Development of a Continuous Process for the Production of Vanillin and Syringaldehyde from Kraft Black Liquor

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by

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And to God, that placed amazing people in my life that helped me to get and stay on the “right path” despite the big rocks along the very same road.

Abstract

Lignin is a byproduct from the pulp and paper industry present in the black liquors from which it can be precipitated. The black liquor, rich in process chemicals of the kraft process, is usually sent to multi-effect evaporators where concentration takes place so that it is burned after and then the process chemicals returned closing the process loop. This action is performed to increase process economy and to reduce pollution, which are the main benefits of the kraft process, and why it became the dominant process in the pulp and paper industry. Lignin, as an undervalued product, is usually valorized through the generation of steam and electricity, significant efforts have been made so that the profitability of the process is increased based on this abundant biomass resource, second only to cellulose. The presence of lignin is at times undesired, since the increase in the capacity of the pulp production of the facility necessarily implies an increase of the capacity of burning liquors.

This work intends to give a contribution for the valorization of lignin by the means of production of chemicals that can be obtained through its oxidation, namely the most investigated one, vanillin but also others like syringaldehyde. The great majority of these industries apply the kraft process to wood of the *hardwood* type, of which an example is the *Eucalyptus globulus* species, very abundant in Portugal and it is primarily composed of syringyl structural units that give origin to syringaldehyde. For the production of vanillin, wood of the *softwood* type, like pine trees, are the ideal since they are rich in the guaiacyl structural units that will originate vanillin. Other lignin oxidation products like the acids vanillic and syringic, and the ketones acetovanillone and acetosyringone are followed. In the concept of biorefinery, it is necessary to have a complete valorization of all the fractions obtained from biomass resulting in the most integrated process as possible.

The valorization of the lignin is primarily related with the oxidation process it must undergo. It is necessary to improve of the oxidation conditions since the yields of this step are vital for the profitability of the whole process downstream (separation/purification). Improvement efforts were made in the reaction unit (structured packed bubble column reactor) and these are summarized.

The separation/fractionation of the lignin by membrane filtration processes (ultrafiltration and nanofiltration) intends to diminish the organic charge, partially oxidized lignin, oligomers, searching for a balance between the number of membrane processes and the economy of the whole process. After this phase of membrane filtration, the successful separation of several compounds with commercial interest in the final permeate by chromatographic processes is performed, obtaining fractions with significantly different composition from the feed, namely phenolic acids, phenolic aldehydes and phenolic ketones; This work has already been published.

The final step of purification, where the crystallization of the added-value phenolic compound vanillin from these rich fractions is obtained was investigated. Extraction with ethyl acetate was performed on the vanillin rich fraction for further purification and the use of pH 6 proved to be essential for the success of the crystallization step. Vanillin with purity of up to 96% was obtained and melting points of up to 79.8˚C as well. Acetovanillone is present as the main contaminant.

Resumo

A lenhina é um subproduto da indústria de pasta de papel proveniente dos licores negros a partir dos quais pode ser precipitada. Os licores negros, sendo ricos em químicos do processo kraft, são normalmente encaminhados para evaporadores ende são concentrados para depois serem queimados e os químicos retornados ao processo, quer por razões de economia do processo, quer pela diminuição da poluição, que são os pontos fortes do processo kraft, e pelas quais se tornou no processo dominante, ao longo dos anos, nesta indústria. Sendo um recurso pouco valorizado, normalmente através da geração de energia eléctrica e vapor, têm sido feito esforços no sentido de retirar mais-valias deste recurso de biomassa abundante, o segundo mais abundante a seguir à celulose, e por vezes indesejado, uma vez que o aumento de produção de celulose implica necessariamente um aumento da capacidade de queima de licores.

Este trabalho pretende contribuir para a valorização da lenhina por via de produtos químicos que podem ser obtidos pela oxidação desta macromolécula, nomeadamente o mais investigado, a vanilina mas também outros incluindo o seringaldeído visto que uma grande parte das indústrias aplicam o processo kraft a madeiras do tipo *hardwood*, do qual é exemplo o *Eucalyptus globulus*, abundante em Portugal e maioritariamente rica em unidades estruturais siringilo. Para a produção de vanilina, madeiras do tipo *softwood*, do qual é exemplo o pinheiro, são as preferidas visto serem ricas nas unidades estruturais guaiacilo, que lhe vão dar origem. Outros produtos da oxidação de lenhina tais como os ácidos vanílico e siríngico, as cetonas acetovanilona e acetoseringona serão também tidos em conta visto que para que o conceito de biorefinaria seja concretizado, é necessária uma valorização de todas as fracções, resultando num processo o mais integrado possível.

A valorização da lenhina está primeiramente relacionada com o processo de oxidação. Para isso é necessária a melhoria das codições de oxidação já que os rendimentos deste passo inicial serão determinantes para a rentabilidade de todo o processo a jusante (separação/purificação). Esforços de melhoria foram feitos na unidade de reacção e serão resumidos.

Numa segunda fase, a separação/fraccionamento das lenhinas por via de utilização de processo de filtração por membranas (ultrafiltração e nanofiltração) visa diminuir a carga orgânica, lenhina parcialmente oxidada e oligómeros, sendo procurado um equilíbrio entre o número de processo de membranas e a economia do processo. Após esta fase de separação por membranas, pretende-se separar os vários composto de interesse comercial por processos cromatográficos obtendo assim fracções significativamente mais concentradas em alguns compostos nomeadamente aldeídos fenólicos, ácidos fenólicos e cetonas fenólicas, como já foi feito com sucesso, originando duas publicações.

A fase final é fase de purificação, onde é pesquisado com maior detalhe a cristalização, a partir das fases ricas nos compostos de interesse comercial. Foi efectuada extracção com acetato de etilo das soluções enriquecidas em vanillina para maior purificação do produto e o uso de pH 6 revelou ser essencial para o sucesso da cristalização da vanilina. Uma pureza de vanilina de até 96% foi alcançada e pontos de fusão de até 79,8˚C foram obtidos. Acetovanilona é o maior contaminante no produto final.

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Résumé

La lignine est un sous-produit de l'industrie des pâtes et papiers présente dans les liqueurs noires à partir desquelles elle peut être précipitée. Les liqueurs noires, riches en produits chimiques du procédé kraft, sont généralement envoyées vers des évaporateurs à effets multiples où la concentration a lieu, de manière à être brulés après quoi lesquels sont ensuite revenus en fermant la boucle de procédé. Cette action vise à accroitre l'économie de traitement et à réduire la pollution, les principaux avantages du procédé kraft, donc il est devenu le procédé dominant dans l'industrie des pâtes et papiers. La lignine, en tant que produit sous-évalué, généralement valorisé par la production de vapeur et d'électricité, des efforts importants ont été consentis pour que la rentabilité du procédé soit accrue sur la base de cette abondante ressource en biomasse, la deuxième après la cellulose. La présence de lignine est parfois indésirable, car l'augmentation de la capacité de production de pâte à papier de l'installation implique nécessairement également une augmentation de la capacité de combustion des liqueurs.

Ce travail veut contribuer à la valorisation de la lignine par la production de produits chimiques obtenus par son oxydation, à savoir le plus étudié, la vanilline, mais également d'autres comme le syringaldéhyde. La grande majorité de ces industries appliquent le procédé kraft au bois de type feuillu, par exemple l'espèce *Eucalyptus globulus*, très abondante au Portugal et composée principalement d'éléments structurels syringyles qui donnent origine au syringaldéhyde. Pour la production de vanilline, on préfère les bois de type résineux, comme les pins, car ils sont riches en unités structurales en guaiacyle qui seront à l'origine de la vanilline. D'autres produits d'oxydation de la lignine, comme les acides vanillique et seringique et les cétones acétovanillone et acétosyringone sont également suivis. Dans le concept de bioraffinerie, il est nécessaire de procéder à une valorisation complète de toutes les fractions obtenues à partir de la biomasse, ce qui donne le procédé le plus intégré possible.

La valorisation de la lignine en produits chimiques est principalement liée à l'oxydation. Il est nécessaire d'améliorer les conditions d'oxydation, car les rendements de cette étape sont essentiels à la rentabilité de l'ensemble du procédé en aval (séparation / purification). Des efforts ont déjà été faits dans l'unité de réaction (réacteur colonne à bulles et à garnissage structuré) et ceux-ci seront résumés.

La séparation / fractionnement de la lignine au moyen de procédés de filtration sur membrane (ultrafiltration et nanofiltration) vise à diminuer la charge organique, la lignine partiellement oxydée, les oligomères, en recherchant un équilibre entre le nombre de procédés membranaires et l'économie de l'ensemble du procédé total. Après cette phase de séparation par membranes, la séparation par chromatographie de plusieurs composés présentant un intérêt commercial dans le perméat final permet ainsi d'obtenir des fractions nettement plus concentrées en certains composés à savoir, les aldéhydes phénoliques, les acides phénoliques et les cétones phénoliques déjà réalisées avec succès; ce travail a déjà été publié.

La dernière étape de purification, au cours de laquelle on a étudié la cristallisation du composé phénolique ajouté, la vanilline, à partir des fractions riches obtenues. L'extraction a été effectuée avec acétate d'éthyle sur la fraction riche en vanilline pour une purification supplémentaire et l'utilisation du pH 6 s'est révélée essentielle pour le succès des étapes de cristallisation. On a obtenu de la vanilline avec une pureté allant jusqu'à 96% et des points de fusion atteignant 79,8°C. L'acétovanillone était le principal contaminant.

Zusammenfassung

Lignin ist ein Nebenprodukt der Zellstoff- und Papierindustrie, das in den Schwarzlaugen enthalten ist, aus denen es ausgefällt werden kann. Die Schwarzlauge, die reich an Prozesschemikalien des Kraftverfahrens ist, wird in der Regel zu Mehrfacheffektverdampfern geschickt, wo die Aufkonzentrierung stattfindet, damit sie anschließend verbrannt und die Prozesschemikalien zurückgeführt werden, um den Prozesskreislauf zu schließen. Diese Maßnahme wird durchgeführt, um die Prozessökonomie zu erhöhen und die Umweltverschmutzung zu verringern, die die Hauptvorteile des Kraft-Prozesses sind und warum er in der Zellstoff- und Papierindustrie zum vorherrschenden Prozess wurde. Lignin, ein unterbewertetes Produkt, das in der Regel durch die Erzeugung von Dampf und Elektrizität verwertet wird, hat erhebliche Anstrengungen unternommen, um die Rentabilität des Verfahrens auf der Grundlage dieser reichlich vorhandenen Biomasse-Ressource zu steigern, die nur Cellulose übertrifft. Das Vorhandensein von Lignin ist zuweilen unerwünscht, da die Erhöhung der Kapazität der Zellstoffproduktion der Anlage notwendigerweise eine Erhöhung der Kapazität von Brennlaugen impliziert.

Diese Arbeit soll einen Beitrag zur Valorisierung von Lignin durch die Herstellung von Chemikalien leisten, die durch Oxidation gewonnen werden können, und zwar das am häufigsten untersuchte, Vanillin, aber auch andere, einschließlich Syringaldehyd. Die überwiegende Mehrheit dieser Industrien wendet das Kraft-Verfahren auf Hartholz an, zum Beispiel auf die in Portugal häufig vorkommende Eucalyptus globulus-Art, die sich hauptsächlich aus Syringyl-Struktureinheiten zusammensetzt, aus denen Syringaldehyd hervorgeht. Für die Herstellung von Vanillin werden Nadelholzhölzer wie Kiefern bevorzugt, da sie reich an Guajacyl-Struktureinheiten sind, aus denen Vanillin entsteht. Andere Ligninoxidationsprodukte wie die Säuren Vanillin und Syringin sowie die Ketone Acetovanillon und Acetosyringon werden ebenfalls verfolgt. Im Konzept der Bioraffinerie ist eine vollständige Verwertung aller aus Biomasse gewonnenen Fraktionen erforderlich, um einen möglichst integrierten Prozess zu erzielen.

Die Valorisierung des Lignins hängt hauptsächlich mit dem Oxidationsprozess zusammen, den es durchlaufen muss. Die Oxidationsbedingungen müssen verbessert werden, da die Ausbeuten dieses Schritts für die Rentabilität des gesamten nachgeschalteten Prozesses (Trennung / Reinigung) von entscheidender Bedeutung sind. Verbesserungen wurden bereits in der Reaktionseinheit (strukturierter gepackter Blasensäulenreaktor) vorgenommen und diese zusammengefasst.

Die Abtrennung / Fraktionierung des Lignins mittels Filtrationsverfahren (Ultrafiltration und Nanofiltration) soll die organische Ladung, teilweise oxidiertes Lignin und Oligomere verringern und ein Gleichgewicht zwischen der Anzahl der Membranprozesse und der Wirtschaftlichkeit des gesamten Verfahrens herstellen. Nach dieser Phase der Membranfiltration wird die Trennung mehrerer Verbindungen mit kommerziellem Interesse im Endpermeat durch chromatographische Verfahren durchgeführt, wobei Fraktionen erhalten werden, die in einigen Verbindungen, nämlich Phenolsäuren, Phenolaldehyden und Phenolketonen, die bereits mit Erfolg durchgeführt wurden, signifikant konzentrierter sind. Diese Arbeit wurde bereits veröffentlicht.

Der letzte Reinigungsschritt, in dem die Kristallisation der wertschöpfenden Phenolverbindung Vanillin aus diesen erhaltenen reichen Fraktionen untersucht wurde. Die Extraktion mit Ethylacetat wurde an der Vanillin-reichen Fraktion zur weiteren Reinigung durchgeführt, und die Verwendung von pH 6 erwies sich als wesentlich für den Erfolg des Kristallisationsschritts. Es wurde Vanillin mit einer Reinheit von bis zu 96% und Schmelzpunkten von bis zu 79,8 °C erhalten. Acetovanillon ist als Hauptverunreinigung vorhanden.

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1 Introduction

1.1 Relevance and Motivation

Added-value compounds like vanillin and syringaldehyde are difficult to manufacture and usually have synthesis routes that give origin to a lot of chemical waste. They are used as precursors in the pharmaceutical industry that could benefit from the fact that the lignin-derived compounds are already highly functionalized molecules (different functional groups, hydroxyl, methoxy, carboxyl, carbonyl); it would save resources in the often long, laborious and wasteful synthesis of active pharmaceutical ingredients. The oxidation of lignin produces highly functionalized molecules like aromatic acids, aromatic aldehydes and aromatic ketones that can be supplied to the fine chemical industries and shorten the *de novo* synthesis routes of more complex structures. Lignin is an undervalued biomass resource with almost all of the lignin ending up used as fuel after being concentrated in multistage evaporators. Its current utilization, as fuel in the pulp and paper industry, will be shifting into a more profitable one, as diversified products are made from lignin, replacing to some degree the oil-derived counterparts.

In the past, lignin has been utilized as a major source for the production of vanillin in the sulfite process, a process for wood pulping that produced a lot of chemical waste and that later was replaced for a more sustainable one, the kraft process, which allowed the recycling of process chemicals, unlike the former. It also results in stronger cellulose fibers, therefore the Kraft designation. Meanwhile, the production of vanillin shifted towards the ONCB (*o*-nitrochlorobenzene) route and nowadays the catechol route [1]. Currently, kraft lignin is not used as a major source of added-value monomers. An exception is the case of Borregaard, which produces vanillin from softwood lignin employing still lignosulfonates from the sulfite process. Vanillin produced in this way is more natural, although it employs several steps of extraction with toxic solvents [2, 3]. This work proposes a way of producing monomers, namely vanillin and syringaldehyde, with the utilization of processes with reduced environmental impact.

1.2 Objectives

The major objective of this work is to obtain vanillin and syringaldehyde from the oxidation of lignins like Indulin AT, Tobacco Butanol and Ethanol Organosolv lignins, also lignin coming from Kraft black liquors, through a series of physical separation operations with reduced environmental impact. These include membrane filtration (ultra- and nanofiltration), adsorption processes, extraction and crystallization, ending up with a final dry purified product.

To produce vanillin and syringaldehyde, the lignin coming from Kraft black liquors must be oxidized efficiently so that the losses along the series of separation steps do not significantly affect the ability of recovering these compounds, preferably in their pure form and in high yields. The oxidation of lignin to monomer molecules requires that the mass transfer apparatus is efficient at delivering the amounts of oxygen necessary for the lignin breakdown. Recently, efforts were made regarding the improvement of the oxygen mass transfer rates by increasing the gas flow or utilizing a gas stream of pure oxygen. The yields can still be further increased by modification of the gas dispersion apparatus before trying to apply selective catalysts and therefore, improvements to the reactor should be a continuous effort.

The separation of the high molecular weight lignin still present in the oxidized mixture is important since it will hinder the separation and purification of the monomers produced. Lower molecular weight lignins are also known for adsorbing into resin adsorbents, this would cause decreases in productivity in the chromatographic phase. A series of ultrafiltration and nanofiltration steps must be performed so that lignin concentration is reduced and the low molecular weight phenolic compounds (LMWPC) like vanillin and syringaldehyde are more easily recovered from the permeate.

The permeate originating from the nanofiltration is then fractionated by chromatography; this resulted in fractions with a very different composition from that of the starting solution and from which the pure compounds can be more easily extracted and purified.

The fractions obtained through chromatography must then be subjected to solvent extraction by known methods so that the vanillin and syringaldehyde produced are *de facto* separated from the other remaining LMWPC and the concept proven. At first, a mixture of both aldehydes is acceptable but obtaining each compound in its pure form is desired. After successful extraction with the solvent, crystallization from the same type of fractions is studied so that a fully environmentally friendly integrated sequence for production of vanillin and syringaldehyde is completed.

1.3 Outline

This thesis contains eight chapters:

Chapter 1, the present chapter, deals with the importance of the current work;

Chapter 2 presents a literature review on the production of vanillin and syringaldehyde from lignin is given;

Chapter 3 shows the advancements made regarding the lignin oxidation in the structured packed bubble column continuous reactor is presented;

Chapter 4 deals with the achievement of the separation of the phenolic compounds produced by families, namely phenolic aldehydes, phenolic acids and phenolic ketones;

Chapter 5 deals with the separation of the phenolic compounds vanillic acid, vanillin, and acetovanillone from the oxidation of Indulin AT Kraft Lignin, a softwood lignin;

Chapter 6 presents the cyclic enrichment/separation of the solution in vanillin/acetovanillone from the mixture with only water as desorption eluent;

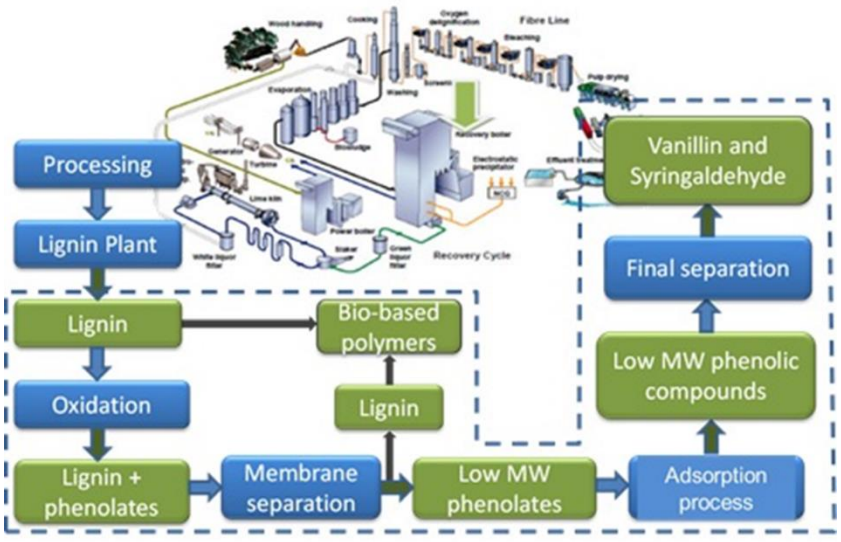
Chapter 7 presents the advancements made regarding the isolation of vanillin from Kraft lignin depolymerization by crystallization;

Chapter 8 closes this thesis with the main conclusions and gives suggestions for improvement and possible continuation of this work.

1.4 References

[1] D. Giannotta, Vanillin: The ONCB route- a sustainable option?, in: IFEAT (Ed.) IFEAT International Conference, Lisbon, Portugal, 2004, pp. 202-211.
[2] M.B. Hocking, Vanillin: Synthetic Flavoring from Spent Sulfite Liquor, Journal of Chemical Education, 74 (1997) 1054-1059.
[3] H. Evju, Process for Preparation of 3-methoxy-4-hydroxybenzaldehyde, in, Borregaard Industries Limited, 1979.

2 State-of-the-Art



Currently, lignin is burned for energetic valorization in pulp and paper mills, the future biorefineries. Lignin holds a great potential for becoming "the" alternative source of phenolic compounds. Two of those potential phenolic compounds that can be a starting raw material for other chemical compounds are vanillin and syringaldehyde. In this chapter, a review of the processes used for the valorization of lignin into vanillin and syringaldehyde is presented.

2.1 Lignin

Lignin is a biopolymer present in biomass along with hemicellulose and cellulose that gives structure and rigidity to the plant material. Lignin is produced in the plant cells by enzymatic polymerization of the three base precursors: *p*-coumaryl, coniferyl and sinapyl alcohols, Figure 2-1. The ratios of these precursors in the lignin vary according to the nature of the wood. For instance, hardwoods (*e.g.* eucalyptus) have syringyl units and guaiacyl units while softwoods (*e.g.* pine) have mostly guaiacyl units.

Lignin has the potential to be the source of aromatic platform chemicals in the future, as an alternative to the petroleum-based source; however, several obstacles are still in the way to that accomplishment by the chemical industry. The major obstacles right now are its depolymerization and subsequent separation of the added-value compounds generated. Due to the different types of bonds present in lignin structure, shown in Figure 2-2, it is difficult to convert it into monomer phenolic compounds. Also, the depolymerization products can be susceptible to repolymerization reactions.

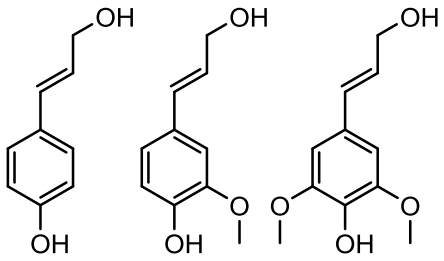


Figure 2-1 – Lignin precursors from left to right: coumaryl, coniferyl and sinapyl alcohols.

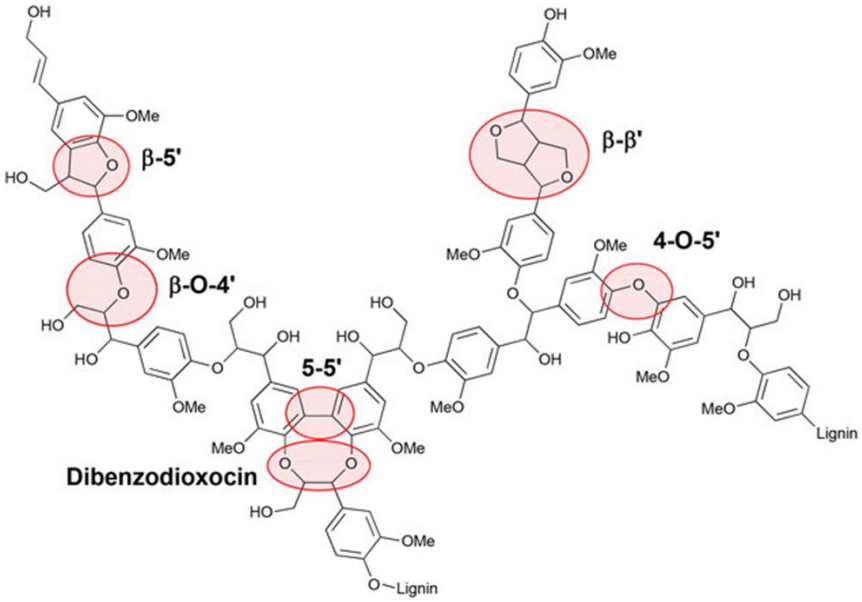


Figure 2-2 – Lignin structure with bonds highlighted. (Image source: W. G. Forsyth et al.2013, [1]).

