Potential and Challenges of Liquid-Phase Adsorption in Fermentative Processes

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Abstract

As the world turns to decrease the environmental impact that it has perpetuated in the last decades, most industries are deciding to switch to bioprocessing to lower their carbon footprint. However, with most bioprocesses counting up to 80% of costs for only the downstream steps, this presents the biggest setback for the choosing of a more environmentally conscious process. Unlike many downstream methods, adsorption can reduce the operational costs of the fermentative processes, while having high efficiencies regarding product recovery. Unfortunately, since this technique has not been yet studied for these conditions, this thesis focus on the research regarding the adsorption of lysine, a fermentation-based product, alongside the single adsorption of medium components commonly found in fermentation media. The study with the highest removal of lysine was performed using activated carbon at a pH of 9.8 and 30℃, reaching an efficiency of 230 mg/g when in the presence of glucose, through polar interactions. The adsorption of most medium components is shown to be dependent of the ionic strength and pH of the environment and mostly independent of the temperature. As adsorption presents a more economically viable with lower operational and energy costs, there needs to be a decrease on the information gap regarding its use for the recovery of fermentation products.

Keywords: Bioprocess, Fermentation, Lysine, Adsorption, Medium Components

1. Purpose of the Research

In today's society, reducing the carbon footprint of industrial processes has become more important than ever. Some industries have been replacing their processes with biorefineries based procedures by, for example, using biomass as an initial resource. Unfortunately, common methods such as distillation and spray-drying, are not suited for aqueous mediums, due to the high working temperatures, bringing the setback of higher costs and energy usage.

The need to improve the downstream steps can take a long time with more different and new processes being tested and implemented than ever before. One of the downstream processes that has yet to be applied at large scale is adsorption, who, at laboratory scale, has been considered simpler and more energy efficient when compared to other methods that are currently being applied. However, up to today the application of adsorbents in fermentative mediums for the product recovery has not yet been studied in detail, neither its process efficiency and setbacks.

2. State of the Art

The first time lysine was industrially produced was back in the 1950s decade by a japanese pharmaceutical called currently *Kyowa Hakko Bio* and it used a homoserineauxotrophic mutant of *Corynebacterium glutamicum* [1]. Later in the 1970s and 80s, mutants were carried out in fermentations adding additional amino acid auxotrophies and resistance to antimetabolites which sprout in a 40 to 50% yield growth in fed-bacth cultures. In the late 1980s and 1990s genetic engineering started to be applied to microorganisms to improve the production of lysine. Thanks to these new techniques, new organisms easier to cultivate, such as *Escherichia coli*, can play a role in the lysine fermentation process [2].

At the year of 2020, over 2 million tons of lysine are produced annually with several hundred thousands tones (approximately 800,000) being exclusively via bacterial fermentations. The market for lysine is still growing every year at an estimate of 10%. In 2018, the lysine market was valued at over 3000 US million dollars and it's even expected to grow at least 29% by 2025 [3, 4]. For the microbial fermentation of lysine using strains of the gram positive *corynebacteria*, such as *Corynebacterium lilium*, *Brevibacterium dicvaricatum* and *Corynebacterium glutamicum*, has been proved to be the most suitable for its production, with the last one being the most adopted bacteria [3, 5].

In most large scale bioprocesses, the downstream steps can constitute up to 80% of the overall cost, making it a major bottleneck for implementation, where in most refineries costs regarding downstream units round up to 45% of the total capital cost [6, 7]. Since fermentation broths are aqueous mediums very rich in many different components, the downstream must be though with care, as to maximize the recovery of the product and exclude the unwanted components [8].

When recovering biomolecules such as proteins and amino acids, many separations methods are being applied in the downstream phase. Some of the techniques used in current

processes include spray-drying and distillation and these are not considered suitable for the downstream of fermentation processes. This is due to the fact that fermentation mediums have a 90 to 95% of water content, making the energy costs necessary to recover the product notably high [9, 10]

Filtration is considered one of the most economical and easier processes to be scaled-up for the clarification of fluids, but finds difficulties with the many particles sizes and flux rates that must be implemented to keep the normal run of the overall process. One of the most used approach in large scale is chromatography, which can range from ion-exchange, affinity, adsorption and HPLC chromatography. It can be one of the most effective separation processes out there, but one of the most expensive too. [11]. The production of salt wastes is also common and can present a contamination risk [12]. A method that can be applied for the extraction of small molecules, like amino acids such as lysine, is an aqueous two phase system (ATPS). While being cost-effective and environmentally friendly by recycling some of the phase components and the used solutions, unfortunately, for these kinds of systems there still is a gap regarding the scale-up, despite the variety of existing works dedicated to improve the method at large scale processes[11, 13].

One method that has been gaining attention is adsorption. This method uses materials that must be able to remove the desired component from the surrounding environment by adhesion to its surface. Today, it is one of the most simple methods to be applied, while being economical, effective and versatile in many different industrial fields [14, 15]. Without counting the expense of the adsorbent materials, reports have shown an economic advantage of 50 to 70%, mainly to the substitution of three to four downstream steps to just one, reducing in half the overall process time and achieving the recovery and purity demanded [6]. Despite, not being the cheaper and neither the most modern, it shows almost no waste production, a higher selectivity and favorable in terms of consumption of energy in the same conditions, concluding that can be used in fermentations processes [16].

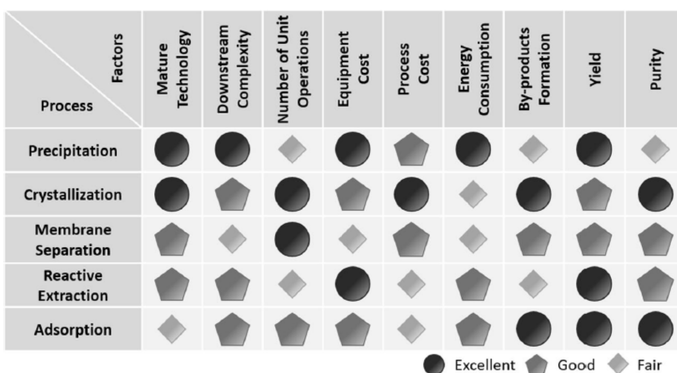

Figure 1: Comparison of advantages and disadvantages of separation methods for itaconic acid in the work by Antonio Irineudo Magalhães Jr group [16].

