side (extractant side), causing the polymer network expansion and an increase in membrane volume.[38] The swelling can lead to a change in the membrane's mechanical properties, such as its stiffness and tensile strength and can alter transport properties, such as its permeability and selectivity [38]. Since the swelling can change the selectivity of the system and additionally lead to lose the membrane hydrophobicity, the feed solution will straightforwardly pass towards the MC and it will be mixed with the extractant. The membrane wetting occurs instead when the membrane in the feed side loses its hydrophobic property and the aqueous phase is allowed to pass towards it.

Table 13: Failed experiments

Code	Type of membrane	Type of solvent	Feed pH	Times of trial	feedback	Feed concentration
T-4-2-5 (B)	PTFE 0.1 µm	Decanol	6	2	Leaking but performed until 24 h	2.5 g/L
T-4-2-6 (A)	PTFE 0.1 µm	Aliq+Octanol	8	1	All the feed has passed into the extractant side in the 1st hour.	2.5 g/L
T-4-2-6 (B)	PTFE 0.1 µm	Aliq+Octanol	4	2	All the feed has passed into the extractant side in the 1st hour	2.5 g/L
T-4-2-7 (A) foam	PTFE 0.1 µm	Aliq+Decanol	6	1	Finished at 4th hour because of membrane wetting.	2.5 g/L
T-4-2-7 (B) no foam	PTFE 0.1 µm	Aliq+Decanol	6	1	Finished at 1st hour because of membrane wetting.	2.5 g/L
T-4-2-7 (C) apolar	PTFE 0.1 µm	Aliq+heptane	5.8	1	All the feed has passed into the extractant side in the 1st hour.	2.5 g/L

T-4-2-8.1 binary feed	PTFE 0.1 µm	Aliq+Octanol	6	1	All the feed has passed into the extractant side in the 1st hour	2.5 g/L
T-4-2-9 (A)	PTFE, 0.02 µm	Decanol	6	1	Worked only for 30 min, because of volume shift. maybe the membrane has been wetted by the DC18, which is well extracted by Decanol.	2.5 g/L
T-4-2-3	PTFE 0.1 µm	Decanol	6	1	All the feed has passed into the extractant side in the 1st hour	2.5 g/L

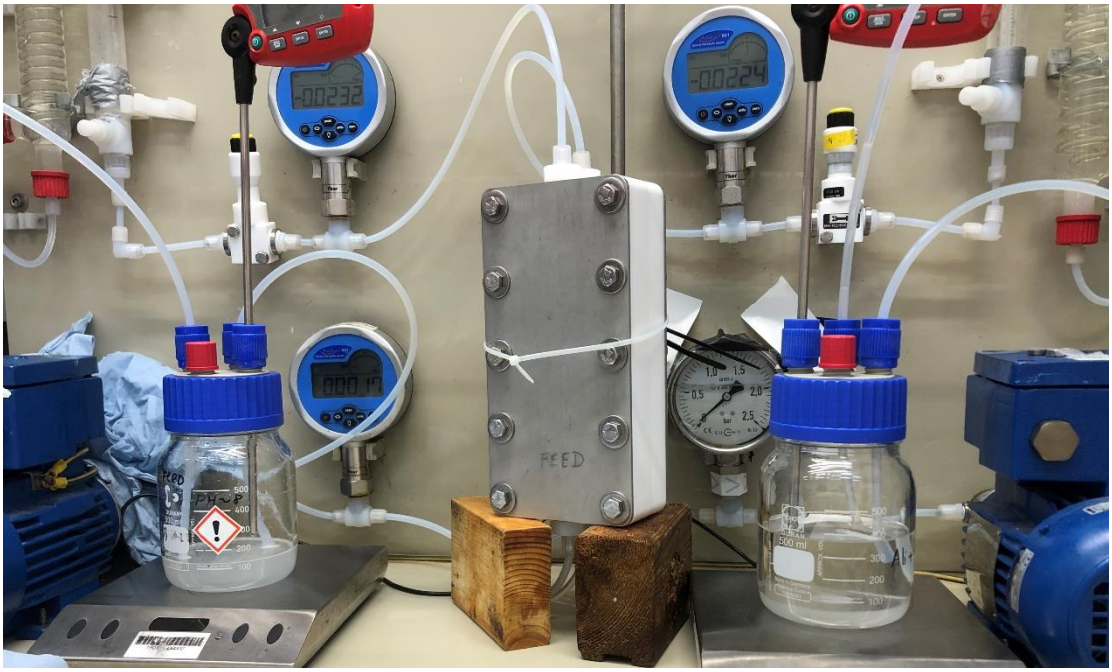

Figure 53: Example of volume shift, T-4-2-6 PTFE 0.1 µm.

The O-ring has caused one additional problem, since it lost its position during the experiment, due to the breaking of its bond, causing the mixing of feed and extractant inside the stuck.

Figure 54: Broken O-ring.

The O-ring used was closed in its ends by a special glue, which was may not compatible with the solvents used. In fact, when the stuck has been opened, the O-ring has been found broken several times; after trials of regluing, the problem has been overcome using a new one-piece O-ring. One other problem has been the leaking. Leaking occurred when the stuck was not enough tightly closed or when the tubes of inlet (positioned at the bottom of the stuck) were not straight, causing the imperfect closing of the inlet screws and consequently the feed/extractant leaking. Contained leaking allows the experiment to work but does not allow to calculate the mass balance.

Figure 55: The closed stuck.