2.2 Vanillin and Syringaldehyde

Vanillin is a flavoring agent that is consumed worldwide in the order of 20 000 t/y with increasing demand every year. Syringaldehyde is also used in the flavoring industry but not to the same extent as vanilla, contributing for smoky aromas in beverages. Currently, vanillin is produced almost entirely from the benzene-derived guaiacol, the main producer being now Solvay (which acquired Rhodia). Vanillin is the main aldehyde produced during the oxidation of softwood lignin, for the case of hardwood lignin, syringaldehyde. Vanillin and syringaldehyde are depicted in Figure 2-3.

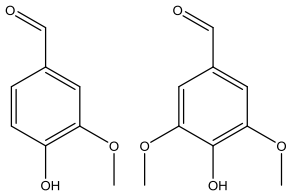


Figure 2-3 – Structure of vanillin (left) and syringaldehyde (right).

Other species are formed during the oxidation of the lignin structure. Figure 2-4 illustrates some of the phenolic compounds that commonly are produced during the oxidation of lignin; these secondary monomer phenolic compounds are also of importance since they also have economic value although present in the oxidation mixtures usually in lower concentrations, by order of abundance phenolic aldehyde > organic acid > phenolic ketone but it can vary according to the lignin oxidation method or use of selective catalysts.

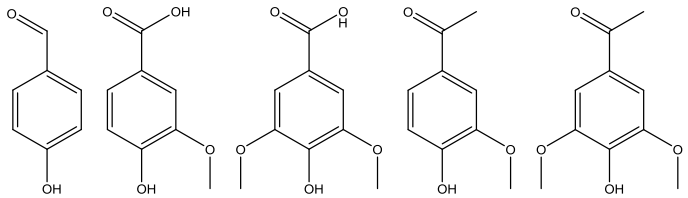


Figure 2-4 – Other phenolic compounds commonly released during the oxidative depolymerization of lignin. From left to right, p-hydroxybenzaldehyde, vanillic acid, syringic acid, acetovanillone and acetosyringone.

2.2.1 Market and Applications of Vanillin and Syringaldehyde

Market – **Vanillin** is one of the most widely used flavoring agents, it is known by its vanilla scent and flavor therefore its use in the food and fragrances industries is much disseminated. The world demand of vanillin is approximately over 20 000 t/y, most of which is supplied by the petrochemical source via the catechol synthesis route. From Figure 2-5 it is clear that near all of the total world demand of vanillin is supplied by 3 companies: Solvay, Anhui Bayi Chemicals and Borregaard; other minor producers are Jilin Petrochemical, Liaoning Shioxing, Camlin Fine Sciences and

Zhonghua Chemicals . Solvay is producing vanillin by the catechol route. Solvay is the lead supplier of synthetic vanillin [2], recent acquisitions of other chinese production facilities have solidified this position. Also, there is a marginal production of vanillin from ferulic acid fermentation by Solvay which is more natural but not cost competitive. There has been a steadily increase in vanillin demand , Figure 2-6 and it is expected to continue to grow motivated by Asian economies.

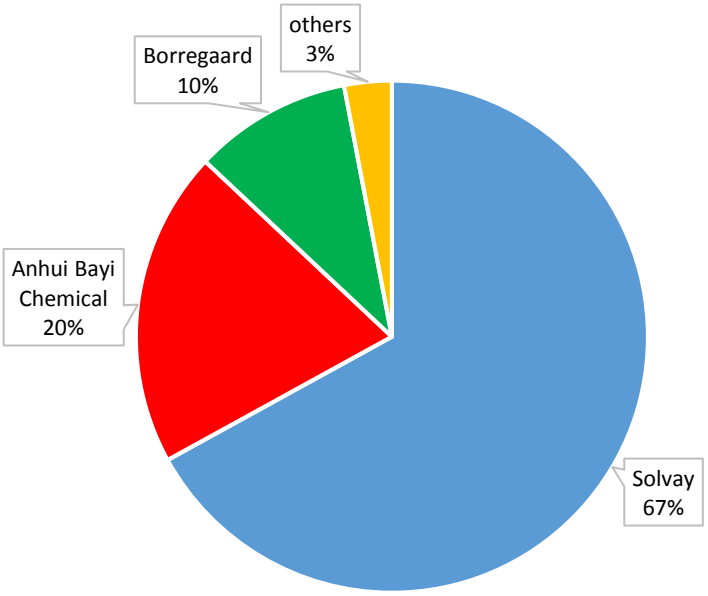


Figure 2-5 – Vanillin market share of the main vanillin producing companies. Data from 2015.

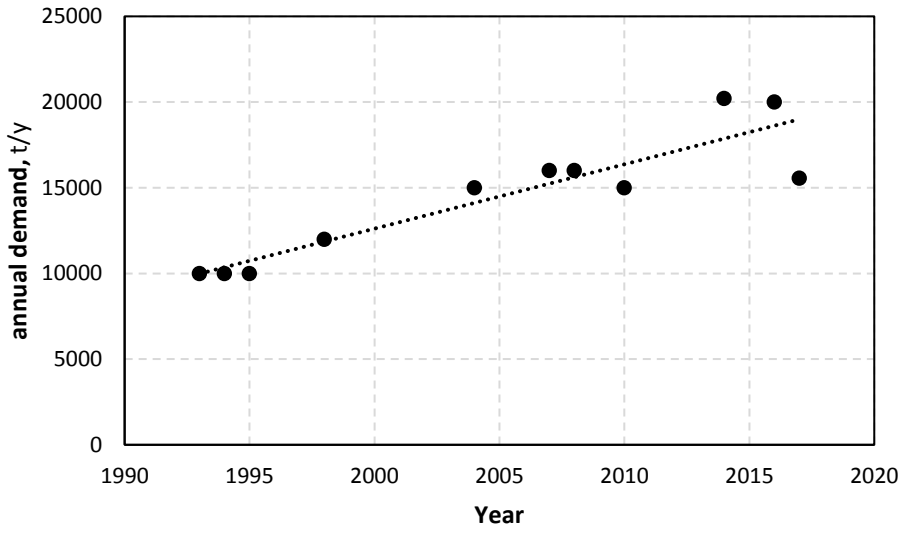


Figure 2-6 – Vanillin demand over the last 30 years.

Applications – **Vanillin** can be a sustainable monomer source for polymers production like polyvinyl acetals [3] and thermoset plastics. Vanillin is also a starting material for several active pharmaceutical ingredients (APIs). Vanillin can be utilized in the production of divanillin, an anti-inflammatory agent [4, 5]. It has several properties like being anticancer [6, 7].

Market – **Syringaldehyde**, on the other hand, is not so widely used and it is found in very small quantities in nature [8] mostly in plant cell walls, although it is an aldehyde with a structure very similar to vanillin. Interest in this molecule is still growing since it was found that it could be obtained from natural sources. Data is scarce which is indicative of the difference in the dimension of the market, or that it is still in development. Since syringaldehyde is also very versatile, the availability of creative or similar applications could push it to market much like vanillin.

Applications – **Syringaldehyde** applications are for the majority related directly with the production of APIs, also in the same ways as vanillin. The most widely known pharmaceutical produced with syringaldehyde as a starting block is trimethoprim, an antibiotic [9] or the related Bactrim or Biseptol [8] which are also bactericides. Since syringaldehyde is structurally very similar to vanillin, the adoption of vanillin for the same purposes but only after an intermediate synthesis step where another methoxy group is added, given that syringaldehyde possesses two. Other pharmaceuticals that can be produced from syringaldehyde are DMU-212, an anti-cancer drug [10], combrestatin (anti-cholesterol) [11], and stilbene derivative drugs, also other anti-cancer drugs [12, 13]. Other uses are as beverages (whisky, rum) fast aging enhancer [14, 15]. Syringaldehyde can also be utilized to produce antioxidants like hydrazones [5, 16]. Syringaldehyde can also be incorporated in sunscreen filter formulations. Recently, interest has been shown in the thermoplastic and resin production with syringaldehyde, as a monomer, for their production as a sustainable source [17, 18]. Like vanillin, syringaldehyde can be employed, in the sustainable production of polyvinyl acetals [3].

2.2.2 Vanillin and Syringaldehyde Production

Synthesis – **Vanillin** is a natural occurring compound extracted from the two vanilla orchids *Vanilla planifolia* or *Vanilla tahitensis* [19] but due to its high cost and high consumption, alternative routes for its production emerged. It was known as early as 1875 that vanillin was present in the waste sulfite liquors produced in the paper mills [20]. But the first synthetic vanillin sold was produced from eugenol or isoeugenol [21] and continued until 1920. After this, the most widely disseminated method of production of vanillin became sulfite softwood liquors by Salvo Chemical Corporation and Marathon Paper Mills Company which released their patents around the same occasion, [22] and [23] respectively. Then, the advent of the Kraft process changed the pulp and paper industry. This forced a shift to the petrochemical synthesis route since Kraft pulping process brought many advantages compared to the sulfite, the most important one being the possibility of recycling the process chemicals, making the pulping process more profitable and environmentally friendly. The shift moved the production of vanillin from sulfite liquors to the *o*-nitrochlorobenzene (ONCB) route and the more benign catechol/guaiacol route, but still starting with benzene [24]. The dominant synthesis routes for the production of vanillin are depicted in Figure 2-7. Nowadays, the most widely synthesis route is the catechol/guaiacol route (in blue). The most toxic and older synthesis route (in red) for comparison is still used by some Chinese manufacturers. The market share of the different sources is presented in Figure 2-8.

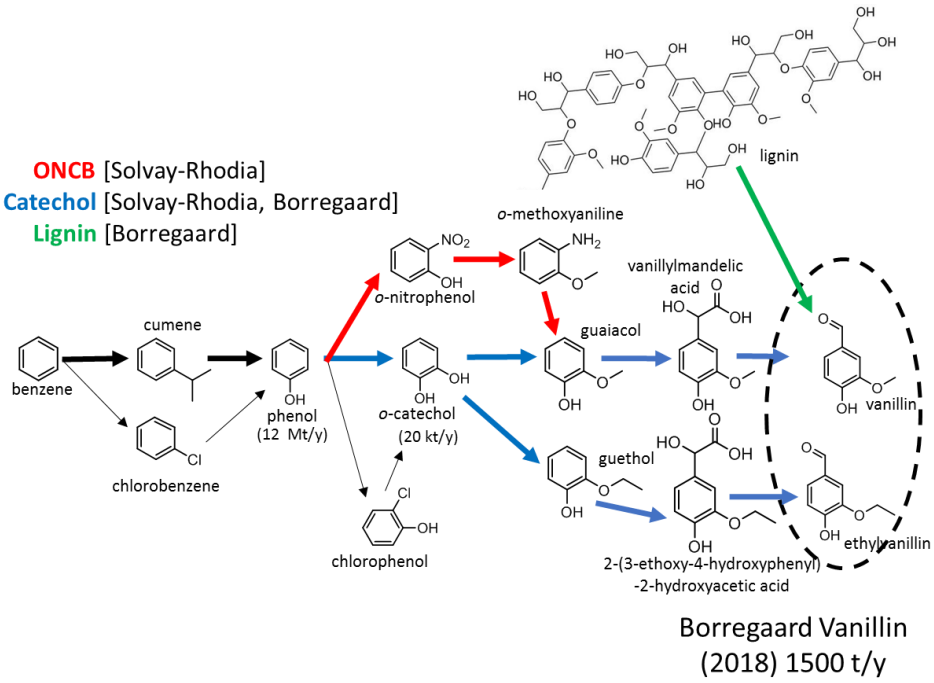


Figure 2-7 – Dominant synthesis routes for vanillin production including benzene and lignin as starting substrates. The old process, the o-nitrochlorobenzene (ONCB) path is depicted in red while the more modern and more sustainable new process, from catechol/guaiacol, is depicted in blue. Lignin-derived synthesis path is presented in green. No separation operations are shown. Dashed circle represents the products that Borregaard sells, ethylvanillin, vanillin derived from petroleum sources and vanillin derived from lignin (1500 t/y).

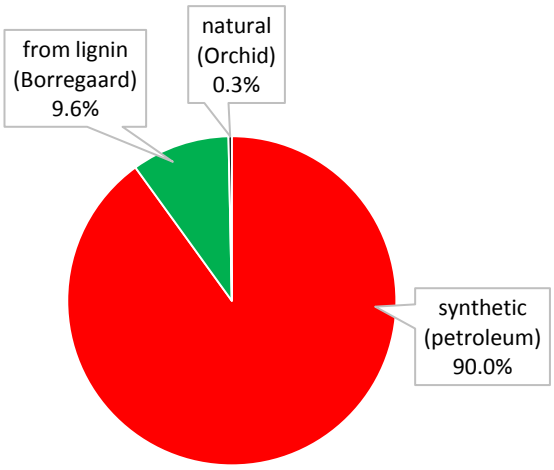


Figure 2-8 – Market share of the main vanillin sources. Data from 2017. .

Synthesis – **Syringaldehyde** is not a very sought chemical since it is also still used by a niche market, pharmaceutical and, only recently, its utilization as plastic/resin precursors has attracted some attention. The synthesis is usually from vanillin where a methoxylation takes place by generating a halide intermediate [9, 25-27].

Borregaard, being the single producer of lignin-derived vanillin nowadays (started in 1962, and ethyl vanillin in 1993 [28]), has benefited from the shift from the sulfite pulping process to the kraft pulping process, since the majority of the vanillin producers at the time halted their production; Ontario Paper had a vanillin production capacity of 3000 t/y and closed in 1988, Monsanto had a capacity of 2000 t/y and closed vanillin operations in 1991, ITT Rayonier had a vanillin production capacity of 1500 t/y and also closed in 1994 [29]. Borregaard maintained its sulfite pulping as a strategic move, betting on niche markets, investing in product diversification, in accordance with their motto: “squeeze maximum value out of each log”.

Along the recent past, Borregaard has been acquiring the competitors closed business branches of lignin-derived vanillin since they shifted their attention to other production routes and adoption of hardwoods as biomass for pulping. This was the case with Monsanto and Marathon Paper that both dropped vanillin production from lignin. Borregaard gathered knowledge from these competitors (acquired) vast number of patents, and to this day still rely on Monsanto’s method of production for instance, (GB695301, 1953) (NO84422, 1954) (US2692291, 1954) even though most of them are expired [30]. Figure 2-9 shows a brief schematic description of some of the patents involved in Borregaard vanillin production process (from lignin).They also have several patents related to several processes regarding biorefinery operations. For instance, they hold a patent on the ultrafiltration of the liquor for enrichment in lignin before the oxidation steps; this aims to increase vanillin yields (US4151207, 1979). They also filed a patent relating to the formulation of the final

product containing several blends of lignin-derived vanillin, synthetic vanillin and synthetic ethylvanillin (EP325152A1, 2017).

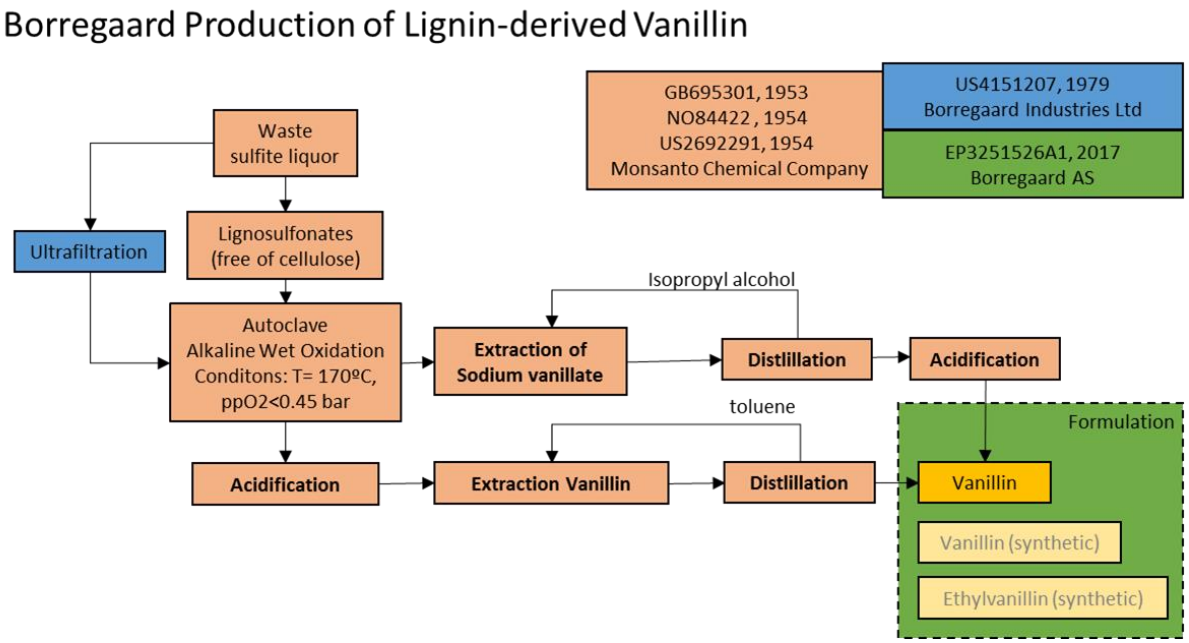


Figure 2-9 – Universe of patents around Borregaard lignin-derived vanillin operations. Patents concerning non-lignin routes are not included in this scheme. Borregaard vanillin production methods is still relying on the "old" Monsanto process.

A more in depth review of the relevant patents in the field of production of vanillin and syringaldehyde from lignin is presented next in Table 2-1.

Table 2-1 – Summary of relevant patents concerning vanillin and syringaldehyde production.

Patent	Essence of the Invention
US2057117 **Process of Making Vanillin** (1936) Guy Howard Co., Marathon Paper Mills Co.	Concentrated lignin material is treated with alkali-metal bisulfite and pH is maintained below 7 by adding SO_2. An organic phase containing vanillin-bisulfite precipitates. This phase is treated with acid to liberate the SO_2 and recycle it. Resulting solution is extracted with benzene. Other compounds extracted by benzene are also present.
US2104701 **Process of Making Vanillin** 1938 Guy Howard Co., Marathon Paper Mills Co.	The patent describes several ways of extracting vanillin from lignin alkaline solutions. The extraction could be accomplished with butyl alcohol or other alcohols which are insoluble or immiscible in water. The following alcohols are proposed: sec-butyl alcohol, benzyl alcohol, n-butyl alcohol, cyclohexanol, normal-amyl alcohol, sec-amyl alcohol or alcohol mixtures.
US2069185 **Manufacture of Vanillin from Waste Sulphite Pulp Liquor** 1937 Howard Smith Chemicals Limited	Vanillin is produced by concentrating the black liquor and heating it (125-160°C) with an excess of NaOH in a pressurized vessel. Vanillin is then in its salt form but liberated by acidification with CO_2. Vanillin is then extracted with solvents like benzene or ethylene chloride and purified by steam distillation, crystallization, sublimation or other well known methods. Insoluble calcium salts are removed and alkali is causticized for reuse in the process.
US2399607 **Process of Making Vanillin** 1946 Marathon Corporation	The patent describes the production of vanillin from alkaline solutions containing large amounts of organic matter with solubilities different from alkali vanillate. It is also proposed to wash the organic phase from the extraction and the non-aqueous solvent, for example, butyl alcohol, can be recovered in practically vanillin-free condition suitable for reuse without distillation. The energetic economy is evident by avoiding the use of steam.
US2434626 **Process for Making Vanillin** 1948 Salvo Chemical Corporation	This invention relates to a process for making vanillin from lignosulfonates by controlled oxidation by gaseous oxygen or air under elevated temperatures and pressures. The best yield was obtained by employing a cooking temperature around 160°C. with a cooking time of 60 to 110 minutes and by arranging for an oxygen consumption of 25 to about 35 g per 100 g of lignin equivalent of the lignosulfonates present in the cooking mixture.

US2576753 **Method of Producing Vanillin** 1951 The Ontario Paper Company	This invention describes the production of vanillin, acetovanillone and other oxidation products from lignosulfonic acid compounds such as waste sulfite liquor after fermentation of sugar content. The yield of vanillin, acetovanillone and other related co-products is substantially increased as the initial concentration of lignin in the reaction vessel is decreased.
GB695301 **Improvement in or relating to Manufacture of Vanillin from Lignin** 1953 Monsanto Chemical Company	This invention relates to an improved process for the production of vanillin by the controlled partial oxidation of waste sulfite liquors by molecular oxygen or atmospheric air under strong alkaline conditions. Another object is to control the partial pressure of oxygen throughout the reaction at optimum levels for maximum yields of vanillin. The objectives are to reduce the cost of manufacturing synthetic vanillin by improving the efficiency of the process and to reduce the total heat input required for the reaction.
US2686120 **Alkaline pulping of lignocellulose in the presence of oxygen to produce pulp, vanillin, and other oxidation products of lignin substance** 1954 The Ontario Paper Company	This invention relates to the pulping of lignocellulosic materials and also to the production of oxidation products, including vanillin during an alkaline pulping operation in one single operation. Identified products formed when softwoods are acetovanillone, vanillic acid, and guaiacol; for hardwoods, syringaldehyde and acetosyringone are formed in addition; fibrous annual crop materials such as bagasse, wheat straw, flax straw, soya bean stalks and oat hulls are employed, both vanillic benzaldehyde have been identified in addition to vanillin.
US2692291 **Manufacture of Vanillin from Lignin** 1954 Monsanto Chemical Company	The invention relates to the production of vanillin in substantially increased yields by controlled partial oxidation with molecular oxygen of lignin form sulfite process (low cost raw material). Efficient utilization of the oxygen content of atmospheric air to provide the necessary reactant for maximum yields of vanillin. Reduce the cost of manufacturing synthetic vanillin by improving the efficiency of the process. Reduce the total heat input required for the reaction.
US3049566 **Vanillin Purification** 1962 American Can Company	The present invention relates to continuous purification of crude vanillin to achieve pure crystalline white vanillin. Crude vanillin distillate containing about 85-90% vanillin and derived from the partial oxidation of lignin in sulfite waste liquor is dissolved in a mixture of 40-50% methanol. The solution is cooled for precipitation of a somewhat purified vanillin-containing solid having a melting range of 82-83˚C. For water crystallization, the vanillin is dissolved in hot water and the solution cooled to produce a water crystallized vanillin.
US3600442 **Method of Recovering Vanillin from Crystallization Liquors** 1971 American Can Company	Vanillin is recovered from aqueous-methanol crystallization mother liquors through selective precipitation by serially treating the crystallization liquors with amounts of alkali-metal hydroxide and a zinc or magnesium salt, preferably a zinc salt, stoichiometrically equivalent to the 5-formylvanillin present, causing the 5-formylvanillin to precipitate, and removing the precipitated 5-formylvanillin, and repeating these steps with the same agents in amounts at least stoichiometrically equivalent to the vanillin and acetovanillone present, thereby precipitating the vanillin and acetovanillone. Vanillin is then separated from acetovanillone by the bisulfite method.
US3686322 **Process for purifying Vanillin** 1972 Sterling Drug Inc.	Crude vanillin in aqueous mixture with structurally related compounds, from the oxidation of lignin liquor, is purified by partial extraction with hot hydrocarbon solvent, cooling to crystallize vanillin therefrom and repeating the procedure, preferably re-using the same portion of solvent, to extract succeeding portions of vanillin.
US4151207 **Process for preparation of 3-methoxy-4-hydroxybenzaldehyde** 1979 Borregaard Industries Limited	The invention comprises the production of 3-methoxy-4-hydroxybenzaldehyde (vanillin) from lignin-containing sulfite waste liquor, wherein the waste liquor is first subjected to ultrafiltration procedure as set forth in Norwegian Pat. No. 127,545 to obtain an enriched lignin-containing fraction. The waste liquor used can be fermented to reduce the sugar content. The enriched fraction is oxidized to produce vanillin. This results in a reduction or elimination in the formation of a crust or scale on the production equipment, and higher yields of vanillin.
US4208350 **Separating Phenols from Alkaline Pulping Spent Liquors** 1980 Boise Cascade Corporation	Phenol, ortho-cresol, meta- and para-cresols, guaiacol, vanillin, acetovanillone and other phenols are separated from alkaline pulping spent liquors by extracting the alkaline liquors with a lower aliphatic alcohol (2-5 carbon) separating the solvent and aqueous phases, and separating the phenols from the solvent phase. The patent states that the phenolic content of spent aqueous liquors derived from the alkaline pulping of lignocellulose may be separated from the liquors without acidification, without any chemical pretreatment preliminary to the extraction leaving the black liquor in a condition suitable for return to the recovery system of the pulp mill.
US4474994 **Purification of Vanillin** 1984 Monsanto Company	The patent provides a process for the purification of crude vanillin contaminated with impurities, containing 70 to 98% (weight) vanillin, by contacting the crude vanillin with a supercritical extraction fluid at supercritical extraction conditions (35-50˚C, 40-100 atm), extracting from the crude vanillin at least some of the impurities, and separating the supercritical fluid containing impurities from the crude vanillin to produce vanillin product with increased purity.
US4652684 **Vanillin Extraction Process using Large Pore High Silica Alumina Ratio Zeolites** 1987 Mobil Oil Corporation	This invention comprises the use of large pore, high silica/alumina ratio zeolites such as Zeolite ZSM-20, Zeolite Beta, or dealuminated Zeolite Y for removing vanillin from various liquid solutions, particularly from fermented spent waste-liquors containing vanillin. Mixtures of the zeolites can also be used.
EP0238575B1 **Vanillin production process** 1987 Fried Krupp AG	In a vanillin production process, a water mixture containing vanillin is extracted with CO_2 (0-110˚C, 30-400 bar), after which the vanillin is separated. The CO_2 containing vanillin is led at the extraction temperature and pressure through an aqueous solution of sulfite or hydrogen sulfite, this solution is then acidified with sulfuric acid to a pH value between 2 and 4, and the vanillin-free CO_2 Is returned to the extraction stage.