When compared with other extraction techniques, adsorption presents more advantages, such as easier operations, lower resources costs, no requirement of chemical additives and it is able to reuse the carriers for later processes [17].

3. Fermentation of *L*-Lysine

Lysine, or more specifically *L*-Lysine ($C_6H_{14}N_2O_2$), is a fundamental amino acid for humans and animals health due to its nutritional value [3]. It is considered a basic amino acid due to having two amine groups, one on the alphaposition and other at epsilon-position [5]. The production of lysine in a large scale can happen by both chemical or biochemical routes, with lysine produced by chemical synthesis methods resulting in racemic mixtures and biochemichal methods in a much bigger production of *L*-lysine. The biochemical method with the highest yield of lysine and most economical efficency is the microbial fermentation [4, 5]. Having found a lot of applications in many different fields, such as supplement of feed stocks, lysine became one of the most comercially important amino acid [18].

The fermentation of lysine must follow established parameters so that the micro organisms in the medium can produce the amino acid at the best rate possible. Since the activation entropy for lysine formation is very low, that means that its formation occurs in an anabolic way [19]. To obtain a successful fermentation, there must also be a special attention to its medium, mainly the carbon and nitrogen sources, vitamins (such as biotine) and salts. The main source of carbon can dictate the overall performance and influence the cost of the process [4].

3.1. Appropriate Organisms

For the intent large scale production of *L*-lysine, a primary metabolite, the used organisms must have two main properties: they must be homoserine auxotrophic (due to a defect of homoserine dehydrogenase) and S-(2-aminoethyl)-*L*-cysteine (AEC) resistent [2]. Experiments done in 70s when the lysine fermentation process was starting to grow, many bacteria were tested to check which one had the best production of the amino acid. *Corynebacterium* showed the highest values at work conditions of 30℃ at neutral pH values of 7 and 7.5, achieving a final lysine concentration of 20.8 and 3.77 g/L, respectively [20]. Currently, the most used organism is *Corynebacterium glutamicum*, with other species such as *Brevibacterium* (with its subspieces of *flavum* and *lactofermentum*) being occasionally used. *E.coli* strains have also been found to be effective in the same type of fermentation when properly engineered [2, 21]. Nonetheless, *C. glutamicum* was found as the better host, due to its physiological properties, its GRAS, having a fast growth rate, being genetically stable and capable of using a variety of different carbon source, making it accessible to manipulation and cultivation in more harsh industrial conditions [22].

3.2. Temperature

When discussing temperature, most processes regarding fermentation of lysine are done in mesophilic conditions, usually below 35℃. Being an exothermic process, studies show that an increase in the temperature of the medium lowers the viability of the entire process and can put it at risk since it affects the growth of the organisms and even the stability of the end product. Many researches are still looking for economically and favourable processes where higher temperatures can be

applied without bringing disadvantages to the process, considering the high costs of the cooling operations [4, 23]. In the study performed by the group of Toshihiro Tateno the overall yields of production lowered from 18.80% to 2.38% when temperatures went from 30 to 40℃, while keeping the pH level constant at 7,0 [24]. J. Ohnishi *et al* proved that a specific mutant of *C. glutamicum* can operate functionally at 40℃ and provide an improvement of 20% in production yield for lysine (reaching a final titer of 85 g/L) when compared to the 30℃ culture, but with a decrease of 75% in the growth rate [23].

3.3. Oxygen Supply

Being an aerobic process, the supply of oxygen to the lysine fermentation is considered critical for the productivity of this amino acid, since the level of oxygen in the medium is directly related to the metabolic activity, verified by a linear production of ATP. Nonetheless, the increase of the oxygen transfer rate (OTR) can later develop to oxidative stress and further decrease in productivity [4]. A study conducted by A Hadj Sassi *et al*, looked into how the oxygen and carbon dioxide could influence the overall production of lysine when using *C. glutamicum*. At 27℃, pH 7.2 and a carbon source of glucose, the highest lysine production happened at 0.75 vvm with a 50% increase, meaning a 30 to 35% saturation of oxygen in the medium. Unfortunately, the authors don't indicate what the stirring rate was. When testing how the presence of CO_2 could influence the process, the ideal oxygen saturation was maintained and increased the concentration of CO_2 in the medium. This increase revealed a reduction in the production of lysine by 17%, with the carbon being redirected to the formation of lactic acid, due to the cell giving more importance to the glycolyic activity, enhacing the necessity of the removal of this component [18].

3.4. Osmotic Pressure

Since C. glutamicum is a gram-positive bacteria, the whole cells tend to contract in hyperosmotic stress instead of plasmolysis when osmotic pressure rises in the medium, mainly due to a higher intracellular pool of amino acids which gives them a higher turgor pressure. O. T. Skjerdal *et al* showed that, in their conditions of 30℃, pH 7.0 and an oxygen saturation of 30%, gram-positive baceria tolerate higher levels of stress than gram-negative bacteria, with *C. glutamicum* tolerated higher levels of stress when compared to *B. lactofermentum*, such as increasing NaCl (2.1 osmol/kg), sucrose (1.6 osmol/kg) and lysine (1.7 osmol/kg) concentrations [25].

3.5. pH

The pH level must be regulated as to facilitate the export rate of lysine to outside the cell, making it easier to collect. This excretion via an export system is highly dependent on the membrane electric potential. It is in this neutral pH values that the carbon source reaches its highest levels of consumption and, eventually, we have the highest yields of production for lysine with the export enzyme carrier activity reaching its higher capacity at a pH of 6.5. When analysing different values of pH, it was noted that the biomass concentration stayed constant at 25 g/L, but the production of lysine decreased notably [26]. Another study supported the idea that the best production of lysine happened at pH 7 (at 30℃), with the culture and its specific rate decreasing for lower pH levels [24].

3.6. Manipulation of the Biochemical Routes

To improve the production of lysine in *C. glutamicum*, the most common approach inside the metabolic engineering has been to reduce the specific growth rate of the organisms by redirecting the carbon source to the synthesis of lysine [27]. The most important precursors are pyruvate and oxaloacetate (OAA), which means that an attempt of lowering their availability for other routes can improve the formation of this amino acid. Ideally, if we increase the replenishment and decreasing the consumption of precursors we improve its availability, thus increasing the lysine final concentration. This can be made possible by genetic manipulation of the expression of enzymes [28].

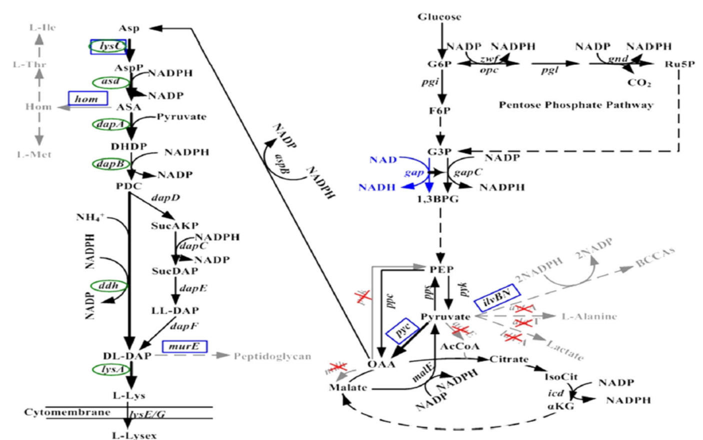

Figure 2: Schematic representation of metabolic engineering strategy for genetically modifying C. glutamicum strain for L-lysine production. Gray arrows represent the interrupted or attenuated pathways, red cross represents the gene knockout, green ring represent the gene over-expression, blue line and box represent the gene replacement, and the thick arrows represent the up-regulated pathway [28].

Jianzhong Xu *et al* [28] decided to approach how the overexpression or deregulation of the enzyme genes could influence the production of lysine with cultivations of *C. glutamicum* at 30℃ and pH 7. A survey of the same study was to increase the quantity and availability of the precursor OAA by manipulating the expression of enzymes. The production of lysine achieved a final concentration of 130.82 g/L, with a glucose conversion efficiency of 47.06%. As a result, the production of lysine reaching its highest production, it left the cells defective due to a lack of formation of cell-wall peptidoglycan. The authors conclude that, except, for the over expression of *lysA*, they obtained a strain capable of highly improving the production of lysine using *C. glutamicum.*

3.7. Mode of Operation

In batch cultures, since the medium is not renewed, the bacteria has a tendency to suffer a catabolic repression and an additionally osmotic pressure, mainly due to the accumulation of the amino acid end product in the medium along other by-products, which can even lead to the deactivation of the overall fermentation. It is no longer considered a preferred

method and only used in smaller fermentation plants. A fed-batch culture can operate as an alternative to batch operations since they can avoid loss of productivity due to product repression or the presence of toxic precursors, thanks to the constant feed of carbon and nitrogen components [4, 18]. The study by A. Hadj Sassi [18] ends up showing the flaws of the fed-batch operation - there is a higher production of by-products, such as lactic and acetic acid, and therefore, a lower productivity. Nonetheless, in the overall process, it reaches a higher final lysine concentration.

At the end of the day, a continuous type fermentation presents the most advantages when compared to the previous modes of operation in large scale productions, since it averts problems like osmotic pressure and end-product repression thanks to its constant nutrient input and product and by-products removal[4]. Unfortunately, since continuous fermentations have longer working time periods, this means that despite reaching higher end-product concentrations, their productivity and process yields are lower. Other disadvantages are how the system has a higher vulnerability to contamination and the genetically destabilization of the used strain, making the work with mutant strains more complex, since it is imperative that these remain invariable throughout the process [4, 19]. From the research of Kiss and Stephanopoulos[19], and what was already talked in the previous topic of biochemical routes, the main nutrients necessary for the production of lysine are also being used for the production of biomass and its cell growth. With the constant feed of carbon and nitrogen to the medium, to improve the yield of the process, the nutrients must be redirect to the synthesis of lysine by adjusting the process conditions or by genetically engineering the organism that will be used.

4. Principles of Adsorption

This phenomenon of adsorption consists in a mass transfer process that leads to the removal of molecules from a fluid bulk phase to a solid surface, usually solutes present from a gas or liquid mixtures and at phase boundaries usually between a solid and a liquid or gaseous phase [29, 30]. The material of the solid surface (the adsorbent) where the molecules will be retained will be characterized by its specific surface area and volume, with the adsorbate accumulating in its surface due to different types of interactions. With the existence of different kinds of forces and interactions, this process can be divided into two categories, namely physical adsorption and chemical adsorption [31]. Physical adsorption tends to form a single or multilayer of adsorbate on the material surface due to the weaker interactions, while chemisorption needs stronger interactions such as covalent bonds, forming only a single layer on the surface [32]. As previous mentioned before, adsorption is considered a mass transfer phenomenon and it can be summed up in three steps, which are film diffusion, pore diffusion and surface reaction (fig. 3) [33].

Typically, adsorption is reversible, with suitable adsorbents having a responsibility to remove the substances but also for their later release by the change of conditions. For most of the cases, this process is described at the equilibrium by means of equations called isotherms models [34]. Many models are being applied to the current researches where the most common ones are the Langmuir (eq. 1) and Freundlich (eq. 2) isotherm models. Between these two models, the main difference resides in the distribution of energy in the adsorption sites - Langmuir considers the surface of the adsorbent material as energetically equivalent, while Freundlich assumes it as energetically heterogeneous [35, 36].