US4847422 **Method for the Production of Vanillin** 1989 Yhtyneet Paperitehtaat Oy	The invention relates to a method for the production of vanillin in the form of a very pure product by oxidizing lignin contained in the wood pulping liquor. The separation and purification of vanillin from the reaction mixture is carried out by means of an extraction at a supercritical pressure and temperature. CO_2 can be used as extraction gas.
CA1013369 **Vanillin Recovery Process** 1994 Canadian International Paper Company	A process for isolating vanillin from alkaline aqueous solutions containing chemically-related phenolic impurities, such as ortho-vanillin, acetovanillone, *p*-hydroxybenzaldehyde, syringaldehyde and others, wherein the alkaline aqueous solution is subjected to extractive bisulfitation. This involves the formation of an alkali metal-bisulfite complex of vanillin in the presence of a substantially water insoluble organic solvent, such as a water-insoluble alkanol (e.g. n-butyl alcohol).
CA1014973 **Vanillin Production from Kraft Black Liquors** 1994 Canadian International Paper Co.	An efficient integrated process for producing vanillin from the thiolignin present in Kraft process black liquor. The Kraft black liquor is subjected to oxidation by treatments with an oxygen-containing gas at elevated temperature and pressure in an alkaline medium, whereby the alkaline reaction liquors, after extraction of vanillin, can be recycled to the Kraft mill for recovery and utilization of sodium and sulfur.
CA483829 **Lignin Oxidation Process** 1995 The Ontario Paper Company	This invention relates to the production of vanillin, acetovanillone and other oxidation products from lignosulfonic acid compounds such as waste sulfite liquor and especially from the same after sugar fermentation. Further increase in vanillin yield is obtained when the oxidation reaction with lime as the active alkali and in the presence of a finely dispersed gas containing free gaseous oxygen is conducted on a substantially continuous basis rather than on a batch basis.
CN1060464 **Method for producing vanillin and syringaldehyde by catalytic oxidation of alkali lignin of sugar cane residue** 2001 Chinese Academy of Sciences of Guangzhou	The method uses acid to precipitate the pulp waste bagasse alkali lignin in the pulp waste liquid. Filtration, and then its basic catalytic oxidation to generate syringaldehyde and vanillin and other low molecular product, which are then extracted with organic solvent and then uses one of the following methods, hydrogen sulfite sodium extraction, distillation, aldehyde ammonia complexation, recrystallization, sublimation to separate and purify syringaldehyde and vanillin. This method is characterized by the alkaline catalysis of alkali lignin oxidation.
CN102115432 **Method for preparing and separating p-hydroxybenzaldehyde, vanillin and syringaldehyde from lignin** 2011 University of Jiangnan	The present invention relates to a method for separation of *p*-hydroxybenzaldehyde, vanillin, syringaldehyde by preparing a (weak) base with lignin. It includes the preparation of $LaB_{(1-x)}Cu_xO_3$, yttrium, ore-type complex oxide catalyst; the centrifugation of the crude aromatic aldehydes, acidified and vacuum dried; extraction with $CHCl_3$ to obtain *p*-hydroxybenzaldehyde and vanillin, and another mixture of phthalic acid and syringaldehyde;. the fraction with syringaldehyde is dissolved in absolute ethanol and ammonia is added. pH is adjusted to give syringaldehyde. The liquid is concentrated under vacuum to give a solid. Distillation is used to separate vanillin from *p*-hydroxybenzaldehyde.
US0089046A1 **Process for the electrochemical cleavage of lignin at a diamond electrode** 2011 BASF SE	The invention relates to a process for the electrochemical cleavage of lignin by means of a diamond electrode and to a process for producing Vanillin and derivatives thereof by electrochemical cleavage of lignin in a solution having a pH ≤ 11.
US0232853 **Method for selective prod. of biobased chemicals and biofuels from plant lignin** 2013 Thesis Chemistry LLC	The present invention is directed generally to a method of production of value-added, biobased chemicals from lignin sources, including waste lignin. A method of using a depolymerization of lignin to create a tiered production of biobased aromatic chemicals and biofuels. Additionally, a reduction of waste products may also be provided from the resent method.
US0316165 **Method for Purifying Vanillin by Liquid-Liquid Extraction** 2014 Rhodia Operations	Purification of vanillin from a solution in a solvent S1 containing impurities comprising the following steps: a) evaporating the solvent S1 in the presence of water from such initial solution to obtain an aqueous solution of vanillin; b) a step pf liquid/liquid extraction by S2, at a pH between 8 and 10, to obtain an organic phase ad the aqueous phase containing vanillin and residual solvent S2; c) precipitation of vanillin at pH between 4 and 7.5; and d) isolation the vanillin.
WO006108 **Method for obtaining Vanillin from aqueous basic compositions containing Vanillin** 2014 BASF SE	The invention relates to a method for obtaining vanillin from an aqueous, basic composition produced in electrolytic oxidation, especially in the oxidation by means of, of aqueous alkaline compositions containing lignin, comprising at least one treatment of an aqueous, basic composition containing vanillin, in particular the treatment of a composition produced in the oxidation, especially in the oxidation by means of electrolysis, of aqueous alkaline compositions containing lignin, with a basic adsorbent, in particular an anion exchanger.
EP3109226A1 **Process for preparing Vanillin** 2016 BASF SE	The patent provides a process for the production of vanillin from vanillic acid in high yields comprising: a) production of 4-hydroxy-3-methoxybenzoic acid chloride, b) hydrogenation of 4-hydroxy-3-methoxybenzoic acid chloride with H_2 in the presence of catalyst. The technical applicability of the process is simple and inexpensive.
US0223438A1 **Electrochemical Conversion of Lignin to Industrial Chemicals** 2018 Ohio University	The patent describes the electrochemical conversion of lignin to a variety of industrial products using a binary catalyst with nickel or cobalt as a first metal and any transition metal as a second metal. Electrochemical conversion of waste lignin from pulping mills allow better reaction energetics than other thermochemical processes. This could generate additional revenue streams and enhance the value of biomass.

2.2.2 Alternative Production Methods

The production of vanillin, and by association syringaldehyde, from lignin nowadays, is still regarded as an alternative since it is not the main supply or the cheapest from of production. Although production from softwood sulfite lignin is dominated by Borregaard, it accounts only for 1500 t/y of the total world demand of approximately 20 000 t/y and growing [31]. Borregaard also seems to be using another complementary alternative route for vanillin employing glyoxylic acid [32].

Alternative Synthesis – Vanillin can also be produced in smaller scale via biochemical synthesis employing microorganism. Examples: vanillin can be synthesized from isoeugenol by utilizing enzymes [33]; it can also be produced from ferulic acid and glucose as substrates by bacteria, fungi and yeasts as production hosts [34-37].

Syringaldehyde production from syringin, a glucoside of sinapyl alcohol has been reported and also a method of production from 1,3-dimethoxy-2-hydroxybenzene [38] or methylated pyrogallol has been patented in [39]. Also another way of producing it from pyrogallol is reported by Mauthner *et al*. in [40, 41]. From gallic acid as well by McCord *et al*. from *p*-cresol by Ji *et al*. [42] and Tripathi *et al*. [43], from *m*-cresol [44] or from the whole class of *p*-cresols by Jiang *et al*. in [45].

2.3 Integrated Process for Lignin Valorization

An integrated process for the production of high added-value products from lignin has been put forward in the past by our group Figure 2-10. The integrated process is meant to be implemented by pulp and paper mills where a portion of the side stream of black liquor is diverted to lignin precipitation and subsequent added-value products manufacture. The integrated process is based on previous works in LSRE-LCM Associate Laboratory. Over the years, this integrated process has suffered several changes along with more recent works that were developed further and contributing for its update. The updated integrated process encompasses several unit operations, namely the oxidation of lignin in an initial phase and then a series of steps of separation, including ultra-/nanofiltration, adsorption and crystallization. The retentate stream of the filtration steps can be directed back to the process stream or they can be directed to the production of polymers namely polyurethanes considering their now narrower molecular weights distributions [46].

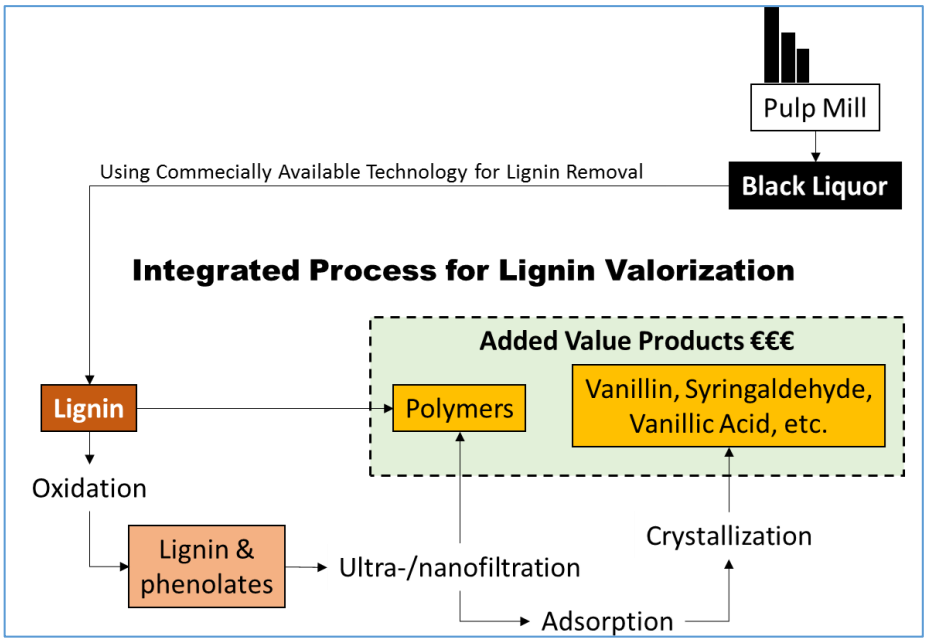


Figure 2-10 – Scheme for the updated Integrated Process for Lignin Valorization (partly based in this work).

The importance of reducing the impact of manufacturing processes by the reduction of the utilization of harmful solvents can be translated on the adoption of a more sustainable and greener process for the production of vanillin, syringaldehyde and other molecules of interest from Kraft lignin.

2.4 Lignin Precipitation Technologies

Lignin is obtained by precipitation from the black liquors from the pulp and paper industry. The liquors are very rich in lignin since it is dissolved during the wood pulping. The composition varies with the specific purpose of the pulp being produced, but it is mostly composed of hardwood (*e.g.* eucalyptus) or softwood (*e.g.* pine) lignin, hemicellulose, sugars, extractives, and process chemicals along with other trace minerals [47, 48]. Acidification with mineral acids or acid gas injection is used to precipitate lignin in the liquors, usually carbon dioxide followed by filtration of the suspended aggregates.

The first patent filled concerning the removal or reduction of lignin from pulping liquors was filled by West Virginia Pulp and Paper Company (US2464828, 1949) [49]. This company later became Westvaco, and after merging with Mead Corporation became MeadWestvaco Corporation. More recently, two simple and similar processes were also patented for lignin removal from pulping liquors, the LignoBoost™ and the LignoForce™ processes. The patented process LignoBoost™ was developed by the Swedish Forest Products Laboratory (Innventia now) and licensed to Valmet (US8486224, 2013) [50]. The competing process LignoForce™ was developed by FPInnovations and NORAM (US8771464, 2014) [51]. Figure 2-11 depicts the overlapping between both commercial solutions sold by Valmet and FPInnovations, respectively [52-54]. The major difference between both is the inclusion of a

non-obvious oxidation step to reduce the sulfur content producing a lignin with weaker odour; both systems can produce two types of lignin one at higher pH and another at lower pH. These precipitation methods can offer great advantages to the pulp mill:

a) Energy efficiency increase of the mill, since the excess fuel (lignin) can be stored temporarily for later use;

b) Capacity of pulp production is increased since the heating value of the black liquor is reduced due to the removal of some lignin; the furnace is able to burn higher volumes of liquor;

c) a new product, lignin can be sold as a commodity for different industries, e.g. concrete and building materials, textile dyes, ceramic products, batteries, mining activities and agricultural and fishery product.

The first demonstration lignin plant employing the LignoBoost™ Process was installed in Sweden by LignoDemo AB in Bäckhammar, 2007 and then two commercial plants: one built by Domtar, in USA 2013; and the other by Stora Enso in Sunila, Sweden in 2015. The first commercial lignin plant for the precipitation of lignin by employing the LignoForce™ process was installed in the WestFraser pulp mill in Alberta, Canada in 2016.

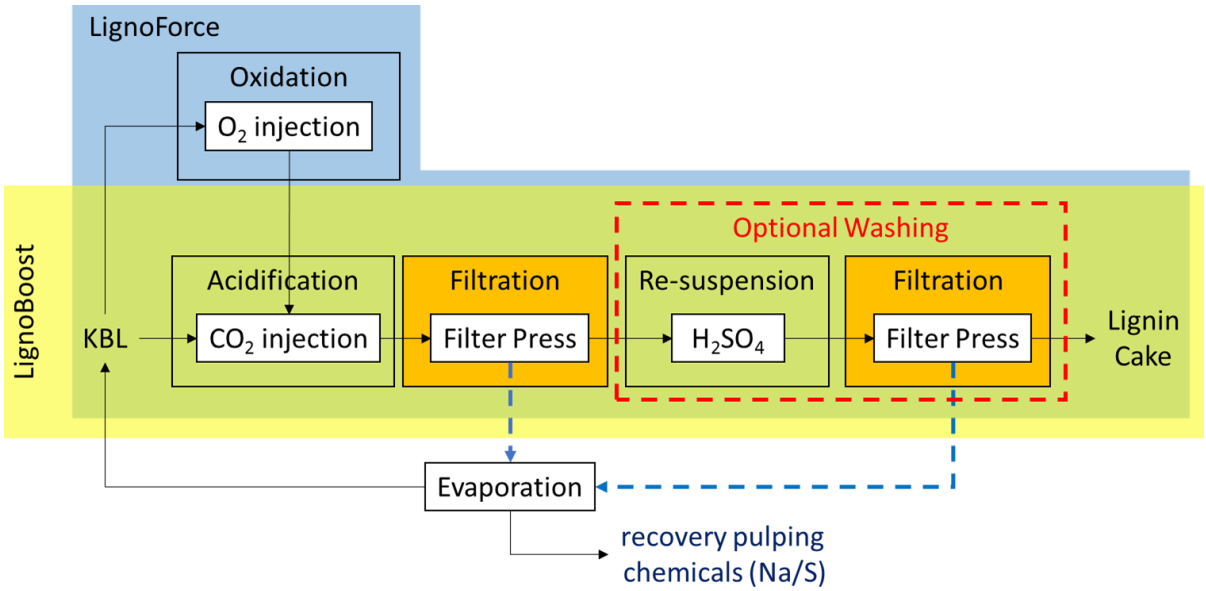


Figure 2-11 – Schematic comparison between the two main commercial processes for the reduction of lignin in black liquors. KBL – Kraft black liquor.

Filtration processes like ultrafiltration and nanofiltration can be used as well for the removal of lignin but they suffer from significant disadvantages, the major one being the great reduction in flux due to the formation of cake in the membrane surface due to contaminants of various molecular weights. Filtration processes can be successfully applied in the removal of lignin that has already been purified or washed like demonstrated in the precipitation technologies above mentioned.

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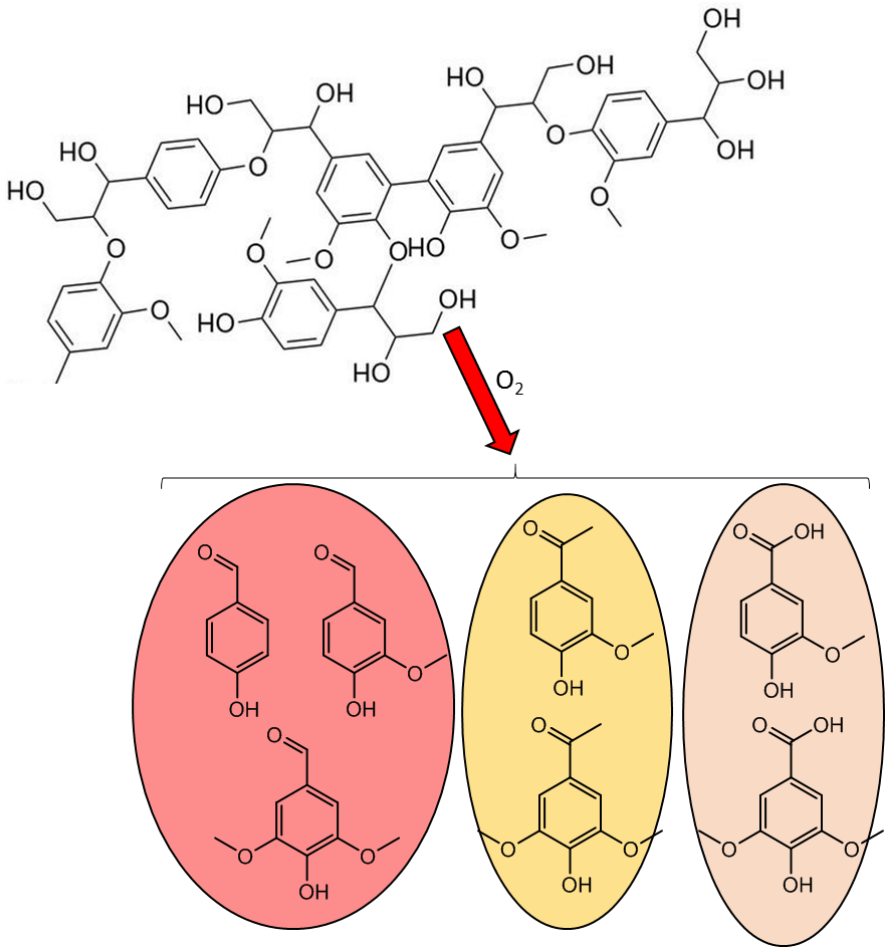
3 Lignin oxidation



The improvement of the depolymerization of lignin by alkaline wet oxidation is still a continuous research challenge. The conditions of the lignin oxidation step were improved to increase yields. This is determinant for the success of the separation processes that follow, since it is necessary to start the separation sequence with sufficient amounts of monomer phenolic compounds ending up with quantifiable amounts. Several efforts were made that resulted in the successful increase of the vanillin yields in the reactor without using any catalyst. A maximum vanillin yield of 4.31% (lignin weight basis) was reached in the structured packed bubble column reactor when employing Indulin AT.

3.1 Introduction

Lignin is a biopolymer with potential application as a source of platform chemicals produced in its oxidative depolymerization. The lignin oxidation generates several species with lower molecular weight among which monomers, dimers, oligomers and also high molecular weight lignin that did not depolymerize; mono- and dicarboxylic acids are also present. Among the phenolic monomers produced, vanillin is the most researched, but other phenolic compounds of interest are present like syringaldehyde, acetosyringone and acetovanillone (apocynin) and vanillic and syringic acids. In fact, the exploration of these other phenolic compounds will bring value to the oxidation stream and thus, a complete valorization of this stream is mandatory for the success of the biorefinery concept.

The degree of depolymerization depends on the final application of the lignin, but generally what is intended is an increase in monomer yields. Depolymerization can be performed under reducing or oxidizing conditions. Depolymerization can occur both in acidic and alkaline conditions, also the reaction can be catalyzed. Alkaline wet oxidation is one of many techniques used for the oxidative depolymerization of lignin. In an extensive review by Schutyser *et al.* (2018) it is shown clearly where alkaline wet oxidation is relatively to other depolymerization strategies available (Figure 3-1) in view of conversion to monomer aromatic compounds [1]. Relatively to the production of vanillin, alkaline wet oxidation might be the more promising, but for other utilizations like bio-oil production and production of aromatics with low degree of oxygenation or even a higher usage of the lignin material in total, strategies like pyrolysis and reductive depolymerization can be more viable. Also, oxidative depolymerization can be used for the production of small organic molecules due to ring cleavage, namely short chain carboxylic and dicarboxylic acids (formic, acetic, succinic, oxalic, malonic, malic and maleic,) with yields ranging from 11% to 56 % (lignin weight basis) [2-4].

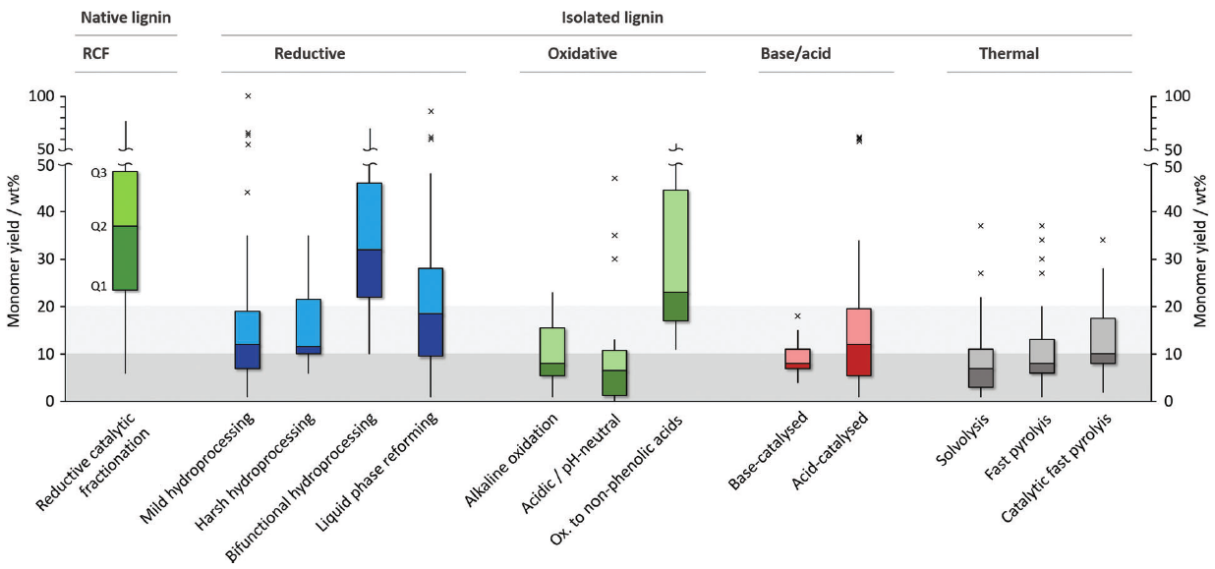


Figure 3-1 – Distribution of monomer yields for different lignin depolymerization methods (adapted from Schutyser et al. (2018).

The oxidation of lignin takes place in a reactor, that was built during another PhD program. Since then, adjustments to the setup were made in an attempt to improve vanillin production but the main focus has been the separation of the added-value compounds from the oxidation mixture containing lignin, even though increasing yields is a continuing struggle in the route to lignin valorization [5-14]. Over the years, several advances were made regarding the valorization of lignin and some contributions were made at the Associate Laboratory LSRE-LCM to this field. Some of the most significant contributions were the biological and chemical depolymerization of lignin by Mathias *et al.* (1995); the recovery of vanillin from standard solution with ion exchange and removal of lignin using membranes by Zabkova *et al.* (2006); the building of the structured packed bubble column continuous reactor by Araújo *et al.* (2009); the proposal of an integrated process for the valorization of lignin by Borges da Silva *et al.* (2009); and several contributions in the field of characterization of different lignins by Pinto *et al.* in the recent past [6, 8, 9, 15-19]. On the separation of the phenolic compounds, several contributions were also made by Mota *et al.* (2016) laying the foundations for the present work with SP700 resin [11, 12].

In this chapter, the efforts made in order to better understand and increase the yield on monomer phenolic compounds from the oxidation of lignin in the continuous reactor, the structured packed bubble column reactor (SPBCR), are presented, namely oxygen solubility experiments and volumetric mass transfer coefficient determination; oxidations of lignins with different origin are presented (not only kraft), summarizing the work that has been developed.

3.2 Material and Methods

3.2.1 Lignin and Lignin solutions

In this work, Indulin AT was used as the main starting raw material. Indulin AT is a widely studied softwood lignin, with average molecular weight (weight average) of 4000 Da [20, 21]. Indulin AT is derived from Kraft pulping mills and sold by MeadWestvaco Corporation (USA). Other lignins were used for comparison: hardwood lignin (*Eucalyptus globulus*) and black liquor obtained from a pulp mill (The Navigator Company, Portugal) employing the Kraft pulping process was used and, tobacco butanol and ethanol organosolv lignins, from an annual plant obtained previously from R. J. Reynolds Tobacco Company (North Carolina, USA). The solutions to be oxidized, with exception of the black liquor which was oxidized directly, are prepared with 60 g of lignin and 80 g of sodium hydroxide (AkzoNobel, Netherlands) per liter of solution. This lignin concentration of 60 g/L intends to mimic the high concentrations of lignin in black liquors, and the sodium hydroxide concentration of 80 g/L was set based on the work of Mathias *et al*. (1995) [16]. For oxidation in the continuous reactor, the preparation of volumes over 20 liters is common.

3.2.2 High Performance Liquid Chromatography

The known products from alkaline lignin oxidation/depolymerization are followed by high performance liquid chromatography (HPLC). A reverse phase column ACE 5 with C18-pentafluorophenyl group ($250\ mm$ x $3\ mm$, $5\ \mu m$) is used. The composition of the two main eluents used in the gradient is water/methanol 95%/5% (v/v) and methanol/water 95%/5% (v/v) both acidified with 0.1% formic acid. Solid phase extraction (SPE) was performed before HPLC analysis. The complete analytical procedure is reported elsewhere [22].

3.2.3 Oxygen solubility measurements and volumetric mass transfer coefficient determination

Oxygen solubility measurements were made at conditions the closest possible to the operating conditions since the sensor is only able to operate at a maximum temperature of 85˚C and 12 bar of total pressure while still being able to tolerate 120˚C for short periods, e.g. cleaning. The sensor used is a Hamilton VISIFERM DO 120 (Yokogawa, Japan) with fluorescence tip (Figure 3-2, left). The sensor is connected to a proprietary software (Arc Sensor DTM 1.5.0) for calibration, on-line measurement and data logging. The sensor includes a housing steel case for external circulation of the reactor contents (Figure 3-2, center). The general installation scheme for the sensor is presented in Figure 3-2, right. The calibration is done at room temperature and pressure; during calibration, the sensor is placed above a beaker filled with water being bubbled with nitrogen (point zero) and then in air. There are limitations to the measurement of dissolved oxygen (DO) due to the saturation of the sensor. Still,

this was the best affordable commercial DO sensor. Oxygen solubility measurements were performed at several temperatures (30, 60 and 80°C) and oxygen gas phase compositions (5, 10 and 20% O_2 (v/v)). The oxygen volumetric mass transfer coefficient was determined for both reactors, the structured packed bubble column reactor (SPBCR) and the batch BÜCHI reactor for comparison. The BÜCHI reactor was used for the solubility measurements as well Figure 3-3.

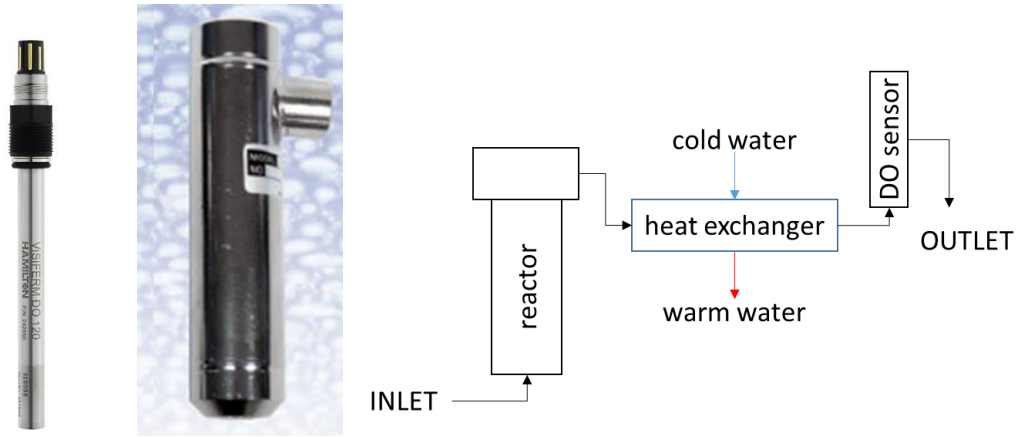


Figure 3-2 Dissolved oxygen (DO) sensor VISIFERM DO 120 (left) and the stainless steel vessel for adaptation (center); simple scheme for the installation of the DO sensor in the continuous reactor (right).

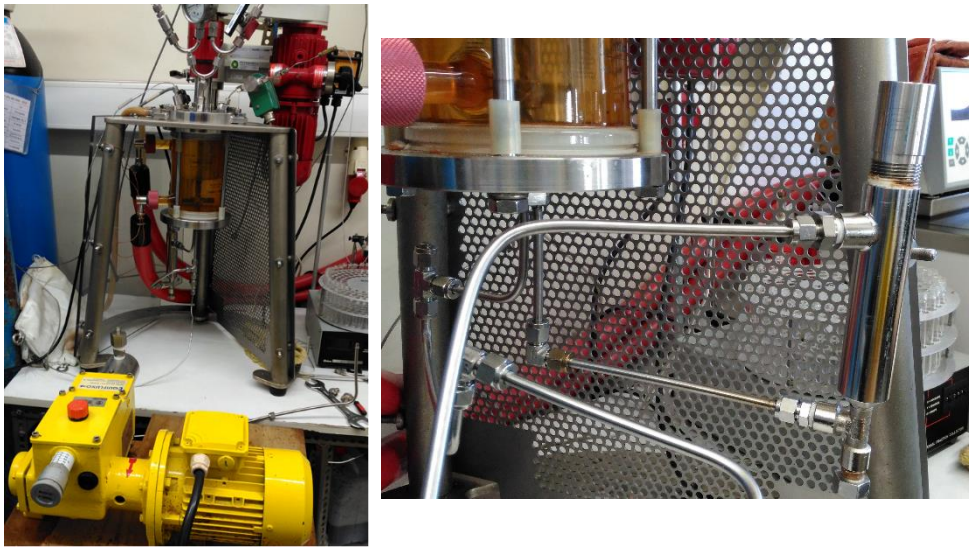


Figure 3-3 – Batch reactor (BÜCHI) setup for the oxygen solubility experiments (left). Detail of the DO sensor vessel installed (right).

The solubility of oxygen is known to be directly proportional to the pressure, and inversely proportional to the temperature [23]. For the calculation of $k_L a$, it is necessary to use equation 1,

$$\frac{dC_L}{dt} = k_L a(C_L^* - C_L) \quad (1)$$

which when integrating from 0 to t gives equation 2,

$$\ln\left(\frac{C_L^* - C_{L,0}}{C_L^* - C_L}\right) = k_L a\, t \quad (2)$$

in which $C_{L,0} = 0$, and C_L^* is the maximum oxygen concentration in the liquid (in mg/L) that would be in equilibrium with the conditions of the gas phase. This value is calculated based on equation 3,

$$C_L^* = \frac{y_{O_2} P}{\mathrm{H}_{O_2}} M_{O_2} \quad (3)$$

where y_{O_2} is the fraction of oxygen in the gas phase, P, the total pressure, M_{O_2} is the molecular mass of molecular oxygen. The Henry constant H_{O_2} for oxygen in water is $756.7\ atm\ L\ mol^{-1}$ at 25℃ and $1\ atm$ [23]; however this value needs to be corrected to take into the account the effect o temperature by utilizing the van't Hoff equation, equation 4,

$$\frac{d \ln H}{d\ (1/T)} = \frac{-\Delta_{sol} H}{R} \quad (4)$$

in which $-\Delta_{sol} H$ is the dissolution enthalpy, which takes the value of 1700 J/mol for oxygen and assuming that this value does not change much with temperature, the integration of the equation gives equation 5,

$$H(T) = H^{ref} \exp\left(\frac{-\Delta_{sol} H}{R}\left(\frac{1}{T} - \frac{1}{T^{ref}}\right)\right) \quad (5)$$

3.2.4 Experimental Setup and Procedure for the reactor operation

The structured packed bubble column reactor (SPBCR) is depicted in Figure 3-4 and the complete experimental setup for the reaction system is shown in Figure 3-5. The installation includes three sections: the feed section, the reactor itself and the gas washing. The feed is the section responsible for holding the mixture that will be oxidized. The system is fed through a positive displacement pump that can pump delivering up to a maximum liquid volumetric flow rate of 10 L/h under pressure. The gases used are nitrogen and oxygen stored in pressurized bottles. The gas volumetric flow rate is controlled by two mass flow controllers. Currently, the maximum mass flow rate controller installed operates at 10 SLPM. The reactor is a column type reactor with no mechanical agitation. The agitation relies only on the movement of the bubbles of gas rising in the reactor column body and also from the structured packing 3 Mellapack 750Y (from Sulzer). The pressure is controlled manually by adjusting a gas valve or by adjusting the liquid flow rate valve on the outlet side. The pressure and three different temperatures are read through a LabVIEW application developed for the reaction setup in a previous work [5]. The scrubber is important to insure that the gases coming out of the reactor do not contain caustic substances. Also, if an unexpected release of gas occurs, with liquid coming from the exit gas line the scrubber will serve as buffer to attenuate the effects of any accidental release. The gases escaping from the scrubber are directed to the fume exhaust. For the oxygen solubility tests and volumetric mass transfer coefficient determination the reactor was heated up to 140˚C and the temperature of the outlet stream is controlled by a heat exchanger/cooling bath.

The batch Büchi reactor (Figure 3-3) was used for comparison of the oxygen volumetric mass transfer coefficient and for the determination of the solubilities in water. The reactor is filled with 700mL of water and the agitation is set to 900 rpm. The gas phase is controlled manually by setting the desired composition of oxygen in the mixture (5 to 20% O_2 (v/v)) and the pressure is increased gradually by steps of 1 bar until 10 bar maximum. The temperature is also allowed to stabilize at the predefined temperatures (30, 60 and 80˚C), before the DO reading is made.

a b c

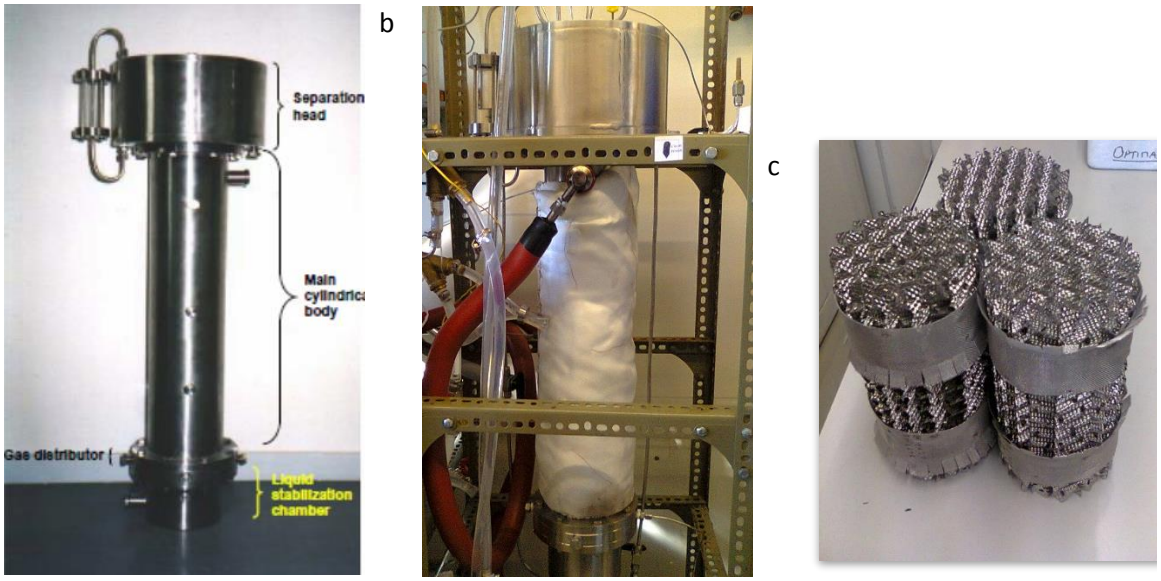


Figure 3-4 Structured packed bubble column reactor (SPBCR) in 316L stainless steel.(a) picture with no insulation; (b) detail of the reactor column with insulation and oil heating tubes connected; (c) 3 modules of structured packing Mellapack 750Y (from Sulzer) used inside the reactor.

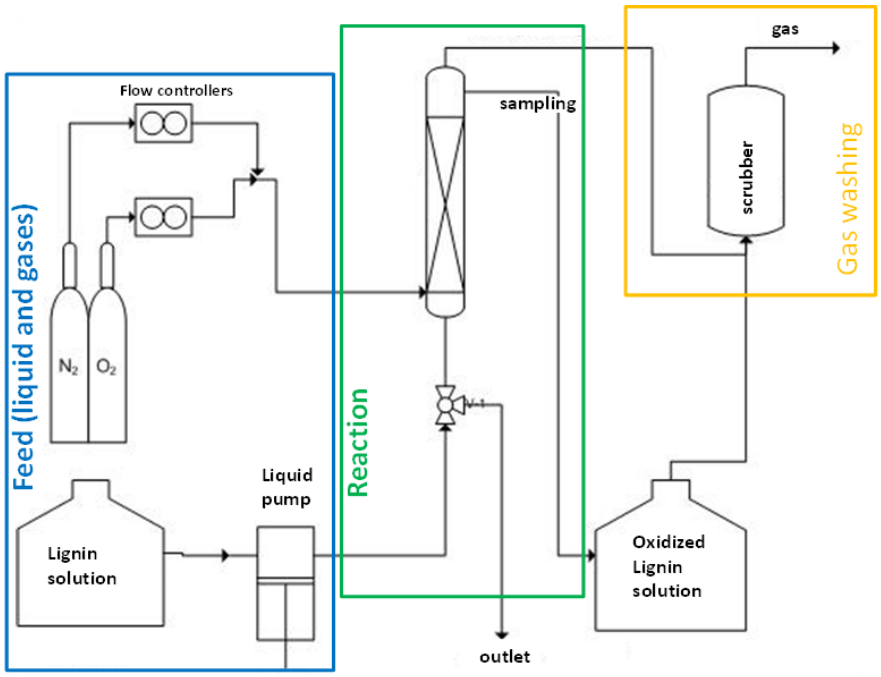


Figure 3-5 – Simplified scheme of the reaction setup for the continuous oxidation of lignin.

Briefly, the oxidation procedure is as follows:

a) A sufficient volume of solution (20 to 40L) is prepared with a lignin concentration of 60 g/L and sodium hydroxide of 80 g/L is prepared;

b) The reactor is purged with nitrogen and after 20min the outlet gas valves are closed;

c) The thermostatic oil bath is set to an intermediate temperature below 100˚C and the reactor body is allowed to heat while the mixture is being fed;

d) When the reactor is filled with lignin mixture the outlet liquid valve is opened and the liquid is directed to the feeding vessel (refrigerated in a cooling water bath);

e) The liquid is recirculated at its intended operation volumetric flowrate until the reactor reaches the desired temperature set point of $140℃$ and pressure of $10\ bar$, also the nitrogen gas is allowed to circulate;
f) The stability of pressure and temperature must be assured at least for 30 minutes;
g) After stabilization of conditions, the gas stream composition setpoint is changed to allow the oxygen to enter the reactor;
h) Operation on the defined conditions for at least 2 h so that a steady state is reached or until all mixture is oxidized;
i) Then the pump is stopped, the liquid flow is closed and oxygen supply is closed, and the temperature of the oil heating bath set point is reduced for slow and safe cooling;
j) Pressure is relieved only after the temperature is below $100℃$.

3.3 Results

3.3.1 Solubility experiments

The solubility of oxygen in water was studied in the batch BÜCHI reactor for a range of oxygen gas phase compositions and temperatures. The oxygen solubility in water at atmospheric pressure and room temperature is about 8 mgL^{-1}. This value is affected by several factors among which temperature, pressure and gas phase composition. Also, the presence of salts has a salting out effect for oxygen but this was not studied. By inspection of Table 3-1, the oxygen solubility is well described by equation 3 since the experimental readings for the simple water system agree with the calculated value. Dissolved oxygen for the points 2.5 and 5.5 bar were collected since at those conditions, and for the total pressure of 3 and 6 bar respectively, the sensor was saturated and the measurement had to be done at a slightly lower pressure so that the sensor was not saturated. Grey boxes are the DO points that could not be read due to sensor saturation.

Table 3-1 – Oxygen solubility in water in the batch BÜCHI reactor (missing values in grey are due to saturation of the sensor). Experiments performed at 900 rpm.

	Dissolved Oxygen/ mg L-1																	
y_{O2}/ %	5%						10%						20%					
T/ ºC	30		60		80		30		60		80		30		60		80	
ΔP/ bar	**Exp**	**Calc**	**Exp**	**Calc**	**Exp**	**Calc**	**Exp**	**Calc**	**Exp**	**Calc**	**Exp**	**Calc**	**Exp**	**Calc**	**Exp**	**Calc**	**Exp**	**Calc**
0	1.95	1.72	1.14	1.06	0.60	0.79	3.97	3.54	2.22	2.10	1.42	1.58	7.15	6.99	4.30	4.22	2.50	2.50
1	3.60	3.43	2.14	2.13	1.55	1.58	7.45	7.00	4.44	4.20	2.92	3.16	14.48	13.98	8.06	8.44	5.41	6.30
1.5													17.94	17.48				
2.00	5.08	5.14	3.28	3.19	2.29	2.37	10.32	10.44	6.34	6.26	4.46	4.74	20.39	20.97	12.12	12.66	8.70	9.41
2.5															13.92	14.77	10.10	10.99
3	6.89	6.86	4.43	4.26	3.08	3.16	13.80	13.92	8.49	8.34	6.21	6.32		27.96		16.87		12.64
4	8.58	8.58	5.37	5.32	3.91	3.95	17.07	17.37	10.55	10.45	7.71	7.90		34.95		21.09		15.80
5	10.08	10.29	6.47	6.39	4.79	4.74	20.43	20.87	12.29	12.54	9.16	9.48		41.94		25.31		18.96
5.5							21.88	22.20										
6	11.72	12.01	7.33	7.45	5.22	5.53		24.47	14.02	14.67	10.79	11.06		48.93		29.53		22.12
7	13.03	13.73	8.45	8.52	6.32	6.32		27.96		16.87		12.64		55.92		33.75		25.28
8	14.66	15.44	9.29	9.58	7.10	7.11		31.46		18.98		14.22		62.91		37.97		28.44
9	15.98	17.16	10.30	10.64	7.82	7.90		34.95		21.09		15.80		69.90		42.19		31.60
10	18.09	18.87	11.29	11.71	8.51	8.69		38.45		23.20		17.38		76.89		46.41		34.76

The objective was to test the solubility of oxygen in water when in contact with oxygen rich atmosphere under high pressure and temperature, similar to delignification process conditions. Since the sensor had limitations regarding the temperature range of operation, the measurements were halted and the volumetric oxygen mass transfer coefficient was studied after.

3.3.2 Oxygen mass transfer coefficient determination (continuous and batch)

The availability of oxygen is crucial for the efficient degradation of the lignin macromolecule. Knowing the rate at which oxygen dissolves into the alkaline solution is essential for the establishment of the right conditions inside the reactor. Although the determination of the mass transfer coefficient was performed in pure water, it is expected that the difference in behavior between continuous and batch reactor have the same magnitude as in alkaline environment. Experiments with the dissolved oxygen sensor in the continuous reactor were performed (Figure 3-6) and also in the batch reactor (Figure 3-7) so that a comparison between both reactor systems could be made.

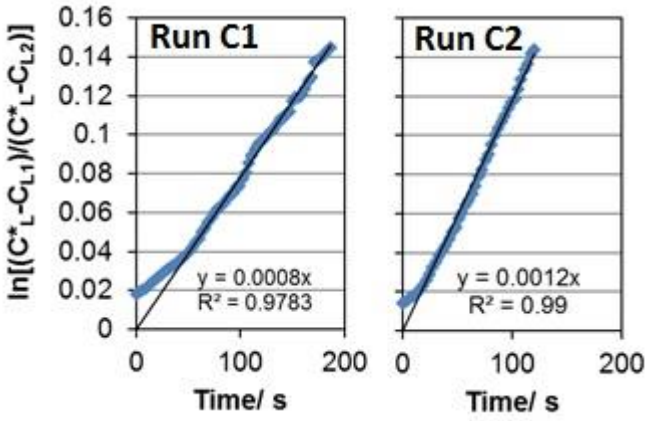


Figure 3-6 – Volumetric mass transfer coefficient determination inside the continuous reactor (SPBCR). 2 experiments performed. , T = 80˚C, Total pressure = 10.5 bar. O_2 gas composition = 50% (v/v); C – continuous.