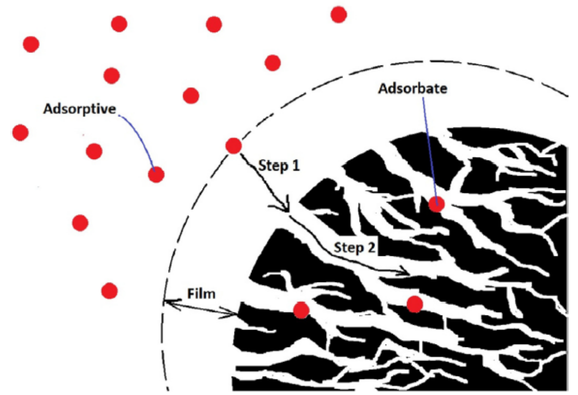

Figure 3: Schematic representaton of the mass transfer process in adsorption. Step 1 - film diffusion; Step 2 - pore diffusion. Surface reaction is not represented [33].

$$q_{\mathsf{e}} = \frac{q_{\mathsf{max}} K_{\mathsf{L}} C_{\mathsf{e}}}{(1 + K_{\mathsf{L}} C_{\mathsf{e}})} \quad (1)$$

$$q_{\mathsf{e}} = K_{\mathsf{F}} C_{\mathsf{e}}{}^{1/n} \quad (2)$$

4.1. Types of Adsorbents

The adsorbent material used must have a great affinity and select conditions for the components present in the solution that are intended to be removed at best efficiency [37]. The most important characteristics that make an adsorbent suitable are its large specific surface area, surface properties such as polarity amd the availability of the adsorption sites [38]. According to the nature of the material, adsorbents can be classified as organic and inorganic, while also being natural, synthetic and waste [35].

The most commonly used material for adsorption is activated carbon (AC) which can be produced by pyrolysis of carbonaceous organic materials such as coal, wood and husks, making them an amorphous carbon-based material [31, 35]. This adsorbent has an excellent adsorption capacity for most pollutants due to their porosity, high hydrophobicity and large specific surface area (ranging from 500 to 2000 m^2 per gram). Regarding average porosity they can be classified as microporous (below 2 nm), mesoporous (2-50 nm) and macroporous (above 50 nm) and have a bulk density range of 400 to 500 kg/m^3 [35, 39]. Nonetheless, to turn these materials into AC adsorbents, they must be subjected to an activation which can happen *via* chemical or physical route [40, 41].

Another adsorbent material typically used are zeolites. Zeolites are hydrated crystalline microporous aluminosilicates, usually amorphous, with cavities of constant molecular dimensions (0.3–1.5 nm diameter), making them very selective [35]. Structurally, three independent parameters must

be considered regarding the classification of each zeolite, namely the aluminosilicate framework (Si/Al ratio), exchangeable cations and zeolitic water [42, 43].

Nanoporous organic polymers are attracting attention by using multi functional organic building blocks and putting them through a number of cross-linking reactions, it results in a variety of amorphous networks with a permanent porosity and high specific surface areas [44, 45]. These porous and organic polymers (POPs) are highly stable physically and chemically, with hypercrosslinked polymers (HCPs) seeing the fastest development in recent times. The synthesis of HCPs is based on Friedel–Crafts chemical reactions [46, 42].

One material that can also be used as an adsorbent is silica gel (SiO_2), which is a porous, amorphous form of silica with an hydrophobic surface area that possess an ample network of connected microscopic pores (6–25nm) [47]

4.2. Biosorption Processing

When applied in bioprocesses, the adsorbent materials are commonly introduced in the downstream phases. In a batch type operation, the adsorbent is added to the solution until the concentration of the pollutant has reached the intend level. The adsorbent is later discarded or regenerated. This mode of operations is useful to study intrinsic kinetics. For a continuous process, the adsorbent is present in a column bed adsorption system. The liquid is fed through the top or the bottom of a stationary bed of adsorbent material curve [35]. In these columns, the rate and kinetic of the process can be followed and determined by the study of the breakthrough, as presented in fig. 4 [33].

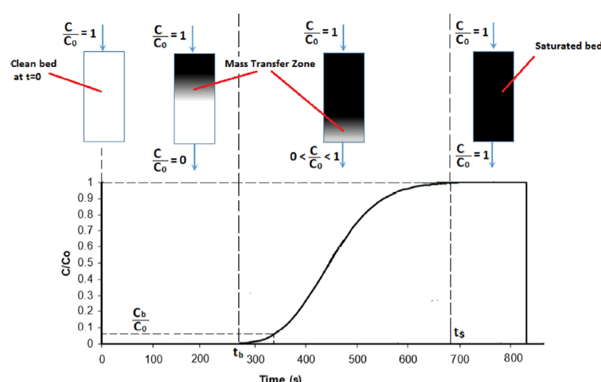

Figure 4: Represented is a typical S-shape breakthrough curve. The corresponding position of the mass transfer zone in the column is shown on top of the curve. C_b corresponds to the breakthrough concentration; C_0 means feed concentration [33]

At higher adsorbate initial concentrations, the driving forces in the column are typically the highest, resulting in a faster transport of the component to remove through the adsorption column. Unfortunately, this also leads to a quicker occupation of the binding sites due to smaller mass transfer zones. With more adsorbent, it's clear that a higher quantity can be removed from the solution due to having more binding sites. With the increase of the bed height comes an increase of both time and breakthrough points, meaning a higher interaction and contact time. At higher flow rates there is a decrease in the contacting time between the surface of the adsorbent and the adsorbate. This means a reduction on the breakthrough point and the rate of the adsorption lowers. Unfortunately, some aspects may find adversities when applied in larger scales. For example, the lower flow rates can translate into process speed problems, especially at an industrial scale with the large amount of medium solution that needs to be processed. The need for a higher quantity of adsorbent material can also lead to monetary complications [48, 49].

As it was mentioned, the fermentation of lysine also produces by-products, most of them being weak carboxilic acids. In the case of acetic acid, since it tends to interact with compounds who are more basic, the adsorption will be facilitate if the adsorbent applied has a basic nature [50]. The same situation is observed when lactic acid is adsorbed. According to the most researches, the removal of acids is very dependent on the pH of the environment, despite showing some different behaviours according to the adsorbent used [51].