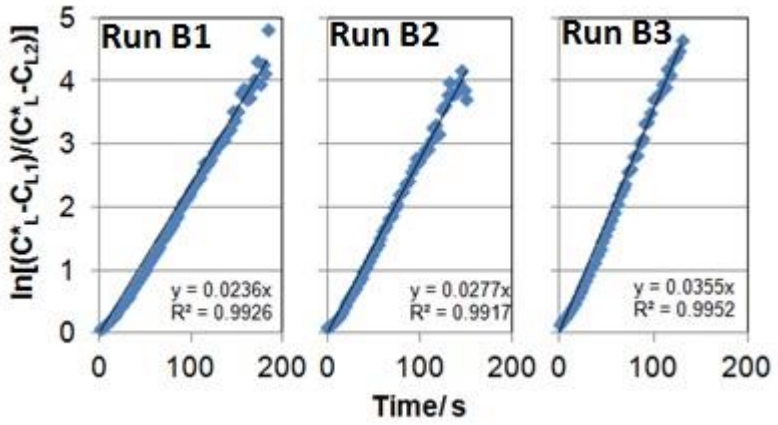


Figure 3-7 – Volumetric mass transfer coefficient determined inside the batch reactor (BÜCHI). 3 experiments performed. General conditions: agitation speed = 900 rpm, T = 31˚C, Total pressure = 10 bar. O_2 gas composition = 5% (v/v); B - batch.

As depicted in Table 3-2, the values obtained for the $k_L a$ from these experiments in the continuous reactor (SPBCR) are in accordance (same order of magnitude) with those adjusted to vanillin and lignin oxidation data by Araújo *et al.* [5]. The $k_L a$ in the batch is approximately 50 times higher than that of the continuous reactor, at its worst-case scenario and, 30 times at its best. For the tested conditions, the dissolved oxygen values in each experiment in the continuous reactor, there is still a margin inside which it is possible, with an appropriate gas-liquid contacting system, to increase

the mass transfer of oxygen and thus increase the current vanillin yields, which are significantly lower when compared with those of the batch reactor [5].

Table 3-2 – Comparison of the volumetric oxygen mass transfer coefficient (k_La) obtained in both reactor systems.

Reactor Type	Experimental/Estimation	k_La, (s^{-1})	Reference
Continuous (SPBCR)	Experiment #C1	8.0×10^{-4}	This work
	Experiment #C2	1.2×10^{-3}	This work
	Estimated	3.8 to 8.1×10^{-4}	Araújo *et al.*
Batch (BÜCHI)	Experiment #B1	2.4×10^{-2}	This work
	Experiment #B2	2.8×10^{-2}	This work
	Experiment #B3	3.6×10^{-2}	This work

3.3.3 Oxidation of Indulin AT Kraft lignin

Several oxidations were performed during this work. Three oxidations of Indulin AT were performed, at different conditions of oxidation in the SPBCR (Figure 3-8). The profiles of those oxidations are combined so that the differences are more visible. In the first experiment, the vanillin concentration reached only 0.93 g/L (yield of 1.55%, lignin weight basis) at steady state operation. Vanillic acid was also produced reaching a concentration of approximately 0.2 g/L and acetovanillone, but in lower amounts (not shown). After the improvement of the reaction conditions, namely the increase in gas volumetric flow rate (which increased the turbulence inside the reactor) and utilization of a pure feed of oxygen (increasing the driving force for the dissolution of oxygen) the concentration of vanillin increased approximately 230%. In the third oxidation, at steady state operation of the continuous reactor, vanillin reached a concentration of 2.16 g/L. Also, vanillic acid concentration was higher than the concentration in the previous experiment reaching 0.9 g/L. This trend should continue to increase with increasing gas mixing efficiency.

As stated previously, and with the experience obtained in the operation of the continuous reactor, a non-static gas distributer would be effective for the increase of oxygen mass transfer since the design of bubble column reactors is highly empirical [24]. Several options are available for the improvement of the contacting efficiency, including oscillatory baffled reactors, or high speed rotary reactors; both types make use of cavitation to break up the gas bubbles increasing the area available for the mass transfer [25]. Impinging jet reactors are also used efficiently for the delivery of oxygen to the aqueous phase in the wastewater treatment field which deals with waters that have a very high demand of oxygen [26].

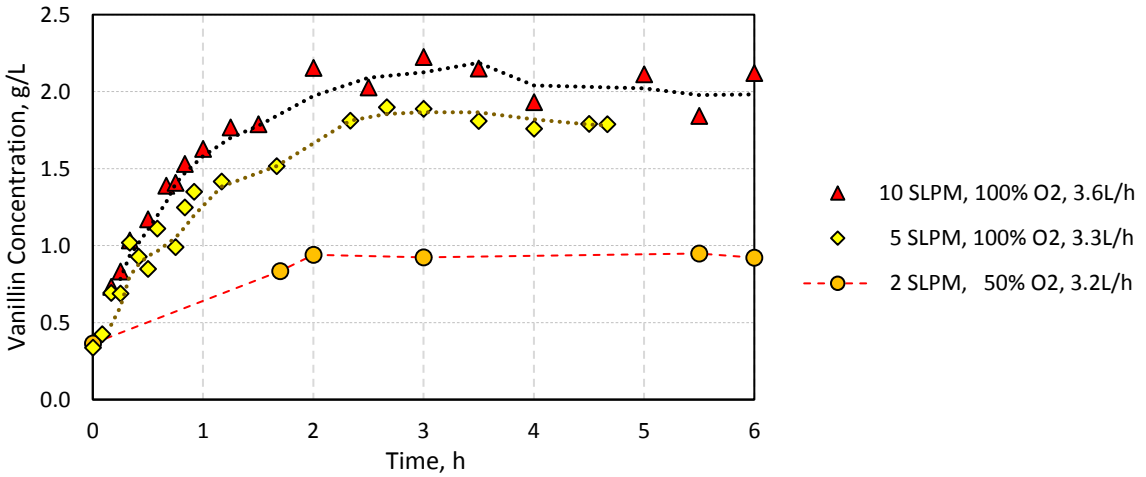


Figure 3-8 – Vanillin concentration histories at the outlet of the continuous reactor (SPBCR) for the oxidation of Indulin AT Kraft softwood lignin at 3 different sets of operating conditions. Conditions of the first oxidation (circles): gas volumetric flow rate 2 SLPM, O_2 (% v/v) = 50% and liquid volumetric flowrate =3.2 L/h; second oxidation (diamonds): gas volumetric flow rate 5 SLPM, O_2 (% v/v) = 100% and liquid volumetric flowrate =3.3 L/h; third oxidation (triangles): gas volumetric flow rate 10 SLPM, O_2 (% v/v) = 100% and liquid volumetric flowrate =3.6 L/h. General conditions: Temperature = 140°C, Total Pressure = 10 bar. SLPM – standard liter per minute.

3.3.4 Comparison of the yields obtained in the oxidations of several lignin types

A brief summary of the most important oxidations already performed is presented in *Table 3-3*. The yields are reported in terms of vanillin on a lignin weight basis. Yields are also given in terms of the total known phenolic compounds for reference. The initial temperatures at which the oxidations took place are similar, 140˚C, except for the ethanol organosolv lignin experiment that was performed at 123˚C.

For the case of Eucalyptus Kraft black liquor, the increase in residence times (or low liquid volumetric flow rates) resulted in an increase of the yields; a yield on vanillin of 0.24% (lignin weight basis) was obtained for the most favorable scenario. The presence of sulfur compounds and sugars are two of the possible compounds present responsible for competing consumption of the oxygen fed resulting in low monomeric phenolic compounds production. This could explain why the increased residence times, result in higher yields. Higher liquid volumetric flowrates result in the lignin not being oxidized efficiently, since other species are still consuming the oxygen. For the case of the oxidation of the lignin obtained from the isolation from the same hardwood Kraft black liquor, it resulted in a higher yield than the direct oxidation of the black liquor. For the same set of operating conditions, the vanillin yield is 0.27% (lignin weight basis), over two times that of the Kraft black liquor oxidation. This may encourage the isolation of lignin from the liquor before oxidation but profitability may be influenced as well by other factors like equipment for isolation and acid costs.

Tobacco butanol organosolv lignin was the lignin utilized that produced the lowest vanillin yields, 0.52% (lignin weight basis) and it is presented to illustrate the impact of the pulping/isolation treatment and the origin of the plant material has on vanillin yields. Ethanol organosolv lignin depolymerization under alkaline wet oxidation conditions led to similar results, although the oxidation conditions differ, resulting in low yields, 0.53% (lignin weight basis). The harsh conditions of the organosolv isolation process alters the lignin and increase the number of condensed units (e.g. bonds of the type 5-5’ or β-5) translating in a larger condensation of the lignin macromolecule and affecting the extent of the depolymerization [27, 28]. Organosolv derived lignins monomer yields can vary a lot when depolymerized. In a set of lignin materials study Costa *et al.* demonstrated that tobacco butanol organosolv lignin had the lowest potential for the production of vanillin (and syringaldehyde) [19].

Table 3-3 – Summary of yields obtained with lignins of different origin. No catalysts used. Experiments performed at 10 bar.

Oxidation of	Gas vol. flow rate, (SLPM)	O_2 gas composition, (% v/v)	Liquid vol. flow rate, (L/h)	Initial Temp. (ºC)	Yields (% lignin weight basis)	
					Vanillin	All*
Indulin AT (Kraft)	**10**	**100**	**3.6**	**140**	**4.31 %**	**6.74 %**
Indulin AT (Kraft)	5	100	3.3	140	3.04 %	4.21 %
Indulin AT (Kraft)	2	50	3.2	140	1.55 %	1.92 %
Kraft Black Liquor (KBL)	2	50	3	147	0.24 %	1.99 %
Kraft Black Liquor (KBL)	2	50	6	143	0.14 %	1.79 %
Kraft Black Liquor (KBL)	**2**	**50**	**10**	**142**	**0.12 %**	**1.56 %**
KBL Lignin	2	50	10	144	0.27 %	2.48 %
Ethanol Organosolv Lignin	2	50	4.4	123	0.53 %	1.61 %
Butanol Organosolv Lignin	2	50	2.4	148	0.52 %	1.75 %

*this yield is calculated taking into account the known phenolic compounds: for softwood the yield comprises only G and H derived units (4 in total: *p*-hydroxybenzaldehyde, vanillic acid, vanillin and acetovanillone), while for hardwood S derived units are also quantified (7 in total: *p*-hydroxybenzaldehyde, vanillic acid, vanillin, syringic acid, syringaldehyde, acetovanillone and acetosyringone).

As demonstrated above, the nature of lignin plays a significant role on the yields, for instance, softwood lignin gives higher yields (Indulin AT experiments). It gave the best yields on vanillin (4.3%, lignin weight basis) ever obtained in this set of reactions performed. The exploration of successively higher gas volumetric flowrates (up to the limit of the installation, 10 SLPM) and increasing oxygen composition led to these increased yields. The oxidation of Indulin AT produced of a final oxidation solution with vanillin concentration of 2.16 g/L, high enough to proceed with separation studies. Other studies including softwood lignins also gave an advantage to lignins containing guaiacyl type of structural units. The Kraft Indulin AT lignin is better suited for the production of the aldehyde vanillin than the hardwood lignins as observed in Table 3-3. Being a widely studied lignin and having reasonable yields on the vanillin production, it was selected as an ideal lignin to further develop the knowledge on vanillin separation processes.

Temperature is another important factor in regulating the yields of aldehydes. The temperature of 140˚C was set based on past experiments, given that at the same initial temperature, the batch produces higher yields. Based on reviewed literature, a possible way to further increase yields without modifying the reactor would be to increase the oxidation temperature to ranges closer to 200˚C [29].

3.4 Conclusions

The continuous efforts to increase the vanillin yield on the continuous reactor resulted in the following conclusions:

- Mass transfer coefficient, k_La in the continuous reactor was experimentally determined to be in the range of 8.0×10^{-4} to 1.2×10^{-3} s^{-1} which is in contrast with the k_La value in the batch reactor ranging from 2.4 to 3.6×10^{-2} s^{-1}, revealing that if the mass transfer of oxygen is improved, the yield of vanillin and other monomer compounds can be increased in the continuous reactor;
- The rate mass transfer in the reactor is influenced greatly by the type of mixing apparatus; currently there is no way of decreasing the size of gas bubbles inside the continuous reactor, which would translate in larger area available for oxygen mass transfer;
- Indulin AT was the best lignin, from the ones tested, to produce vanillin;
- The increase of gas volumetric flowrate and oxygen composition led to increased yields; the maximum vanillin yield obtained with the current best conditions is 4.31 % (lignin weight basis);
- Improving the monomer yields is mandatory for the increased viability of the integrated process.

3.5 References

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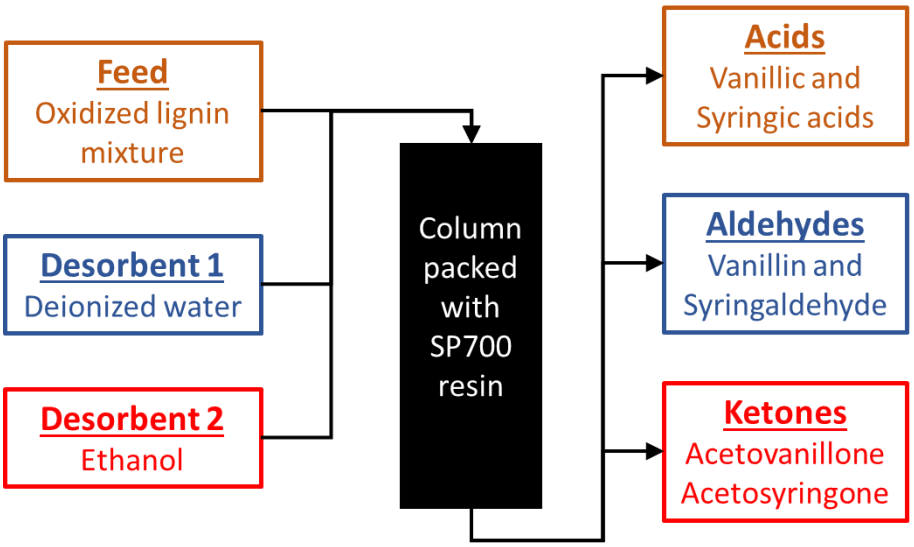
4 Fractionation of acids, ketones and aldehydes from alkaline lignin oxidation solution with SP700 resin



The separation of the products from the alkaline oxidative depolymerization of lignin is currently very laborious, generally, requiring several steps of solvent extraction and precipitation. The separation of the different species was achieved by adsorption with nonpolar SP700 resin, which is able to fractionate the real solution in families of chemicals namely phenolic acids, aldehydes and ketones. The main fractions obtained during adsorption, desorption with water followed by desorption with ethanol are very distinct in composition; the first is richer in vanillic (VA) and syringic (SA) acids, the second, mostly vanillin (V) and syringaldehyde (S) and the third one concentrates acetovanillone (VO) and acetosyringone (SO).

Fractionation can be adjusted by choosing the pH of the feed mixture which was studied in the range of 9 to 12. Depending on the fixed feed pH, the recoveries of aldehydes (V+S) in the water desorption step were almost complete (>80% at pH 10 or above) with only traces present in the fraction desorbed with ethanol. It was found that pH 12 was better for concentrating aldehydes and ketones at the same time, and that pH 10 was better at maximizing the fractionation of aldehydes and ketones since the majority of aldehydes desorb in the water phase (87%) while there is also a decrease on the amount of the ketones desorbed (from 53% to 25%). Ketones are desorbed preferably in the ethanolic phase.

Chapter published in the article *"Fractionation of acids, ketones and aldehydes from alkaline lignin oxidation solution with SP700 resin"* in Separation and Purification Technology, Volume 194, 2018, Pages 256-264.

4.1 Introduction

Lignin is a biopolymer that binds cellulose and hemicellulose in the plant cell walls granting rigidity and support. Plants can have a lignin composition up to 30 % (w/w), thus making it the second most abundant biopolymer next to cellulose [1]. Side streams from the pulp and paper industry are very rich in lignin and the common route employed for the valorization of these streams are the production of steam and electricity, making the pulp and paper industry a net seller of energy [2]. A more noble route for the valorization of lignin involves its oxidative depolymerization into highly functionalized monomers like vanillin and syringaldehyde, among other compounds [3].

One of the aspects hindering the adoption of lignin and other biomass sources as platform chemical feedstock is the difficulty on the separation of the added-value compounds produced during the oxidative depolymerization without resorting to complex sequence of processes that employ harmful solvents [4-6]. Alternative approaches have been tried in the recent past, ranging from membranes, precipitations and solvent extractions or any combination of these. A useful review is available elsewhere [7].

There are real advantages to the usage of nonpolar resins including their versatility, easy regeneration and great stability at wide pH ranges. Additionally, resins have been applied to numerous studies for the recovery of other phenolic compounds, namely antioxidants from olive mill wastewater [8] and winery wastes [9], or removal of toxic phenol derivatives from wastewater [10].

The majority of the adsorption studies related to vanillin with nonpolar resins are performed with synthetic mixtures [7, 11-15], and only a few of these studies were performed with real mixtures [16]. Wang *et al.* (2010) [16] studied the adsorption of vanillin and syringaldehyde in oxygen delignification spent liquor on the nonpolar resin D101 in the pH range from 4 to 6.5, performing the desorption steps with ethyl ether. The authors succeeded in recovering the aldehydes vanillin and syringaldehyde with high contaminations of acetovanillone and acetosyringone and trace amounts of other phenolic compounds. Purification of the compounds could be achieved by chromatographic processes or by selective crystallization. Tarabanko *et al*. (2012) [17] have proposed the separation of synthetic mixtures of vanillin and syringaldehyde by crystallization, based on the different solubility of the ammonium salts of these aldehydes in solutions saturated with potassium carbonate with recovery of 95% of vanillin and purity of 98% in just one step of crystallization.

Several authors proposed the ion exchange resins for the recovery of the phenolic compounds in oxidized spent black liquor or solutions with lignin, and although ion exchange resins have been able to adsorb monomer phenolic compounds [18-20], the resins employed were unable to fractionate the monomer phenolic compounds in families like the nonpolar resins used in this work.

In this work, an oxidation mixture containing several phenolic monomers, among other compounds, is treated by an adsorption process onto SP700 resin after ultrafiltration with a membrane with an average molecular weight cut-off of 5 kDa. This preprocessing is performed to remove some of the higher molecular weight lignin structures that were not depolymerized during oxidation. Experiments at four different pH values (9, 10, 11 and 12) were performed in order to understand the influence of the pH on the adsorption of the phenolic monomers of interest and take advantage of the presence of compounds in the mixture with different p*Ka* values [21]. The phenolic monomers quantified were *p*-hydroxybenzaldehyde (H), vanillic acid (VA), vanillin (V), syringic acid (SA), syringaldehyde (S), acetovanillone (VO) also known as apocynin and acetosyringone (SO).

The compounds are adsorbed and then desorbed using a two eluent technique, first employing deionized water and then ethanol. The desorption step with deionized water is able to recover the aldehydes and the desorption step with ethanol is able to recover the ketones along with the aldehydes that might remain. This approach is flexible and the degree of fractionation can be adjusted by using a determined pH in the feed solution.

To the best of the authors' knowledge there are no studies evaluating the influence of the pH of the feed (lignin oxidation solution) during adsorption, and at the same time, assessment of the differences in desorption phases in view of the fractionation of the acids, aldehydes and ketones in a single adsorption process.

4.2 Experimental Methods

4.2.1 Production of the Feed Mixture

The real mixture was prepared with 60 g/L of butanol organosolv lignin (tobacco stalks) and 80 g/L of sodium hydroxide. The mixture was oxidized in a structured packed bubble column continuous reactor previously built in our laboratory [22]. Oxidation was performed at an average temperature of 144^{o}C and partial pressure of oxygen of 5 bar and total pressure of 10 bar. The liquid mixture was fed at a volumetric flow rate of 10 L/h and the gas was fed to the reactor at a rate of 2 SLPM. Afterwards, the oxidized mixture was filtered with an ultrafiltration membrane with a molecular weight cut-off (MWCO) of 5 kDa (PLEAIDE, from Orelis) in a GE Osmonics SEPA CF II filtration system. The ultrafiltration was performed in recirculation mode at a transmembrane pressure (TMP) of 20 bar and temperature of 25^{o}C. For each experiment, the pH of the feed mixture was adjusted to the desired value with sulfuric acid and sodium hydroxide and was then allowed to stabilize overnight. After resting overnight, the pH of the feeding solution was measured again. The mixture was then fed to the adsorption column without further treatments.

4.2.2 Resin Activation

The nonpolar SP700 resin (resin properties in Table 4-1) was activated by performing several washes with acidified deionized water and methanol to remove all material adsorbed in the resin from origin as described elsewhere [12]. First, the resin is placed in a beaker with excess water and after settling, the floating dry resin is removed. Then, the resin is filtered by vacuum and placed in a flask with excess water and is left to stir in the orbital shaker for up to one hour. This step is repeated once more and afterwards the resin is rinsed with methanol, filtered and placed in a flask with methanol acidified with 0.1 % (v/v) of formic acid and left in the orbital shaker for another hour. After this the resin is filtered out, rinsed with water acidified with 0.1 % (v/v) of formic acid, and shaken for one hour with excess acidified water. Finally, the resin is rinsed several times with deionized water and is ready to be used/transferred to the column.

Table 4-1 – Physical and chemical properties of SP700 resin.

Property	Value/Designation
Resin denomination	**SP700**
Manufacturer	Mitsubishi Chemical Corp.
Matrix	polystyrene-divinylbenzene (PS-DVB)
Density of wet adsorbent, (gL^{-1})	1010
Moisture content, %	60-70
Pore Volume, (mLg^{-1})	2.1
Average Porosity of Particle	81
Average Particle size, (μm)	450
Specific surface area, (m^2g^{-1})	1200
Average pore radius, (nm)	9

4.2.3 Experimental Installation

Experiments were carried out in a glass column (Merck–Darmstadt) with diameter of 26 mm and 446 mm of total height, packed with 151.6 g of nonpolar SP700 resin previously activated. The column cooling jacket was maintained at 25^{o}C by a water thermostatic bath. The pump delivering the liquid to the column is a Smartline P1000 from KNAUER. The installation has also a Smartline UV 2500 detector from KNAUER which is useful during desorption and resin cleaning/regeneration in order to monitor if there are still components being desorbed. Detector wavelength set at 280 nm. The parameters describing the column were determined by simple tracer experiments with blue dextran as described elsewhere [23]. A mean residence time of 540 s, Peclet number of 555 and a bed porosity of 0.37 were obtained at a liquid volumetric flow rate of 10 mL/min.

4.2.4 Adsorption

First, a breakthrough experiment was performed, during which the mixture was fed for a period of 8.5h. On the remaining experiments, adsorption phase is performed during 3h at a liquid

volumetric flowrate of 10 mL/min of filtered oxidized mixture (permeate from the 5 kDa membrane). This feed time duration was selected taking into consideration the breakthrough experiment. At 3h of feed, phenolic compounds other than acids start to elute in very low quantities so this time was defined as the duration of the feed step for the other experiments at different pH values. This time interval depends on the concentration of phenolic compounds in the feed. For more concentrated solutions, this interval should be shorter, since the resin will reach equilibrium faster.

4.2.5 Desorption

Desorption was performed following a two eluent technique. Desorption with deionized water was performed first during 1h and then, a second desorption step was performed with ethanol (97% v/v) during 1h. Previous works at our group [12, 13] showed that using ethanolic solutions as the desorption eluent results in the recovery of concentrated solutions of phenolic compounds from the SP700 resin, which is also in agreement with other authors [9, 24].

4.2.6 Sampling and Sample Preparation

Throughout the experiments, samples were collected at times based on early exploratory experiments and also the information taken from the breakthrough experiment. The frequency of the sampling is increased at times when the peaks are eluting from the column. When important peaks are eluting the samples are collected every 50 seconds. A single complete experiment takes 5h and over 90 samples are generated. Along the breakthrough experiment, samples were analyzed to check if the column was already saturated.

Samples obtained before ultrafiltration were prepared by solid phase extraction (SPE), following a method developed and reported elsewhere [25]. It was observed that after the ultrafiltration, the acidification of samples did not originate significant precipitation of lignin avoiding thus the SPE extraction step. Samples collected at the outlet of the chromatographic column were diluted in eluent (95% water / 5% methanol, 0.1% formic acid, (v/v)), with dilutions starting from 20 up to 200 times before being injected in the HPLC.

4.2.7 Analysis Method (HPLC)

Each sample was analyzed by high performance liquid chromatography (HPLC) employing a reversed phase column ACE 5 C18-pentafluorophenyl group (250 x 3.0 mm, 5 μm). The eluents compositions are water/methanol: 95%/5% (v/v) containing 0.1% (v/v) formic acid and methanol/water 95%/5% (v/v) acidified with 0.1% formic acid. The complete analysis procedure is reported elsewhere [26].

4.2.8 Total Solids Content

Analysis of the solids contents was performed to effectively assess the reduction of the lignin in the solution after the ultrafiltration step. Crucibles with sand were prepared, and placed in the oven at 750˚C for 4h. Then 20 mL of solution was added, and placed in the oven at 105˚C overnight for determination of the total solids content. Then the crucibles were placed again at 650˚C for 6h for determination of the ashes content. Organic solids content is determined by the difference between total solids content and ashes.

4.3 Results and Discussion

4.3.1 Initial compositions for the different experiments

The solution (permeated solution from the 5 kDa membrane) being fed to the column had a total solids composition of 129.4 g/L, of which 18.3% (23.5 g/L) were organics, and 81.7% (105.8 g/L) in ashes. A significant decrease in the organic solids contents was due to the ultrafiltration step since the original organics content of the mixture subjected to oxidation was 60 g/L of lignin and 80 g/L of sodium hydroxide. The increase in the inorganics composition was due to the addition of sulfuric acid during the acidification, previous to the ultrafiltration. The ultrafiltration ended with a pH of 9.41.

The first experiment performed was the breakthrough experiment, to assess the duration of the feeding phase, to be used in the other experiments performed to study the effect of the pH in the fractionation of the phenolic compounds. For the experiments at different pH values, the pH of the oxidation mixture was first adjusted to the desired value with sulfuric acid and sodium hydroxide and was then allowed to stabilize overnight. After resting overnight, the pH of the feeding solution was measured again. Since the experiments were performed at different dates, some decomposition of the monomers in the mixture was verified. Although multicomponent adsorption varies with composition, the aspect that is being studied is not the interaction between components but instead the fractionation reached of the families of compounds between both desorption steps performed, the first with deionized water, the second with ethanol. The initial feed concentrations for each experiment are given in Table 2. Afterwards, the different mixtures were fed to the SP700 resin bed.

Table 4-2 – Feed concentrations for the adsorption experiments performed at pH values between 9 and 12. Values are averages of 3 samples.

Compound	Concentration, g/L				
	Breakthrough pH = 9	pH = 9	pH = 10	pH = 11	pH = 12
H	0.014 ± 0.001	0.015 ± 0.001	0.014 ± 0.001	0.011 ± 0.003	0.011 ± 0.001
VA	0.152 ± 0.005	0.151 ± 0.006	0.165 ± 0.012	0.153 ± 0.009	0.146 ± 0.002
V	0.138 ± 0.006	0.114 ± 0.005	0.056 ± 0.003	0.095 ± 0.007	0.101 ± 0.006
SA	0.170 ± 0.006	0.155 ± 0.008	0.142 ± 0.009	0.155 ± 0.004	0.154 ± 0.010
S	0.103 ± 0.007	0.057 ± 0.003	0.073 ± 0.004	0.017 ± 0.002	0.026 ± 0.003
VO	0.038 ± 0.003	0.036 ± 0.002	0.040 ± 0.002	0.037 ± 0.003	0.036 ± 0.002
SO	0.037 ± 0.002	0.033 ± 0.002	0.035 ± 0.003	0.033 ± 0.002	0.034 ± 0.000

H – *p*-hydroxybenzaldehyde, VA – vanillic acid, V – vanillin, SA –syringic acid, S – syringaldehyde, VO – acetovanillone, SO – acetosyringone.

4.3.2 Typical chromatogram of feed solution

In *Figure 4-1*, it is depicted the chromatogram of the feed mixture used in the experiment at pH 12. A total of seven monomer phenolic compounds are quantified, representing the monomeric species in higher concentration in these oxidation mixtures; by order of elution, *p*-hydroxybenzaldehyde (H), vanillic acid (VA), vanillin (V), syringic acid (SA), syringaldehyde (S), acetovanillone (VO), and acetosyringone (SO). There are also other unidentified monomer phenolic compounds present in lower amounts, as well as lignin fragments or oligomers that were formed during the oxidative depolymerization and have permeated the 5 kDa membrane.

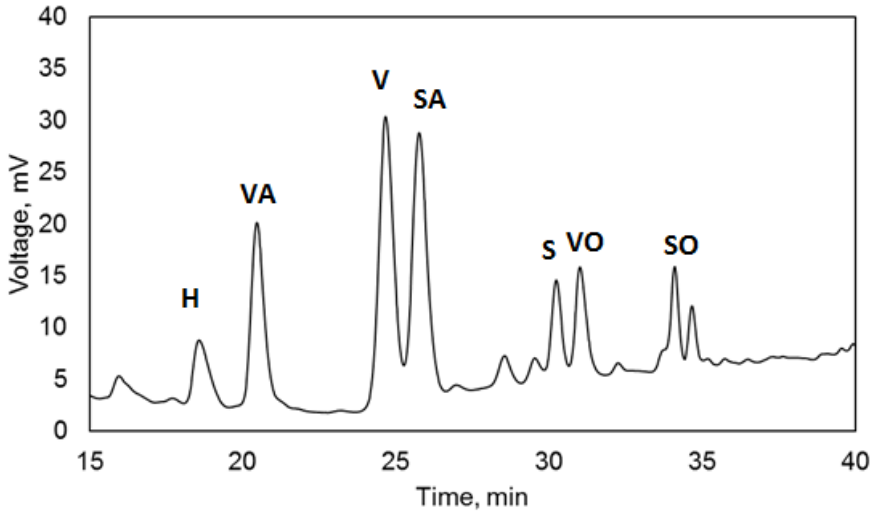


Figure 4-1 – Typical HPLC chromatogram from the feed mixture. H – p-hydroxybenzaldehyde, VA – vanillic acid, V – vanillin, SA – syringic acid, S – syringaldehyde, VO – acetovanillone, SO – acetosyringone.

4.3.3 Breakthrough Experiment

In order to study the adsorption of the real mixture in a column packed with SP700 resin, a breakthrough experiment was performed and is shown in Figure 4-2. The feed step of the mixture was extended long enough so that the outlet concentration of the 7 compounds studied matched those of

the feed solution. The feeding step was extended up to 8.5 h during which a total volume of 5.080 L of real mixture was fed to the column. As can be seen in Figure 4-2, the first species to elute from the column are the vanillic (VA) and syringic (SA) acids, followed by the aldehydes *p*-hydroxybenzaldehyde (H), vanillin (V) and syringaldehyde (S) and at last the ketones acetovanillone (VO) and acetosyringone (SO).

The acids VA and SA are detected first in samples collected at 0.42 h and 0.55 h respectively. At 2.5 h of feed, the first studied aldehyde, H, is also detected at the outlet of the column. At 2.58 h of feed, traces of V are detected, and S is detected for the first time at 3.75 h. The time at which the aldehydes start to elute, approximately at 3h of feed, is used later as reference for the adsorption experiments at different pH values. The ketones are detected first at 4.02 h of feed.

At 7.64 h of feeding, the concentrations of the different compounds are equal to the feed solution and it can be considered that for the seven compounds studied, equilibrium was reached inside the adsorption column.

The roll-ups are very pronounced in the case of the aldehydes and ketones. These roll-ups indicate that among the feed mixture with many compounds there are other unknown species also being adsorbed and causing the displacement of the already adsorbed species. The resin shows more affinity for these other unknown species and gradually, in the adsorption sites, species with less affinity are replaced with species with more affinity [27]. The studied compounds continue to be displaced until the equilibrium is reached inside the column and the concentration returns to the value of the feed solution. In the case of the acids, VA and SA, overshoots were also observed but to a much less extent since they are less strongly adsorbed. This would be attributed to the fact that due to their lower pK_a values, 4.42 for VA and 4.34 for SA [21], they are almost completely in their deprotonated state and therefore are less strongly adsorbed. The other compounds are partially deprotonated since they have pKa values near the pH of the experiment and for that reason are more strongly adsorbed. There is a need of identifying the compounds that bind more strongly to the adsorbent and that are displacing the ketones, but that was not the scope of the present work. An analysis technique like GC-MS might be useful for the identification of other monomers and dimers that might interfere with the separation.

Zabkova *et al.* (2006) [11], in experiments with pure vanillin solutions reported that that the deprotonated form of vanillin was not adsorbed in the SP206 resin, used in their study. More recently, it has been referred elsewhere [16] also that no adsorption of sodium salts of these phenolic compounds was observed. The experiments at higher pH performed in this work show the opposite, these ionic forms of the phenolic compounds can be retained by the resin SP700. At best, it can be inferred that the phenolic salts are less adsorbed in the nonpolar resins at higher pH, due to the fact that some species are deprotonated/ionized but there is adsorption. While the samples are being

prepared for analysis, they are acidified and diluted so that ionic forms of the phenolic compounds are transformed to their respective non-ionic forms and the compounds are quantified properly in their protonated form. Operating in acid medium, where species would not be deprotonated would result in faster saturation of the column due to the adsorption of more species, for instance the vanillic and syringic acids, and thus shorter breakthrough times.

During the overshoots, the concentration of aldehydes H and V reached approximately 2 times the feed concentration, while S reached 2.5 times the feed concentration. The ketones VO and SO reached approximately 3 times the feed concentration. Also worth mentioning, is the order at which the guaiacyl derived compounds (VA, V, VO) elute from the column which is always before the syringyl derived compounds (SA, S, SO). This could be partially explained by the different affinities of the compounds for the active sites of the resin and also by the fact that guaiacyl derived compounds are present in higher concentrations in the feed mixture, with the exception of SA which is somewhat higher than VA.

Depending on the concentration of the species, the breakthrough curves will be different, obviously, due to the adsorption of various other compounds. There are many compounds in the real mixture other than the seven ones studied that are not analyzed as observed in the chromatogram presented in *Figure 4-1*. The species that permeate the 5 kDa membrane are comprised of molecules of different sizes due to the incomplete depolymerization of the macromolecular structure of lignin like lignin oligomers. It should be noted that oligomer structures not identified might also be adsorbed in the nonpolar resin, decreasing the available adsorption sites for monomer recovery [28-31].

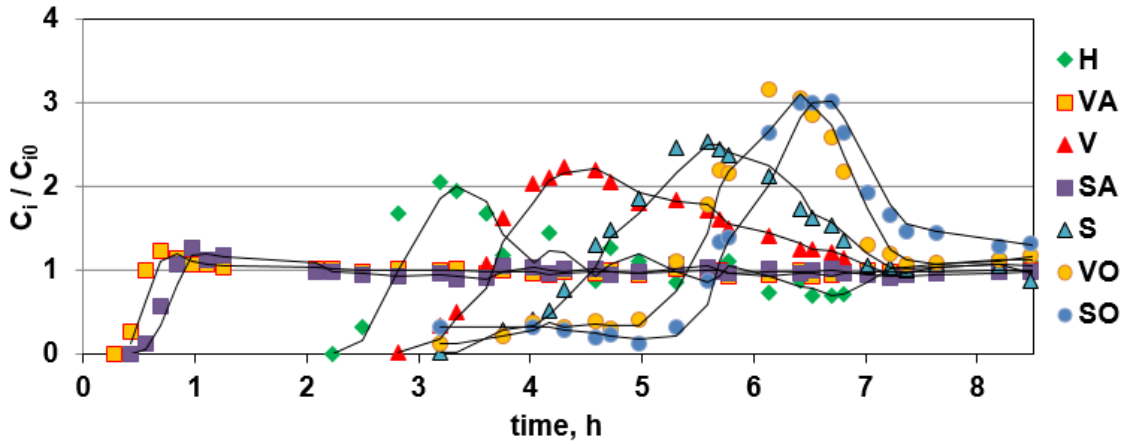


Figure 4-2 - Representation of the normalized concentration histories of the compounds studied in the breakthrough experiment. pH of the feed: 9.21. $\bar{t}_r = 0.15\ h$, where $\bar{t}_r$ is the mean liquid residence time $\bar{t}_r = \frac{\varepsilon V_{bed}}{Q_L}$. $T = 25$°C, $Q_L = 10\ ml/min$. H – *p*-hydroxybenzaldehyde, VA – vanillic acid, V – vanillin, SA – syringic acid, S – syringaldehyde, VO – acetovanillone, SO – acetosyringone

4.3.4 Comparison of the concentration histories at different pH values

Concentration histories of the experiments performed at pH 9, 10, 11 and 12 are presented in Figure 3. The experiments contain 3 different steps: adsorption, region A; desorption with deionized water, region B; and desorption with ethanol, region C.

During the first 3h of adsorption (Figure 3, regions A), or feed, the concentration histories of all experiments are similar and only the acids VA and SA eluted from the column, and around the interval of θ = 2.5 to 3, VA and SA reach the respective feed concentrations. This indicates that the acids are practically not being adsorbed while the other compounds are being adsorbed. After 3h of feed, a desorption step with deionized water is initiated and during this phase, the acids leaving the column reach null concentrations and the aldehydes adsorbed start to desorb and to elute (region B).

During the desorption step with deionized water, the differences due to the variation of pH value of the feed mixture become evident. Selective elution was not achieved by varying the pH of the feed during the adsorption step. During the adsorption steps, pH was fixed and only between each experiment the pH varies. Selective elution is provided by the desorption of water (which desorbs less strongly adsorbed species) and ethanol (which desorbs the remaining strongly adsorbed species that were not desorbed in the water desorption step). At the lowest pH tested, 9, the aldehydes are not completely desorbed with water. This might be attributed to the fact that more aldehydes are in their protonated form causing them to be more strongly adsorbed in to the resin. For instance, at pH 9, the vanillin (V) is not desorbed completely, much like for the syringaldehyde (S). At pH value 10, the aldehydes are mainly desorbed in the water desorption step and for higher pH values the aldehydes are completely desorbed in the same water desorption step.

The desorption with ethanol is also indicative that the modification/adjustment of the pH of the feed mixture influences the fractionation of the complex mixture. It was observed that the higher the pH, the lower were the desorbed amounts of ketones in the ethanol desorption step. On the other hand, a decrease in the pH is able to dislocate the ketones from the water desorption step to the ethanol desorption step. For instance, at pH 12 and 11, the two ketones, of the compounds followed, are present exclusively in the ethanol fraction (*Figure 4-3*, pH = 11 and 12, regions C) while all of the aldehydes were completely removed in the previous step of desorption with water (*Figure 4-3*, pH = 11 and 12, regions B). At pH = 10 there is still good fractionation of the aldehydes from the other families of compounds with only trace amounts of syringaldehyde being lost (*Figure 4-3*, pH = 10, region C).

This set of experiments confirmed the fact that the phenolic acid species, when operating at alkaline conditions, are not strongly adsorbed, similarly to what happened during the breakthrough experiment. Operating at pH 11 has the additional advantage of separating the aldehydes, V and S,

that are mainly collected in the water desorption step, from the respective ketones, VO and SO, which are mainly obtained in the ethanol desorption step. Therefore, besides the advantage of fractionating the acids by operation at alkaline conditions, tuning the adsorption pH can be important to further fractionate the aldehydes from the ketones during adsorption/desorption with a nonpolar resin.

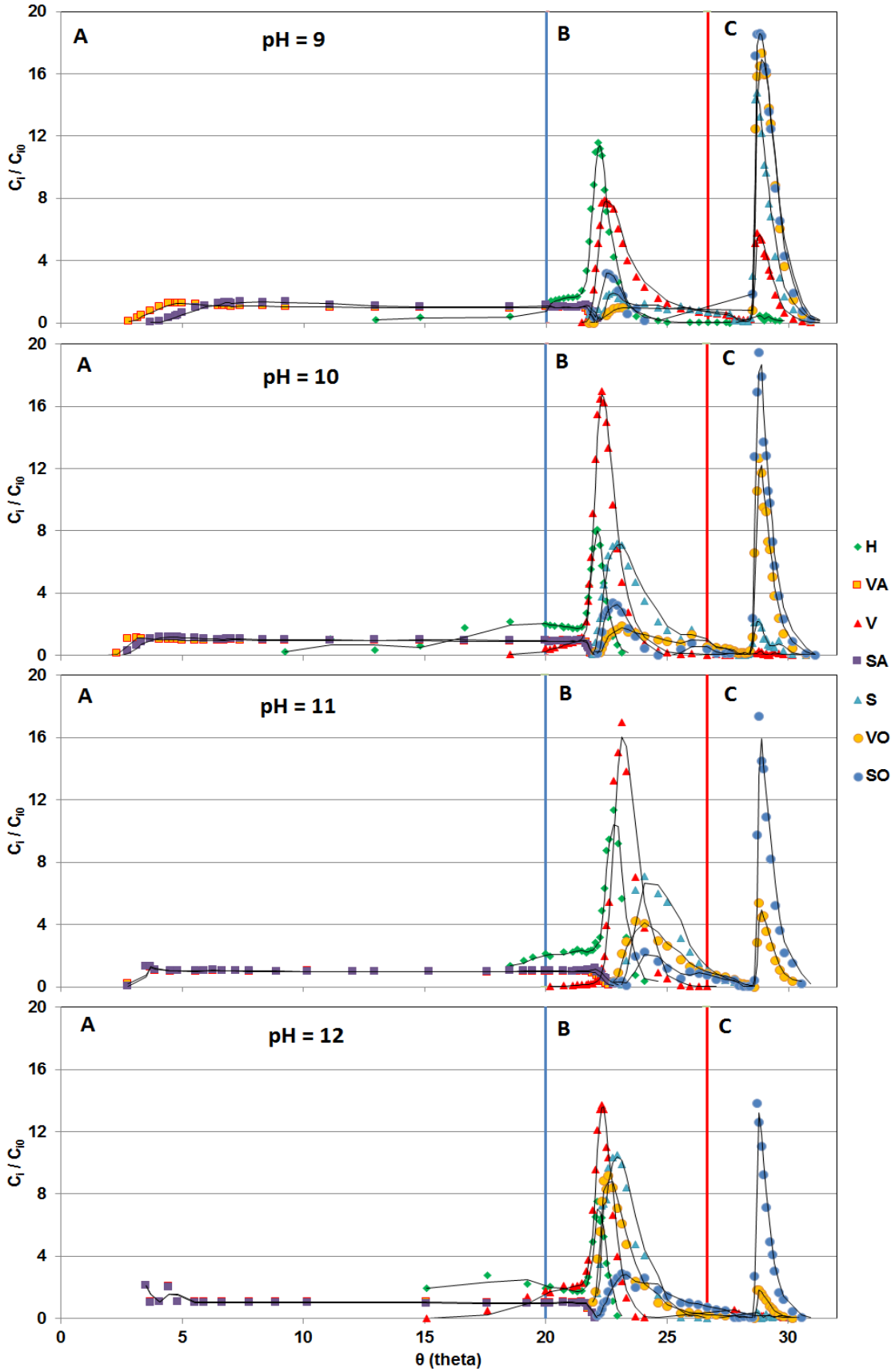


Figure 4-3 - Adsorption and desorption normalized concentration histories of the experiments conducted at pH = 9 (top) and pH = 11 (bottom). A – feed step, B – deionized water desorption step (starts at blue line), C – ethanol desorption step (starts at red line). $\theta = t/\overline{t}_r$, $\overline{t}_r = 540\ s$. $T = 25$℃, $Q_L = 10\ ml/min$, column of 446 x 26 mm and porosity of 0.37. Feed concentrations presented in Table 4-2.

4.3.5 Representation of acids, aldehydes and ketones separately

The three families of phenolic compounds desorbed are presented in *Figure 4-4* in separate graphics to further illustrate the degree of fractionation reached. The experiment at pH 10 is presented with detail of the different phases of feed (first eluate) plus desorption with water and desorption with ethanol individually.

First the aldehydes are presented (*Figure 4-4*, top). At the working pH presented, 10, the majority of the aldehydes, V, S and H, is desorbed with deionized water. Only a remaining small amount of S is desorbed in the ethanol desorption step along with traces of V. Also the ketones (*Figure 4-4*, middle) VO and SO are preferably desorbed during the ethanol desorption step, with very small amounts of these being desorbed during the water desorption step. The acids (*Figure 4-4*, bottom) VA and SA are removed from the mixture earlier during the feeding step since these are much less adsorbed. The ethanol fraction obtained has no acids, and also the water desorption fraction has very low concentration of acids compared to the initial phenolic acid loading. The water desorption fraction can be fed again to the chromatographic column to further remove the remaining acids as well as the ketones, leaving, ideally, a water fraction containing only the aldehydes.

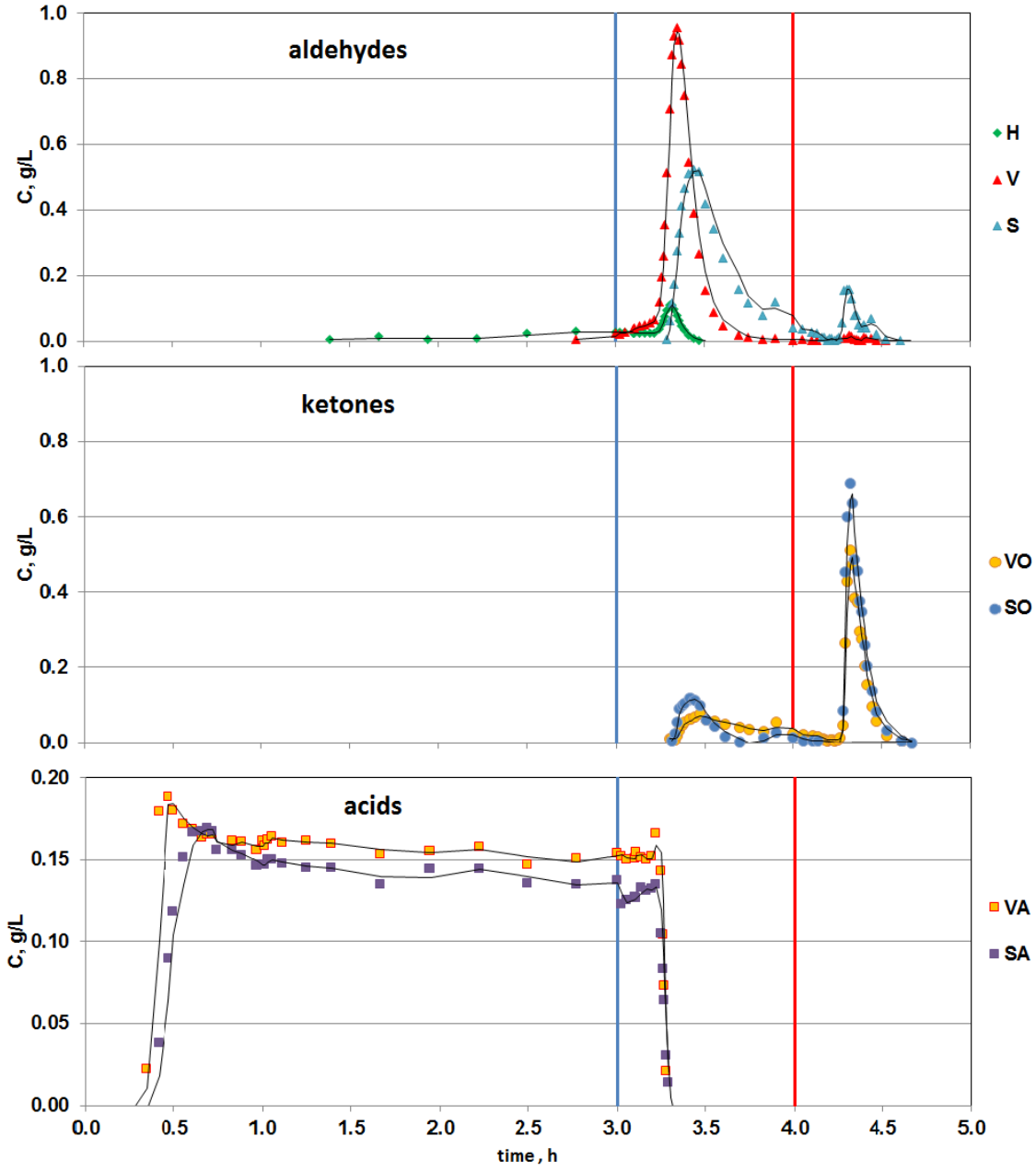


Figure 4-4 – Representation of the concentration histories at the column outlet in detail for the aldehydes (top), ketones (middle) and acids (bottom). Blue line – start of desorption with deionized water, red line – start of desorption with pure ethanol. Conditions: feed volumetric flow rate: 10 mL/min, pH of feed mixture: 10, column jacket temperature : 25°C, H – p-hydroxybenzaldehyde, VA – vanillic acid, V – vanillin, SA – syringic acid, S – syringaldehyde, VO – acetovanillone, SO – acetosyringone. Note the different scale used for the acids.