4.3. Adsorption of Lysine

Since our desired product is an amino acid, we need to comprehend how these types of biomolecules act when subjected to this method of recovery. The most common forces in amino acid adsorption are electrical attraction/repulsion, hydrogen bonding and polar interactions. Lysine has a pI of 9.8 and has an amine group on its R side chain, translating in a third pka and a third electric state [52].

When using silica as adsorbent studies showed higher adsorption at neutral pH values, where the silica surface was negatively charged and lysine existed in a predominant state of a monovalent cation. In the established pH range of 7.1 to 9.8, adsorbed lysine showed unchanged dissociation states of 81% cationic and 19% zwitterionic, both with a 5% error margins. With the increase of pH from 7.1, it does not come as a suprise that the adsorbed cationic lysine increases and the same non-adsorbed state decreases, since the silica surface becomes more negatively charged [52]. The study of Andrea J. O'Connor *et al* [53] reached a maximum adsorption capacity of 0.21 mmol/L, at pH 6 and room temperature. Yanli Yang [54]concluded that the presence of cations with higher positive charges such as calcium, could dramatically reduce the adsorption of lysine, specially in neutral and acidic pH values (4 and 7, respectively) at a constant ionic strengh of 0.01 M of NaCl. The addition of sodium chloride also led to a decrease in the adsorbed lysine since it boosted the electrostatic shielding and competition for the adsorption sites.

L. Stievano *et al* [55] took in consideration the adsorption of lysine onto silica in aqueous medium, in the presence of glycine. Both amino acids were tested using equimolar concentrations. It was observed that at pH 7, silica reached its maximum adsorption rate for lysine, since at that pH it is in its cationic state while glycine presents no electric charge. The authors then conclude that it is the electrostatic interactions that rule the adsorption of different amino acids for the case of silica as an adsorbent.

Jeff Deischter *et al* [56] used AC (a hydrophobic and non-polar material) as adsorbent. The non-competitive adsorption of lysine shows its maximum at pH 10 with 294 mg/g, where lysine is a zwitterion, with AC *A SUPRA EUR* present-

ing the higher amount of lysine adsorbed, meaning it had the best affinity to the zwitterionic form of lysine. In a competitive adsorption with glucose, *CW 20* presented a removal of 230 mg/g and a separation factor of 4.4, with lysine relying more in the interactions between its amine groups and the oxygen surface groups. Some AC have different surface characteristics, such as coconute shell derived AC. In a non-competitive adsorption of lysine, the highest adsorption amount was reached at pH 6, by removing 84% of the existing lysine. This suggests that the surface of the coconut shell AC is negatively charged, making the adsorption a process led by electrostatic interactions.

Temperature was not seen as an influential parameter in none of the mentioned studies.

4.4. Adsorption of Medium Components

The recovery of lysine by adsorption will happen when its still immersed in the fermentation medium. This presents some setbacks, since applying adsorbents in this environment will probably result in the parallel removal of other components that are not the wanted product. To improve the recovery of lysine, we need to understand how adsorption happens for each medium component under similar conditions and adsorbents as to prevent it from occurring.

4.4.1 Microorganisms

In the comparison study by Jan Feuser *et al* [57], gram-positive bacteria showed a higher adsorption to an ion exchange adsorbent in all tested pH values, with gram-negative organisms such as *E. coli* exhibiting the lowest adsorption rate. This supports the hypothesis that most cells are indeed negatively charged at neutral pH values and their adsorption is ruled by electrostatic interactions. For *E. coli*, the group of J Rivera-Utrilla [58] tested its adsorption in AC, reaching its higher rate when the pH was located in between the pI values of both cells and adsorbent materials. The addition of electrolytes such as Fe^{3+} increased these rates up to 87.8%, by reducing the electrostatic repulsion interactions.

In *C. glutamicum* cells, the consumption rate of phosphate can affect its adsorption to a surface [59]. At 30 ℃ and pH 7.2, phosphate saturated cells were compared with phosphate depleted ones. A difference between saturated and depleted cells are the lipoteichoic acids in the external membrane, who are synthesised in the presence of phosphate and reduce the polarity of the cell wall due to their hydrophobic nature. It is expected that electrostatic interactions are the main reason for this process, making the adsorption heavily dependent on the pH. For the case of glass carriers, the adhesion is favored by a higher hydrophobicity of the cells (displayed by the saturated ones).

Xiufen Li *et al* [60] tested the adsorption in polysulphone membrane (an anionic material), the adsorption reached its peak at the pH of the pI of the cells (3.2). This was similar to previous studies and suggesting a binding regulated by electrostatic interactions and an affinity between the positive charges of the cell and a more negatively charged adsorbent. The effect of ionic strength was also observed, with results showing a decrease on adsorption on lower pH values but a rise on higher ones.

As to avoid the eventual adsorption of cells, specially *C. glutamicum*, an established pH different than the pI of the cells and lower ionic components concentrations will retard this removal and allow other components, such as lysine, to be recovered.

4.4.2 Sugars

María Francisco *et al* [61] tested the adsorption of *D*-Glucose in zeolites in the presence of aqueous ionic liquids at room temperature. Reaching an approximately removal of 50% of glucose for every applied zeolite, this is explained by the weakened interactions between glucose and the water molecules, which favours the attraction between the OH groups and the active sites of the adsorbents. The research group of Ziwei Liu attempted a separation of a mix of eight different types of saccharides at 30 ℃ using AC. The increase of carbons in the molecules makes them more heterogeneous and therefore, more distinctive, explaining the better separation between trisaccharides, disaccharides and monosaccharides. This separation using AC is dominated by the electrostatic interactions between the hydroxyl groups of sugars, with hydrogen bonds complementing it.

An attempt on separating a mixture of glucose and fructose was studied by Guido Schroer *et al* [62] using, as adsorbent, crosslinked boronic acid polymers. at pH 10, the adsorbent shows a swelling of 212 and 178% in fructose, for 20 mol% of DVB and EDMA respectively, where in glucose the swelling is negligible when compared to the solution with no buffer. The recovery of fructose must happen at acidic pH since that's where the sugar complexes are most unstable. As the ratio of sugar to polymer increased, so did the selectivity toward the adsorption of fructose. the adsorption of fructose using this kind of material reached a yield of 540 and 407 $mg_{Fru}/g_{polymer}$ for DVB and EDMA 20mol%, respectively.

For saccharides, temperature and pH are not seen as a parameters that adsorption might depend on. The adsorption of sugars are then very dependent on the ionic strength of the medium, since they tend to strongly interact with water molecules.

4.4.3 Phosphates

In the removal of phosphates anions, most studies show that the cover of the surface of the adsorbent with metals components tend to attract, and remove, this types of components.

Yinhai He *et al* [63] used zeolites who were modified with lanthanum in their surface. With the adsorbent's pI of 7.02, below this value the adsorption efficiency reaches values above 80%, since the phosphate species are anionic. This evolution is represented in fig. 5. The increase of temperature show a rise on amount of adsorbed phosphate as well. The same conclusions were drawn out from a study using lanthanum doped AC by Jianyong Liu *et al* [64]. Weiping Xiong *et al* [65] still applied AC nanofibers to test adsorption of phosphates, but this time modifying it with iron-zirconium.

Like the previous researches, the highest adsorption rate was achieved at acidic pH of 4, reaching 26.3 mg/g. This was tested at room temperature.

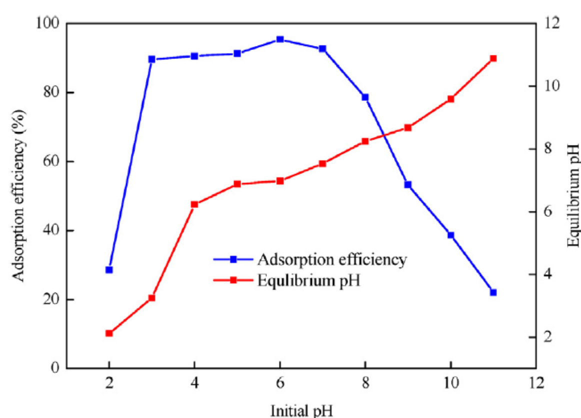

Figure 5: Adsorption efficiency with evolution of pH in lanthnum covered zeolite [63].

This addition of metals on the adsorbents surfaces improves the removal of phosphates, since the electrostatic interactions between the components and the metals were much noticeable. As a large anionic and heavily charged component, it can present a considerable competition to the adsorption sites when present in fermentation broths. The use of adsorbents not modified with transitioning metals can so help to avoid the excessive adsorption of phosphates.

4.4.4 Sulphates

Evgenia Iakovleva *et al* [66] there is a focus on removing sulphate and chloride from an aqueous medium. The alkaline adsorbents showed a preference for the smaller chloride anions, exhibiting a low competition. Nonetheless, the adsorption of sulphate anions had its lowest value at 54% and reached 99%. As expected, the main interactions that ruled this process were electrostatic ones.

Cristiane da Rosa Oliveira *et al* [67] drew the same conclusion when applying zeolites. With working conditions established at 25 ℃ and pH 6, the adsorbent reached a capacity of 1.36 m_{eq}/g. A competition study between four anions, which included the sulphate anion, was done by the research group of Houssine Sehaqui. In all the tests performed, at room temperatur in different pH values using cationic cellulose nanofibers, it was sulphate who reached the highest amount adsorbed, meaning a preference for multivalent anions.

4.4.5 Vitamins

The removal of vitamins must take in consideration their polar properties, since they are divided by their solubility in fat or water.

Sergey N. Lanin *et al* [68] tested the adsorption of water soluble B vitamins in different adsorbents (such as silica, HCP and AC). The vitamins had weak interactions with silica based adsorbents, while presenting stronger ones with HCPs and AC materials. Mainly, the increase in hydrophobicity of the adsorbent surfaces led to a lower rate of removal of vitamins. Zorica Basic *et al* [69] putput to observation the adsorption of B vitamins (1, 2 and 6) in zeolites. At 37 ℃, it was found that, at the acidic pH of 2, the adsorbent showed the best adsorption efficiencies of 21.87, 20.15 and 4.53%, respectively. This shows that zeolites have very small adsorption capacities for water soluble vitamins. Rishabh Saraswat *et al* [70] tested the adsorption of B_{12} in AC. At neutral pH and 30 ℃, the AC beads showed a higher adsorption efficiency with a maximum of 300 mg/g.

The adsorption of water soluble vitamins is then under the control of the overall solvent and the ionic strength that it may present. In aqueous environments, these molecules show a higher tendency to interact with the water molecules rather than the surface of the adsorbents.

4.4.6 Urea

With a very low pk_a of 0.1, urea is considering a cation for most common environments.

V. Wernert *et al* [71] tested the removal of urea in zeolites. At 37 ℃, with initial concentrations of 8.6 and 41 mM at 6 and 5.4 pH, respectively, the adsorption reached efficiencies above 30 mg/g and 140 mg/g for each initial concentration. Urea interacted with the adsorbent material by establishing hydrogen bonds between the amine groups and the existing surface oxygen groups. Tomohito Kameda *et al* [72] observed the adsorption of urea in AC. With initial concentrations in the range of 50 to 2000 mg/L, the highest adsorbed amount was found to be at 10 ℃, with a value of 1.1 mg/g at a rate of 7.8 mg/(g.min), and decreased with the raising of temperature. The adsorption itself was explained by dipoledipole interactions between the NH_2 group of the urea and the existing carbonyl and hydroxyl groups in the surface of the adsorbent.

Safwat M. and Minerva E. Matta [73] decided to observe the influence of pH. The adsorbents (activated alumina and AC) were put to test at three different pH values - 5, 7 and 9 - with the maximum removal efficiencies were both at the highest pH, reaching 24 and 31% for granular AL and granular AC, respectively. In lower pH values, the adsorption removal decreases due to the electric repulsion.