4.3.6 Comparison of the recoveries at different pH values

The biggest difficulty lies on the separation of the aldehydes from the respective ketones. The acids VA and SA are left out since their recovery is easily made. During the adsorption phase, fractions containing only these two acids can be recovered because aldehydes and ketones are being adsorbed. Once again, other unknown aromatic acids might be eluting during this step along with VA and SA. Also, *p*-hydroxybenzaldehyde (H) was left out since it is present in very low amounts compared to the other compounds monitored.

In Figure 4-5, it is shown the degree of recovery of the compounds V, S, VO and SO in the deionized water and ethanol desorption steps. Additionally, in Figure 4-6, the fractionation reached between each desorption step at a specific pH is presented for aldehydes (V+S) and ketones (VO+SO) recovered. The set of experiments performed is able to illustrate the differences that occurred during both desorption steps due to the influence of the pH. The experiments covered the pH values of 9, 10, 11 and 12. From the analysis of the summary bar graphs in Figure 4-5 and Figure 4-6 it is possible to observe the following:

- For vanillin (V), the recovery is always higher than 80% at pH values above 10. Additionally, at these high pH's (from 10 to 12, the recovered amount of vanillin decreased in the ethanol desorption step: at pH 9 the recovery of V was 26% but then, only traces of vanillin were detected at higher pH values. The majority of vanillin is recovered in the step of desorption with deionized water.
- For syringaldehyde (S), also starting at pH 10 the majority of the aldehyde is recovered in the water phase. At pH 9, the syringaldehyde is strongly adsorbed and therefore, it is desorbed at a higher extent later with ethanol. The same type of behavior as observed for vanillin.
- For acetovanillone (VO), the transition from pH 9 to 10 is not so outstanding as in the case of V and S but still a higher displacement of the ketone to the ethanolic phase occurs. The lowest recovery of VO is in the water desorption step, for pH 9 with the majority of the VO being desorbed on the following step with ethanol. On the other hand, if the feed is at pH 12, the ethanol desorption phase has a very low recovery of VO because the majority of its desorption occurs in the water desorption step.
- Acetosyringone (SO) is recovered to a higher extent in the ethanol desorption step for the lowest pH tested, 9. As it was observed for VO, SO has its highest recovery in the ethanolic phase. Additionally, SO is the only compound with high recovery in the ethanolic phase (over 40%) when working at pH 12.

It was possible to observe that according to the pH of the experiment different and interesting outcomes can be obtained and that the pH can be chosen according to the desired separation. Moreover, the purification stage to perform afterwards will also be a factor which needs to be taken into account for the selection of the working pH. For instance, if the separation of the aldehydes from the ketones is desired in a first phase, then it is preferable to operate at the pH of 10 since the fractionation of aldehydes is almost complete, these being recovered in the step of desorption with deionized water. Although at pH 10 some traces of syringaldehyde (S) are present in the recovered ethanol phase, the amount of ketones removed in the ethanol desorption step is considerable making this pH more suitable if the increase in fractionation is worth the loss or contamination by aldehydes. If vanillin is the sole compound in interest, pH 10 can also be utilized since all of the vanillin is desorbed in the water desorption phase, and at the same time the recoveries of the ketones VO and SO are still high in the ethanol phase, thus increasing the fractionation of these two families.

If the purpose is to concentrate the mixture of aldehydes and ketones higher pH values should be used. In the experiment with feed mixture with pH value 12, all of the compounds achieved great recoveries in the water desorption step, the majority of all compounds are recovered in the aqueous phase which in turn can be subjected to other processes but at higher concentrations thus avoiding excessive costs for the acidification step. Given the results obtained, operating at higher pH like 13 or 14 would make the ketones family to desorb at the same time as the aldehydes family, but since maximum fractionation between families was the objective these pH values were not tested.

The separation of the phenolic acids VA and SA from the feed mixture was always achieved in the range of pH used. The majority of the acids is recovered in the feed phase since they are not strongly adsorbed. Although the desorption step with deionized water still has some contamination by acids (*Figure 4-4*, acids, region B), these can be removed in a second step of adsorption of the same type since they are in aqueous solution. These acids leave the column in the step of desorption with deionized water in the totality since no traces of acids are detected later in the ethanol desorption step. These phenolic acids (VA and SA) can be recovered from the aqueous fraction that leaves the column during the feeding step. Two of the ways that this can be achieved would be by acidification of the solution and adsorption in the same type of resin, or by means of ion exchange chromatography (anion) without acidification, but alkaline solutions would be needed for regeneration.

Although the results are preliminary, an industrial process to recover/concentrate the molecules with highest added-value can be envisioned. This knowledge would benefit the implementation of a biorefinery plant where processes need to be well integrated and maximum valorization of all compounds/streams must be attained. If the recovery of vanillin and other aldehydes is made almost entirely in the desorption with deionized water, this aqueous phase can then be easily submitted to another similar adsorption step so that the aldehydes are further purified and obtained

again in an aqueous matrix. On the other hand, the presence of ethanol will not be beneficial for a purification stage with polymeric resin since the ethanol will compete for the available sites of the resin. It has already been shown in previous work [13] that the adsorption of V and S is greatly diminished in the presence of ethanol which is one of the reasons why it is used in desorption. The most important reason to use pure water and ethanol was to simplify the process as much as possible while utilizing environmentally friendly eluents. Other reasons to seek utilization of pure eluents is the ease of recycling by simple evaporation.

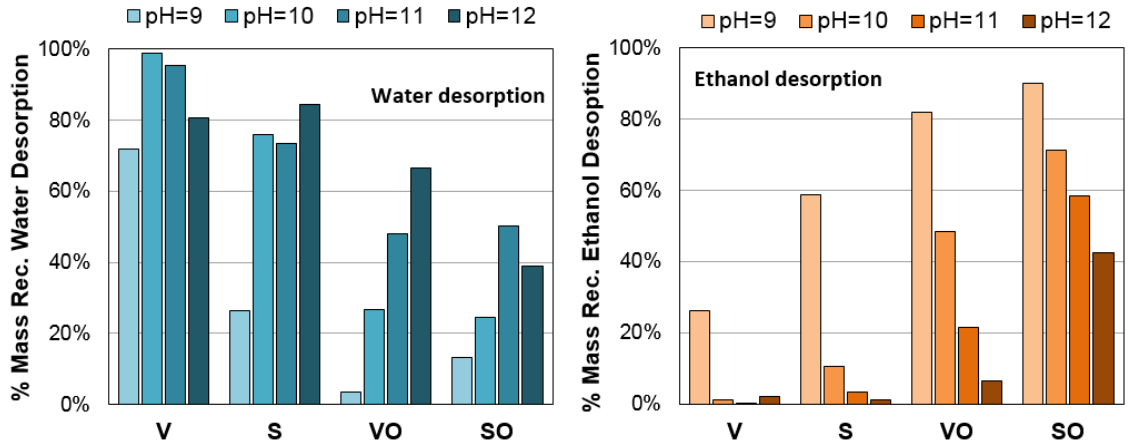


Figure 4-5 - Comparison of the recoveries in the set of experiments at different pH values from 9 to 12. Recoveries in the water desorption phase (left) and recoveries in the ethanolic desorption phase (right).

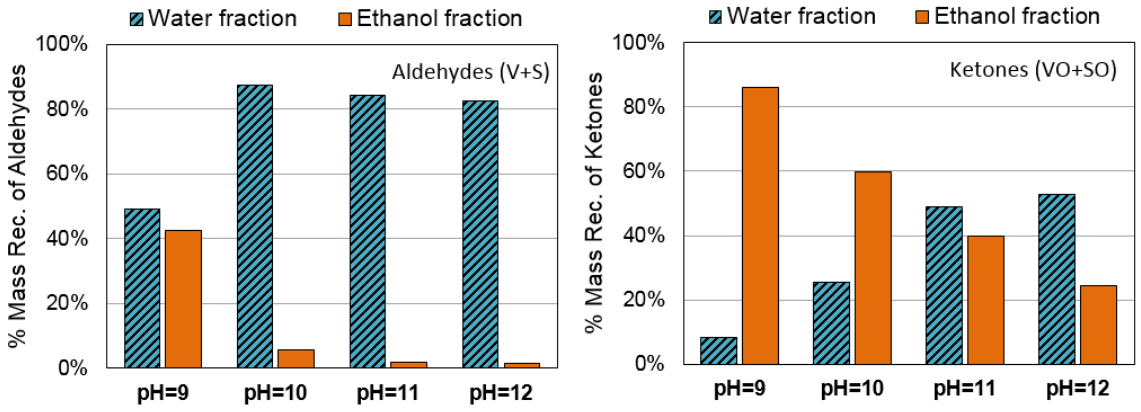


Figure 4-6 Comparison of the recoveries of aldehydes (left) and ketones (right) obtained in each desorption step (deionized water and ethanol) at each individual pH tested. V – vanillin, S – syringaldehyde, VO – acetovanillone, SO – acetosyringone.

4.4 Conclusions

Alkaline lignin oxidation mixture was successfully fractionated by adsorption with SP700 resin into families of compounds namely the acids (VA and SA), aldehydes (V and S) and ketones (VO and SO) based on a two eluent technique with deionized water and ethanol. The vanillic and syringic acids (VA and SA) were the compounds less adsorbed and leave the column first during the feed step; the aldehydes (V and S) are mainly desorbed in the step of deionized water desorption while the ketones (VO and SO), the compounds more strongly adsorbed, leave the column mainly in the step of ethanol desorption. The amount of vanillic (VA) and syringic (SA) acids present is low in the deionized water desorption step and completely absent from the ethanol desorption step.

The higher the pH, the less aldehydes (V and S) are lost to the ethanol desorption step. At pH 9 the V and S are present in the ethanol fraction recovered; at pH 10, syringaldehyde (S) is still present in the ethanol desorption step but vanillin (V) is only detectable in trace amounts; at pH 11 or 12 the aldehydes (V and S) desorb completely in the water desorption phase leaving only the ketones (VO and SO) for the ethanol desorption step. Acetovanillone (VO) and acetosyringone (SO) decrease in the ethanol desorption step as the pH increases while the ketones content increases in the deionized water desorption step.

The remaining aqueous fraction rich in aldehydes from desorption with deionized water can then be subjected to other steps of adsorption/desorption to further decrease the content of ketones in the aqueous fraction. The ethanolic fraction obtained in the ethanol desorption step can also be enriched in ketones and the ethanol evaporated to reutilize. The aqueous solution rich in acids resulting from the feeding step can also be reprocessed for concentration and purification of the vanillic and syringic acids by lowering the pH and adsorbed onto SP700 and recovered with ethanol.

Although the desorption amounts of V and S in aqueous phase increases with pH, the ketones content also increase and thus, the best compromise in pH must be found in order to obtain V and S in the aqueous fraction with the lowest content in ketones possible. A similar approach must be conducted as well in order to find out the best pH value to concentrate the ketones in the ethanolic desorption phase. The experiments performed at different pH values (9 through 12) can provide guidelines in the elaboration of a separation process where concentration of the aldehydes and ketones is desired or, in an application where separation of aldehydes and ketones is to be maximized.

4.5 References

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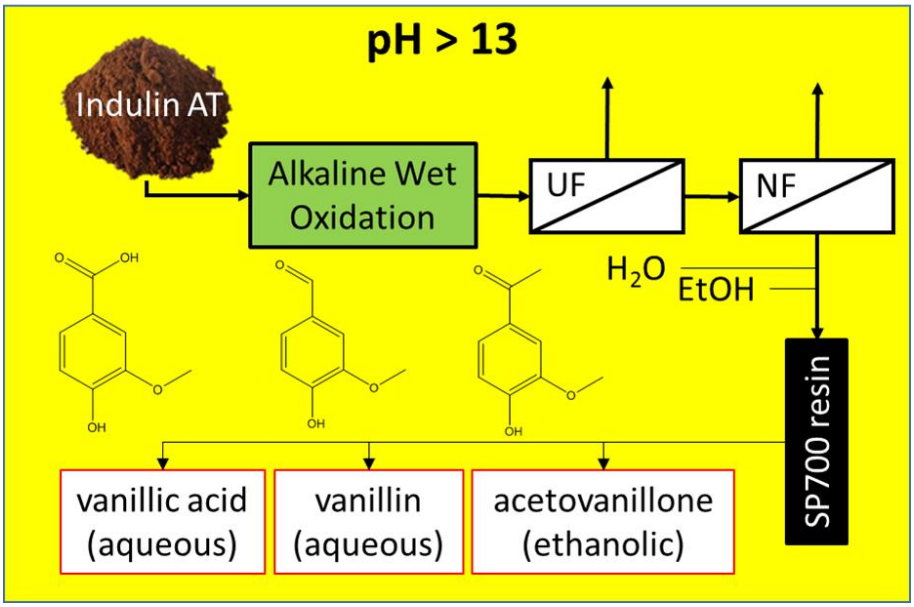
5 Lignin Biorefinery: Separation of Vanillin, Vanillic Acid and Acetovanillone by Adsorption



Vanillin (V), acetovanillone (VO) and vanillic acid (VA) were fractionated after alkaline depolymerization of lignin. The feed solution had an initial pH of 13.7 and the work was performed always under alkaline pH, reducing chemical waste. The organic charge of the solution was reduced from the initial 58.8 (±0.4) g/L to 31.0 (±1.3) g/L by membrane filtration. Permeate from the last membrane, was fed to the chromatographic column, resulting in fractions with different composition. From the total amounts fed to the column, 3.21(±0.25) g of V, 1.48(±0.10) g of VA and 0.35(±0.04) g of VO, it was possible to recover 94.1% of VA in the first eluted fraction, 96.2% of V and 43.4% of VO in the second fraction by water desorption and, the remaining VO, 52.7%, in the third fraction by ethanol desorption. Cyclic operation can be implemented without deep regeneration of the adsorbent as the 4 cycles performed revealed.

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5.1 Introduction

Lignin is an undervalued byproduct of the pulp and paper industry that due to its phenylpropane derived structure has a great potential to become a resource for platform chemicals production, suitable even to compete with petrochemicals as source of oxygen-free aromatics[1]. Figures from 2016 indicate that the dimension of the lignin market is around 1.1 million t/y for the lignin precipitated from the different pulping processes, with only 10% coming from Kraft processing [2]. Lignin present in the black liquors produced is usually burned for steam generation due to its high energy content, with higher heating values ranging from 23.3-25.4 MJ/kg [3-5]. Sometimes, limitations to the increase in annual pulp production occur due to the maximum capacity of the recovery boiler to burn the liquor. Lignin depolymerization into low molecular weight phenolic compounds (LMWPC) would result in a reduction of the heating value of the liquor enabling the burning of more black liquor and thus, an increase in pulp production [6, 7].

Lignin depolymerization strategies are diverse ranging from biodepolymerization with fungi, bacteria and laccases [8-10], or through modified/enhanced metabolic pathways in genetically engineered organisms [11]; depolymerization can also be facilitated by genetically altering the starting biomass [12-15]. On the other hand, examples of routes to depolymerization by chemicals are alkaline wet oxidative depolymerization [16-18] and acidic depolymerization [19, 20]. Nitrobenzene oxidation is efficient in depolymerizing lignin but separation of resulting products is very difficult, so instead, it is used as a reference yield for other depolymerization protocols [21-23].

The depolymerization of lignin gives several highly functionalized molecules as decomposition products. Among the most abundant ones, phenolic aldehydes vanillin (V), syringaldehyde (S) and *p*-hydroxybenzaldehyde (H), phenolic ketones acetovanillone (VO) and acetosyringone (SO), phenolic acids vanillic (VA) and syringic (SA) and other small organic acids from further aromatic ring degradation [24]. The relative amounts of each compound depend mostly on the lignin origin, for instance, the syringyl-derived moieties S, SA and SO, are generally absent in the case of depolymerized softwood lignins [25]. Indulin AT is a softwood lignin well studied with a weight average molecular weight (Mw) of approximately 3500 Da; lignin molecular weight depends greatly its origin and on the delignification process in use, in the case of Indulin AT, the Kraft process which is the most widely used process to this time [26, 27].

Fractionation of lignin in Kraft black liquors (KBL) is a known process with several fractions of different molecular weight lignins being obtained. This can be achieved by utilizing membranes with different molecular weight cut-off (MWCO) [28-30]. The alternative to membrane fractionation is solvent fractionation [31, 32]. Lignins with specific range of molecular weight can be applied to several uses including the incorporation in polyurethane foams [33] or in other polymeric materials [34].

Membranes are also being utilized to reduce the organic charge of the mixtures coming from a lignin oxidation process and to facilitate the separation of LMWPC downstream [35].

The recovery of phenolic compounds from lignin oxidation solutions remains a challenge and needs to be simplified so that lignin can be used as a viable source for highly functionalized chemicals [36]. Successful approaches to the separation/recovery of the monomers obtained after depolymerization applied non-polar resins [37], ion exchange resins [38, 39], combination of Sephadex, to remove high molecular weight lignin and silica gel, to recover monomers [40], chemical derivatizations with ammonia [41], sulfur dioxide (carbonyl-bisulfite adduct formation) [42], and octylamine (Schiff base formation) [43]. More resources on the separation of vanillin and syringaldehyde are given by Mota *et al*. [44].

Resins have been used to recover several phenolic compounds in different processes [45-47], but there is a lack of studies evaluating the recovery of phenolic compounds from lignin oxidation mixtures employing resins, with the majority of the ones available being performed with model solutions [44, 48-53]. So far, studies with ion exchange resins proved to be unable to separate phenolic acids, ketones and aldehydes [54-56]. More recently, a simulated moving bed chromatographic process for the separation of standard solutions of V and S was proposed, but failed at addressing the difficulty in obtaining the mixture of only those two aldehydes from lignin oxidation mixtures [57]; that difficulty is mainly due to the presence of lignin oligomers and other molecular structures very similar to V and S.

In this work, an alkaline lignin oxidation mixture containing the main lignin depolymerization products vanillic acid (VA), vanillin (V) and acetovanillone (VO) is treated by an adsorption process with SP700 resin after a series of filtration steps with three membranes with average molecular weight cut-offs of 5 kDa, 1 kDa and 600 Da. The filtration steps are necessary to remove non-depolymerized lignin so that it would not interfere with the separation of the monomer products. The three main depolymerization compounds, VA, V and VO are adsorbed and then fractionated in the desorption phase using a two-eluent technique (deionized water and then ethanol) previously described [37]. The acid VA is weakly adsorbed and elutes first, the desorption step with deionized water allows the recovery of the aldehyde V and the following desorption with ethanol allows the recovery of the ketone VO. A breakthrough experiment and four cycles of chromatographic fractionation were performed, so that the separation of VA, V and VO could be assessed in cyclic operation while under extreme alkaline conditions.

To the best of our knowledge there are no studies evaluating the cyclic operation of a chromatographic column, fed with alkaline solution from softwood lignin depolymerization, for the separation of vanillic acid, vanillin and acetovanillone in a simple adsorption procedure.

5.2 Experimental Methods

5.2.1 Alkaline Wet Lignin Oxidation

The initial lignin alkaline solution was prepared with 60 g/L of Indulin AT (MeadWestvaco Co.) and 80 g/L of sodium hydroxide (AkzoNobel), based on the average content of lignin in liquors [58]. This mixture was oxidized in a structured packed bubble column continuous reactor (SPBCR) previously built in our laboratory [17, 26]. Oxidation performed at an average temperature of 140^{o}C and total pressure of oxygen (pure) of 10 bar. The liquid mixture was fed at a volumetric flowrate of 3.3 L/h and the gas was fed to the reactor at a volumetric flowrate of 5 SLPM. Total duration of the continuous oxidation was 280 minutes.

5.2.2 Filtrations (Ultra- and Nanofiltration)

Filtration steps are necessary to remove the portions of lignin that did not depolymerize. All filtrations were performed in a GE Osmonics SEPA CF II filtration system that is capable of handling pressures up to 69 bar and with a useful membrane area of 140 cm^2. Filtrations are performed in recirculation mode where the retentate returns to the feed tank until a specific volume reduction factor is achieved. The order for the scheme for the series of filtrations performed was 5000 Da, 1000 Da and 600 Da. The permeate from one membrane is homogenized and fed to the next membrane. The final permeate is then fed to the next separation step, the chromatographic column. A summary of the properties of the membranes employed in this work is presented in

Table 5-1.

Table 5-1 – Properties of the polyethersulfone membranes used in the filtration sequence.

Property	Membranes		
	1st	2nd	3rd
Type	UF	UF	NF
Manufacturer	Orelis	Synder	Microdyn Nadir
Model	PLEAIDE	XT	NP030
Polymer Composition	PES	PES	PES
Max. Pressure, (bar)	10	8.3	40
Max. Temperature, (^{o}C)	50	55	95
pH range	3 to 14	2 to 11	0 to 14
MWCO, (Da)	~5000	~1000	~600

The first ultrafiltration step was performed at a pressure of 10 bar using a membrane with molecular weight cut-off (MWCO) of 5 kDa (PLEAIDE, from Orelis). The second ultrafiltration membrane has an average MWCO of 1 kDa (from Synder, bought from Sterlitech). The second ultrafiltration step was performed at a pressure of 8 bar. The membrane utilized in the nanofiltration step was a membrane with a MWCO of 600 Da, the NP030 (from Microdyn Nadir, bought from Sterlitech). This membrane is frequently applied for the treatment of water since it possesses a 30%

rejection to NaCl. The nanofiltration was performed at 30 bar. All membranes are polyethersulfone (PES) based. The system tends to produce heat particularly at higher pressures, thus temperatures were kept in the range of 25 to 35˚C with the aid of a water thermostatic bath (LAUDA, model RE 206). The pH of the feed solution was not corrected and so filtrations were performed always at pH higher than 13. Before filtrations, the membranes were compacted first with deionized water and after with 80 g/L NaOH solution under a pressure slightly above the operation pressure that is to be used (8.4 bar for PLEAIDE, 12 bar for XT and 34 bar for NP030) for 60 minutes.