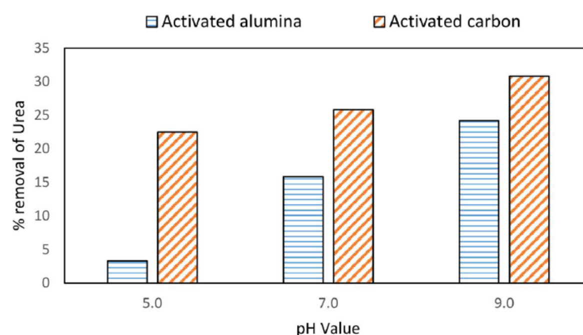

Figure 6: Removal efficiency of urea at different pH values using activated alumina and activated carbon [73].

This substance ends up showing a competition for the re-

covery of lysine due to its small size and higher adsorption at pH values near the pI of the amino acid. Nonetheless, the interactions decrease with the increase of the temperature.

5. Modulation and Simulation

5.1. Adsorption of Itaconic Acid

In this first section, we have a hypothetical case of production and recovery of itaconic acid (IA) by microbial fermentation and adsorption, respectively, in fed-batch mode. A simplified block diagram of the process is represented in fig 7.

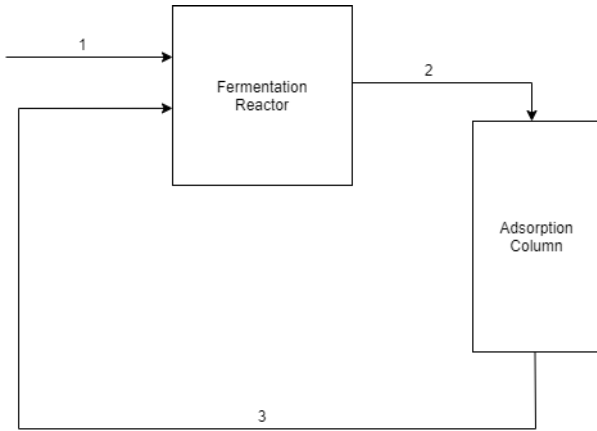

Figure 7: Simplified block diagram for the overall process of fermentation of IA and its recovery through adsorption in a column.

Knowing that the working volume of the reactor is 1.5 L and the process has a productivity of 5 g/(L.h), this means that, per hour, there is a production of 7.5 g of lysine. Assuming the fermentation is happening with an ideal mixing, this means that the concentration of IA (and glucose) is the same inside the reactor and on the outlet.

We can know calculate the volume rate that exits the reactor by dividing the produced mass with the concentration of IA that exits in the outlet, having a result of 0,3 L/h. Since we want to keep the working volume of the adsorption column constant, the volume rate in flow 2 and 3 must be the same, meaning that the volumetric flow rate in the external loop must also be 0,3 L/h.

In each cycle, 7.5 g of IA are produced, meaning that for each cycle we need, at least, 16.16 g of adsorbent material, meaning that after 10 cycles there must be 16.16 g of adsorbent in each column.

$$16.16(1-0,20)^{10} = x \tag{3}$$

By solving equation 3, we find out that to maintain the adsorption capacity to its maximum up to the 11]th cycle, we need to implement 150.5 grams of adsorbent material in the column at the beginning of the process.

5.2. Dimensioning of Lysine Fermentation Reactors and Adsorption Columns

In a similar working process to the previous section, we now have a production of lysine in two scales, laboratory and industrial with each one having a daily production of 25 g and 420 kg, respectively. The following deductions are intend to set the dimensions for both the reactors for fermentation of lysine and its adsorption columns, in a fed batch operation.

Assuming the medium composition from example number three of a patent of lysine fermentation [74], it was found that there was a 32.3% of sugar conversion into lysine, with 54.7% of it being located outside the biomass and able to retrieve. Assuming a total recovery of the intended 420 kg of lysine, there would need to be available approximately 2380 kg of sugar up to the end of the fermentation step. The same example uses a 10 m^3 reactor for the fed-batch fermentation. This reactor is used as to hold the mass of the initial sterile medium of 3980 kg, alongside the total feed that will be receiving from the feed flow (2171 kg), totaling 6151 kg of broth. By applying the same mass compositions and conditions for the intended production of 420 kg of lysine, the initial sterile medium and final quantity of feed would now be 7181 and 3845 kg, respectively, adding to a total of 11027 kg. Assuming a linear expansion, the new reactor would need to have a volume of approximately 18 m^3.

As for lab scale, the analogous calculations were made: an initial sterile medium of 427,8 g and a total feed of 227,6 are necessary to provide 141.5 g of sugar and therefore a production of 25 g of lysine. Since the reactor would need to be able to hold 655.4 g, its volume must be settled at roughly 1.07 L.

To achieve a total removal of 420 kg of our product, the column would have to be filled with 1830 kg of adsorbent (using AC *CW 20*, with an efficiency of 230 mg/g). With an average density of 450 kg/m^3, the volume of the bed ends up being 4.06 m^3. Since the volume of the column must 1.5 time bigger than of the adsorbent bed [75], we can adjust the total volume of the adsorption column to 6.06 m^3, translating into a column with a diameter of 80 cm and an 8 m height [76]. For the lab scale production of 25 g, the amount of adsorbent needed is around 108.7 g, making its bed and total column volume of the column 2.42×10^{-4} m3 and 3.62×10^{-4} m3, respectively. This translates in a column with a diameter of 3 cm and a height of 31 cm.

5.3. Flow Sheet Design for Lab Scale Process

The prepared medium for the fermentation is based upon the recipe used by the AVT laboratory for the fermentation of lysine using *C. glutamicum*.

The fermentation reactor has a designated working volume of 1.5 L, with the cultivation happening at 350 rpm. The carbon source is being controlled by a valve, as is the oxygen. We establish the conditions to a pH at 7.00 $\pm$ 0.02 and a temperature of 30℃, since according to Kiss and Stephanopoulos [19], this conditions provide a lysine production of 0.8 g/L in glucose, with a productivity yield of about 0.075g/(gglucose.h), when at equilibrium. After the reaction, there will be a filtration step, as its considered typical for fermentation broths to separate bigger components, like the *C. glutamicum* cells [43]. For this step, we will assume that all produced lysine was able to pass through the introduced membrane and into the adsorption column. The adsorption column will be an expanded bed and it will use the modified AC *CW 20* as the adsorbent for this process, with a previous addition of NaOH to raise the solution pH to 10 and to achieve a removal of lysine up to 230 mg/g. The software *SuperPro*

Designer was used to represent the process in a schematic and simple approach (fig. 8).

With the established parameters, we can calculate that there needs to be at 15 grams of glucose in the medium for the fermentation to occur without complications. Applying the productivity, we know now that in each hour it is produced 1.125g of lysine [44]. In the downstream, to recover the product with the same efficiency as the presented by the CW 20 adsorbent, 4.89g have to be present in the adsorption column. The recovery is a result due to the application of an acidic solution through the column.

6. Conclusions and Future Prospects

As stated before, this thesis focus on the specific case of the application of adsorbents to recover lysine from its fermentation medium, as to display the advantages of this method (economically and operational).

Parameters such as temperature, pH, oxygen supply and osmotic pressure have been well studied for the production of lysine, using *C. glutamicum* as host. Mesophilic temperatures, neutral pH values, oxygen saturation at a range of 30 to 35% for the gram-positive bacteria translate into the higher production yields for the amino acid. Studies dedicated to advance the production of lysine are currently focusing on the genetic and metabolic manipulation of the bacteria capable of producing the amino acid, as a way to better employ the available carbon source towards the target product and create more resilient strains. Researches have proven that these manipulations in bacteria, specially in *C. glutamicum*, show an increase in the production yields of lysine, alongside a reduction in the final concentration of by-products and cellular growth.

When it comes to the recovery step of lysine, adsorption has only been applied to laboratory prepared solutions that don't accurately resemble a fermentation broth. The conclusions that this adsorption happened due to electrostatic and polar interactions (being a physical adsorption), even when using other materials for adsorbents, are also supported by the current literature. Unsurprisingly, this types of studies are not loyal to a real fermentation broth, where there are many more components competing for the same adsorption sites, with most papers displaying the same conclusion that electrostatic interactions are the main reason for the adsorption of medium components on different materials. This presents a clear competition for the adsorption sites when working to recover lysine from the fermentation medium, since most components were adsorbed under the same conditions.

As a simpler downstream method who is more economically viable due to lower operational and energy costs, adsorption has the potential to produce similar or better results for product recovery than most of the methods currently in usage. This calls for extra research for the application of this technique for product recovery, mainly for industries still relying in petrochemical processes and intend to lower their carbon foot-print by switching to biorefinery ones (such as fermentative processes).

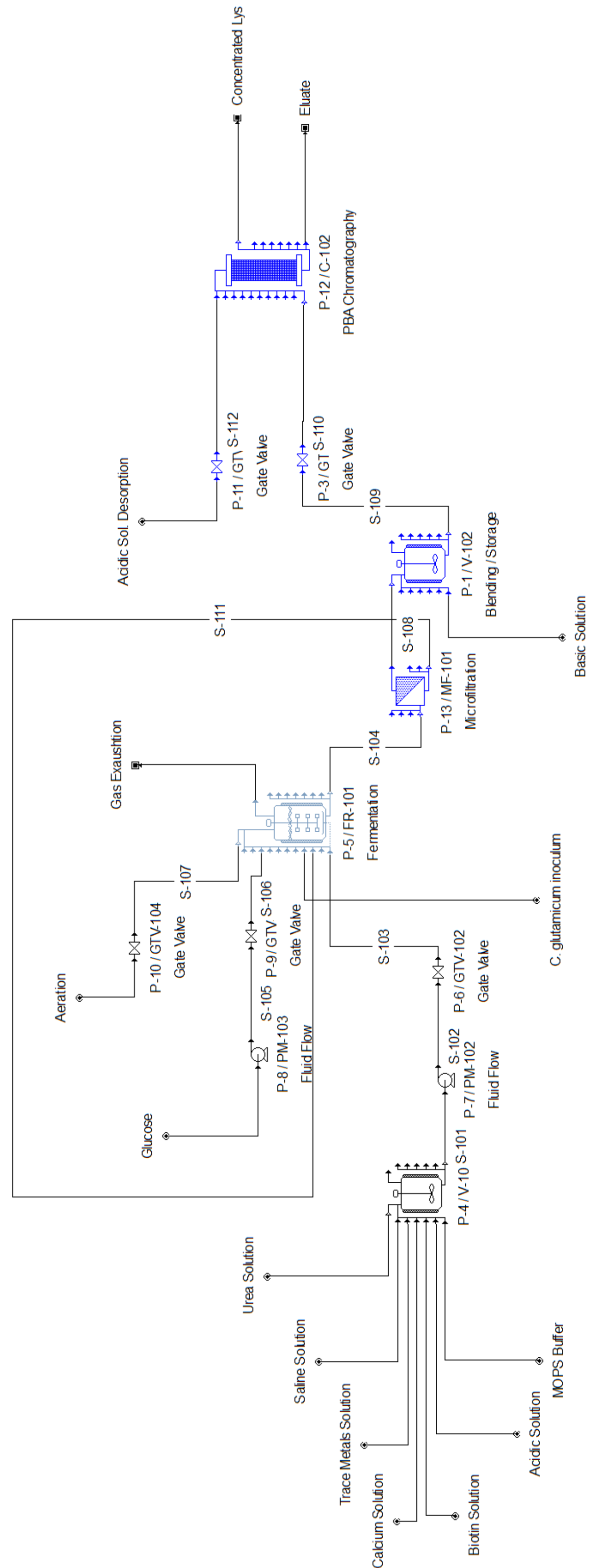

Figure 8: Schematic representation for a lysine production in continuous operation, including the medium preparation (in black), the fermentation reactor (in light grey) and the downstream steps (in dark blue) that include a microfiltration and an adsorption. The valves and pumps in the flowing streams allow a better control of the process as to maintain its long-term viability. The used icons are a simple representation of the unit equipments.

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