Permeability tests also performed at several pressures, in the range of the operation pressure of each membrane. For each pressure tested, five measurements of volume permeated were made. Tests carried out with NaOH solution with concentration of 80 g/L and temperature was maintained at 25^{o}C with thermostatic bath (POLYSCIENCE, model 462-0228).

5.2.3 Chromatographic Separation

The experiments were performed in a simple chromatographic installation composed by a glass column (Merck–Darmstadt, diameter of 26 mm and length 446 mm), connected to a preparative chromatography pump with maximum volumetric flowrate of 50 mL/min (KNAUER, model Smartline P1000). The column was packed with 151.6 g of nonpolar SP700 resin previously activated. The jacketed column was kept at 25^{o}C by a thermostatic bath (LAUDA, model RE 206). An auxiliary UV detector (KNAUER, model Smartline UV 2500), set at 280 nm, is also connected to ensure the complete regeneration of the column. Tracer experiments performed previously at 10 mL/min allowed for the determination of a mean liquid residence time of 540 s, Peclet number of 555 and porosity of 0.37.

5.2.4 Resin activation

The nonpolar SP700 resin (resin properties in Table 5-2) was activated by performing several washes with acidified deionized water and methanol to remove all material adsorbed in the resin from origin as described elsewhere [49]. First, the resin is placed in a beaker with excess water and after settling, the floating dry resin is removed. Then, the resin is filtered with vacuum and placed in a flask with excess water and is left to stir in the orbital shaker for up to one hour. This step is repeated once more and afterwards the resin is rinsed with methanol, filtered and placed in a flask with methanol acidified with 0.1% (v/v) of formic acid and left in the orbital shaker for another hour. After this, the resin is filtered out, rinsed with water acidified with 0.1% (v/v) of formic acid, and shaken for one hour with excess acidified water. Finally, resin is rinsed several times with deionized water and is ready to be used/transferred to the column.

Table 5-2 – Physical and chemical properties of SP700 resin.

Property	Value/Designation
Resin denomination	**SP700**
Manufacturer	Mitsubishi Chem. Corp.
Matrix	Polystyrene-divinylbenzene
Specific surface area, (m^2g^{-1})	1200
Density of wet adsorbent, (gL^{-1})	1010
Moisture content, %	60-70
Pore Volume, (mLg^{-1})	2.1
Average Particle size, (μm)	450
Average pore radius, (nm)	9

5.2.5 Breakthrough Experiment and Adsorption Cycles

The interaction between the lignin oxidized mixture and the stationary phase of the chromatographic column needs to be studied by performing a breakthrough experiment. The column was fed during 8 h with the oxidized mixture (permeate from 600 Da membrane) at a volumetric flowrate of 10 mL/min. During the breakthrough experiment, after 2 h of feeding, aldehydes *p*-hydroxybenzaldehyde (H) and vanillin (V) start to elute in low concentrations; the concentration fronts of H and V are reaching the end of the column and since no V should be lost, this time interval is set to be the feed time for the cycles of adsorption performed after. The adsorption cycles are then performed by feeding the same mixture during 2 h followed by desorption steps. In total, four cycles of adsorption/desorption were performed.

5.2.6 Two-Eluent Desorption

The desorption step is divided in two phases, the first one in which deionized water is used and then ethanol (97% v/v). Water is used to desorb less strongly adsorbed species while ethanol is used to desorb more strongly adsorbed species. Both desorption eluents were fed for 1 h each, at the same volumetric flowrate of 10 mL/min, similar as the feed phase. This same desorption technique was successfully applied in our previous work where the effect of feed pH was studied [37].

5.2.7 Sampling and sample preparation

Before the filtration steps, samples coming from the oxidation step need to be prepared by solid phase extraction (SPE) due to the presence of high molecular weight lignin. SPE is performed as reported elsewhere [59]. Filtration steps (ultra and nanofiltration) remove the majority of the lignin in solution, thus, the samples collected after do not need special treatment since the dilution solvent is sufficient to reduce the pH of the sample before analysis by high performance liquid chromatography (HPLC). Samples collected at the outlet of the chromatographic column were diluted in HPLC eluent (95% water / 5% methanol, 0.1% formic acid, % (v/v)), with dilutions starting from 20 up to 2000 times before HPLC analysis. Throughout the chromatographic experiments, samples were collected at times

based on early exploratory experiments and also the information taken from the breakthrough experiment. The frequency of the sampling is increased at times when the peaks are eluting from the column. When important peaks are eluting the samples are collected every 50 seconds. A single complete experiment takes 5h and over 90 samples are generated. Along the breakthrough experiment, samples were also analyzed to check if the outlet concentration had reached feed concentrations.

5.2.8 High Performance Liquid Chromatography

Samples were analyzed by HPLC, with a reverse-phase column ACE 5 with C18-pentafluorophenyl group (250 mm x 3.0 mm, 5 μm) in a KNAUER Smartline HPLC setup equipped with a diode array detector (DAD). The eluents compositions' are water/methanol: 95%/5% (v/v) containing 0.1% (v/v) formic acid, and methanol/water: 95%/5% (v/v) containing 0.1% (v/v) formic acid. Quantification wavelength set to 280 nm (auxiliary detection wavelengths also obtained at 225, 260 and 300 nm). The development of the HPLC analysis was reported elsewhere [27].

5.2.9 Total solids content

The reduction of dissolved lignin concentrations is assessed by total solids content analysis. Crucibles with sand were prepared and placed in the oven at 750˚C for 4h to remove water. 20 mL of solution to be analyzed is then added, and crucibles are placed in the oven at 105˚C overnight for determination of the total solids content. The crucibles undergo calcination at 650˚C for 6h for the determination of the ashes content. Organic solids content calculated as the difference between total solids content and ashes.

5.3 Results and Discussion

5.3.1 Oxidation of Indulin AT in the continuous reactor

Alkaline lignin solution was oxidized in the continuous structured packed bubble column reactor. The oxidation without catalyst was able to depolymerize the lignin into monomer phenolic compounds, the most representative being vanillin (V), vanillic acid (VA) and acetovanillone (VO) (or apocynin). Samples collected at the reactor outlet were analyzed and the concentration histories of the three major compounds found were obtained, presented in Figure 5-1. For the set of conditions used, maximum yields were reached at 120 min of oxidation.

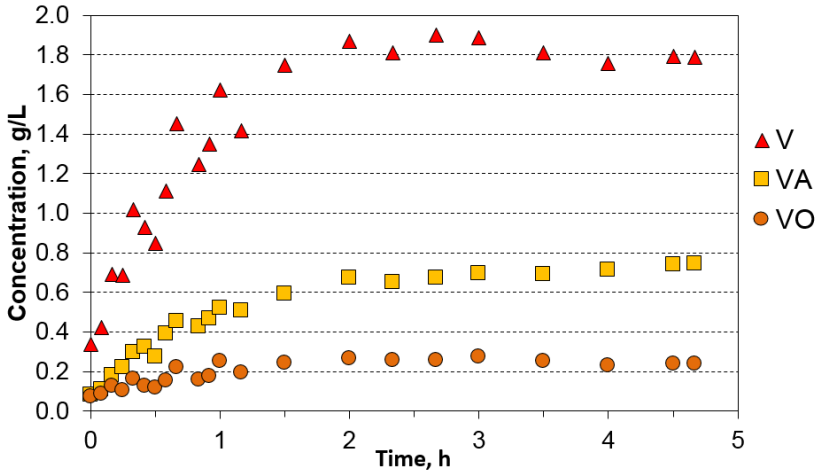

Figure 5-1 Concentration histories of vanillin (V), vanillic acid (VA) and acetovanillone (VO) at the continuous reactor outlet for the oxidation of Indulin AT. Lignin concentration = 60 g/L; Sodium hydroxide concentration = 80 g/L; T = 144˚C, P = 10 bar; pH = 13.7; liquid volumetric flow rate = 3.3 L/h; gas volumetric flow rate = 5 SLPM; gas composition = 100% O_2.

The increase in concentration resulting from the oxidation is evident in Figure 5-1. At steady state, the concentration of V increased over 18 times, from 0.101 (±0.006) g/L to 1.817 (±0.051) g/L, VA concentration increased 4.7 times, from 0.146 (±0.002) g/L to 0.698 (±0.033) g/L and VO concentration increased 6.9 times from 0.036 (±0.002) g/L to 0.252 (±0.015) g/L.

The vanillin yields obtained in this work from the oxidation of Indulin AT lignin are compared with past experiments in the same reactor and also with oxidations performed in the batch reactor in Table 5-3. The best yield of vanillin obtained by Araújo *et al* [26], in oxidations performed in the same continuous reactor (SPBCR) was 1.1% on a lignin basis, which translates to a maximum concentration of vanillin of 0.65 g/L at steady state operation. For comparison, the works of Araújo *et al.* [18] obtained vanillin yields of 3.3% on a lignin basis, for the same type of lignin, with vanillin concentration peaks in the reactor reaching 2 g/L in the batch reactor (BÜCHI). In the continuous reactor (SPBCR), the conditions of pressure and temperature are similar, the major limiting factor affecting the vanillin yields in the continuous reactor is the lack of agitation or the existence of limitations to the oxygen mass transfer as Araújo *et al.* pointed out [26]. In this work, the gas volumetric flow rate was significantly increased while, at the same time, the gas composition was changed to pure O_2 feed. This led to an increase in the yields of vanillin when compared to previous work and the decrease of the differences between the maximum experimental yields obtained in the batch reactor. Nitrobenzene oxidation usually gives maximum reference yields for vanillin from Indulin AT around 10% on a lignin weight basis [60], so there are still improvements to be made to the oxidation conditions, especially at the gas-liquid mixing level.

Table 5-3 – Comparison of yields obtained from the oxidation of Indulin AT lignin with previous oxidations in the same reactors.

Reactor	T, ˚C	p_{total}, bar	Q_G, SLPM	O_2 comp., % (p_{O2}/p_{total})	Q_L, L/h	agitation, rpm	[V], g/L	$yield_V$, % ($w_{vanillin}/w_{lignin}$)	Ref.
Batch	123	9	-	~44%	-	1100	2.22	3.7%	[26]
	123	9.4	-	~68%	-	1100	2.04	3.4%	
Continuous	140	10	2	50%	2.12	-	0.65	1.1%	
	140	10	5	100%	3.3	-	1.8	3.0%	this work

Since lignin can be adsorbed in polymeric adsorbents with PS-DVB matrix [61], The oxidized lignin solution is then submitted to the series of three membrane filtrations so that the lignin content is reduced and interference from the adsorption of lignin in the chromatographic step is minimized.

5.3.2 Filtration Series

Permeability of the membranes was tested with NaOH 2M solution. Results of the permeability test for each membrane presented in Figure 5-2. From the three membranes tested, the ultrafiltration membranes have very similar permeability, although PLEAIDE withstands slightly higher operating pressures thus allowing higher fluxes. PLEAIDE membrane has a permeability to the alkaline solution of 27.02 L $m^{-2}h^{-1}bar^{-1}$, the XT a permeability of 25.72 L $m^{-2}h^{-1}bar^{-1}$, and the NP030 a permeability of 1.43 L $m^{-2}h^{-1}bar^{-1}$. The permeability of the nanofiltration membrane, NP030, is very small when compared with the ultrafiltration membranes, as expected

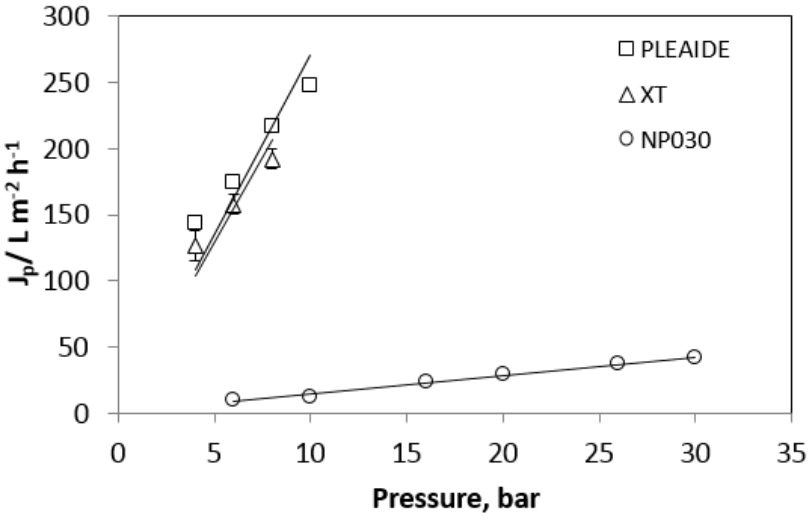

Figure 5-2 – Permeability test to the membranes used in the reduction of the lignin charge. Test performed at 25˚C.

The alkaline oxidative depolymerization is able to break lignin into the desired low molecular weight phenolic compounds (LMWPC) but also gives origin to oligomeric lignin structures. These lignin structures have molecular weights between the initial lignin and the molecular weight of the aromatic monomers, typically around 150 g/mol. Preferably, the solution going to the chromatographic column would contain only the LMWPC but this is not possible since current depolymerization approaches are incapable of complete depolymerization. Either due to *i*) limitations to the oxygen mass transfer [26,

62] or, *ii*) the degree of condensation and presence of strong covalent bonds between phenylpropane units (β-5' and 5-5'), which are more resistant to breakage than the ether β-O-4' linkages [63, 64].

In order to minimize the quantity of the oligomeric structures in solution filtration is applied. A sequence of three membranes was employed to reduce the amount of oligomeric lignin in solution. The sequence is composed of three steps of filtration with MWCO of 5000, 1000 and 600 Da. A summary of the conditions involved in each step are presented in Table 5-4.

Table 5-4 – Filtration sequence conditions.

Parameter	Membrane steps		
	1st	2nd	3rd
Average Operation Pressure, (bar)	10	8	30
Average Operation Temperature, (ºC)	29.0	29.6	29.1
Maximum Permeate Flux, (L h^{-1} bar^{-1} m^{-2})	131.12	5.21	0.80
Minimum Permeate Flux, (L h^{-1} bar^{-1} m^{-2})	95.95	0.41	0.08
Final Volume Reduction Factor (VRF)	15.53	4.42	8.72
Experiment Duration, (h)	19.0	118.9	123.72
Permeated Volume, (L)	17.0	13.9	12.7

The change in organic solids content over the three steps of filtration is represented in *Figure 5-3*. Both the evolution of the permeated and retentate fraction are given. Permeates had a noticeable decrease of the organic solids at each step of filtration. In general, this suggests that the higher molecular weight lignin structures are being retained and removed in the retentates along the sequence of filtration. The increase in the organics content of retentate fractions was then expected. The retentate organic solids content is highly dependent on the VRF reached at the end of the ultrafiltration; values presented in Table 5-4. These polyethersulfone membranes, when in alkaline medium, are negatively charged, thus the charge repulsion to negatively charged molecules will play a major role in separation since also lignin at high pH is negatively charged; but it is also highly dependent on the MWCO of the membrane and to a less extent molecular shape and hydrophobicity [65].

The membrane with highest MWCO, the 5 kDa membrane, does not have a significant impact on the retention of lignin that did not depolymerize since the organic content remains unchanged, 58.8 (±0.4) g/L before and 56.0 (±1.3) g/L after ultrafiltration, which is also very close to the organic solids content in the retentate, 62.8 (±1.0) g/L. This is in accordance with the Indulin AT weight average molecular weight, which is lower than the MWCO of 5 kDa of this membrane (PLEAIDE from Orelis). The membrane showed a minimal decrease in the flux permeate from the initial 131.12 $Lh^{-1}bar^{-1}m^{-2}$, to a final permeate flux of 95.95 $Lh^{-1}bar^{-1}m^{-2}$, which is indicative of a very small retention of lignin and thus a 27% membrane permeability decrease. In addition, this is reflected in the ultrafiltration time which was concluded in 19 h (continuous), after 17 L of permeate were collected. The results obtained with this membrane with MWCO of 5000 Da, indicate that the exclusion of this membrane, for the particular case of Indulin AT depolymerization mixtures, from the sequence of filtration will have

minimal impact on the next membrane filtration step. This membrane might only be useful in cases where a pre-filtration stage is necessary for the removal of fines (cellulose fibers, small-undigested wood chips). The application of membranes with higher MWCO is not beneficial; in this case, it would only increase process costs.

The membrane with intermediate MWCO, the 1 kDa membrane, had a higher impact on the fractionation of the high molecular weight structures. The decrease on the organics solids content is clear, from 56.0 (±1.3) g/L in the feed to 36.8 (±1.8) g/L in the permeate, but also the increase on the retentate, the concentration on organic solids content more than doubled, from 56.0 (±1.3) g/L in the feed to 128.3 (±8.7) g/L. In this ultrafiltration step (with membrane XT from Synder), the permeate flux had a more significant decrease, from the initial 5.21 $Lh^{-1}bar^{-1}m^{-2}$, to a final permeate flux of 0.41 $Lh^{-1}bar^{-1}m^{-2}$, accounting for a 92% decrease in flux permeation. This reduction in permeate flux together with the reduction in organic solids indicates that this membrane is more suitable for the retention of the lignin structures in solution than the previous one. The higher flux reduction/retention of lignin by this membrane is also reflected in the duration of this ultrafiltration step that lasted approximately 119 h (continuous), during which 13.9 L of permeate were collected.

The last membrane with lower MWCO, the NP030 from Microdyn Nadir, was able to reduce the organic solids load even further, from the feed to this nanofiltration step with 36.8 (±1.8) g/L to 31.0 (±1.3) g/L in the permeate. Again, the retentate had its organic solids content increased from the same feed with 36.8 (±1.8) g/L to 152.1 (±14.5) g/L. This nanofiltration step had a duration of about 124 h (continuous), and 12.7 L of permeate were collected. Permeate flux was very low with initial value of 0.80 $Lh^{-1}bar^{-1}m^{-2}$, and final value of 0.08 $Lh^{-1}bar^{-1}m^{-2}$, accounting for a 90% reduction on the permeate flux. This membrane has been applied successfully in the separation of phenolic acids from monosaccharides (sugars) [66] but also in the fractionation of polysaccharides and monosaccharides [67] and concentration of polyphenols from jussara ethanolic extract and artichoke brines [68, 69], showing that this membrane is able to reject, in practice, dimers of guaiacyl units. Werhan *et al.* was successful in using membranes in this MWCO range to fractionate monomers and dimers from a model solution in an organic solvent nanofiltration step while studying the acidic depolymerization of lignin [35].

The evolution of the concentration in organic solids (Figure 5-3) is useful to highlight which membrane had the highest impact on the removal of organic content form the mixture, the 1 kDa membrane. This membrane is responsible for the highest difference in organic solids content between the feed and the permeate. While the 5 kDa membrane could be excluded from the sequence of filtration with minimal impact, the 1 kDa membrane can only be excluded with some compromise in

the operation conditions. The major drawback of nanofiltration is the small permeate flux, so the elimination of a previous membrane that removes a significant part of the lignin that may build up on the next membrane will further decrease the operating permeate fluxes and will lead to longer processing times [70]; these are already long due to the low membrane area, 140 cm^2, but easily managed in an industrial scale by increasing the membrane areas.

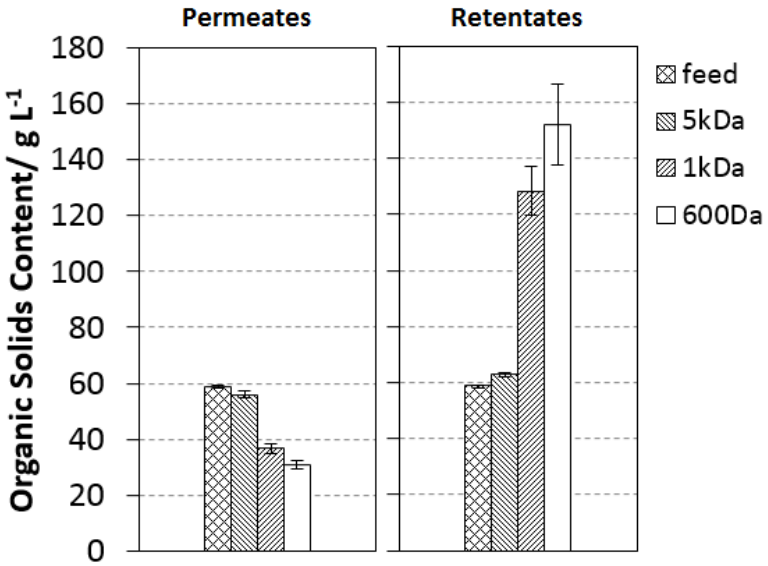

Figure 5-3 – Evolution of organic solids content in permeate (left) and retentate (right) fractions along the filtration sequence. The feed solution originated in the oxidation step is represented in both graphs for reference. Values presented are averages and standard deviation of three separate samples.

5.3.3 Chromatographic Experiments

Chromatography is used to fractionate the most representative compounds present in the complex mixture obtained from the initial oxidation of vanillin. The feed is composed mostly by vanillin (V), vanillic acid (VA), acetovanillone (VO) and *p*-hydroxybenzaldehyde (H). There are also other compounds in lower concentration but the identification of new compounds was not the objective of this work. The concentrations of the most representative compounds in the solutions fed to the chromatographic column are presented in Table 5-5, by order of abundance. V is clearly the component with highest concentration, at a concentration above 2.7 g/L; H is the second phenolic aldehyde in the mixture present in concentrations around 0.1 g/L; VA is also present in relative higher concentrations, at above 1.2 g/L; VO, or apocynin, is the ketone present at a concentration around 0.3 g/L.

Table 5-5 – Feed concentrations of the solutions prior to the adsorption experiments performed. Values given as averages and standard deviations of three separate analysis. V – vanillin, VA – vanillic acid, VO – acetovanillone, H – p-hydroxybenzaldehyde.

	Initial Concentrations, g/L	
Compound	**Breakthrough**	**Cycles**
V	2.753 ± 0.147	2.916 ± 0.345
VA	1.218 ± 0.115	1.333 ± 0.177
VO	0.301 ± 0.014	0.333 ± 0.012
H	0.094 ± 0.005	0.111 ± 0.018

5.3.3.1 Breakthrough Experiment

Permeate obtained from the nanofiltration step with membrane NP030 was fed to the chromatographic column. The solution was fed at a volumetric flow rate of 10 mL/min until outlet concentrations were considered equal to the feed concentrations (Table 5-5). The breakthrough experiment (Figure 5-4) lasted 8h, in total, since VO was present in low concentrations in the feed it took longer to reach the feed concentration. Among the species monitored by HPLC, vanillic acid (VA) is the first species to elute and reach inlet concentration, at t = 0.22 h. *p*-hydroxybenzaldehyde (H) is the second species to elute at t = 1.84 h, followed by the second aldehyde vanillin (V), at t = 2.08 h, and then acetovanillone (VO). As stated before, and presented in our previous work, the order of elution is: 1) phenolic acids, 2) phenolic aldehydes and, 3) phenolic ketones.

Interesting to note is the peak of H obtained before the elution of V, where the H concentration reaches over 3 times its feed concentration. This phenomenon is known as roll-up effect, which is commonly observed with competitive multicomponent adsorption [71], as is the case. The aldehyde H is being displaced by the aldehyde V and then its concentration stabilizes at its feed value. At the adsorption sites, H is being rapidly replaced by V, which causes a sharp increase in the concentration of H. There are also other species being adsorbed, namely species in the range of molecular weights below the average MWCO of the nanofiltration membrane, this is expected to contribute to the reduction in the capacity of the resin to adsorb V. For the purpose of the identification of other diluted species, a technique like GC-MS would be necessary.

Comparison of this breakthrough experiment with previous breakthrough performed with tobacco lignin where syringyl derived moieties are also present, (syringic acid, SA, syringaldehyde, S and acetosyringone, SO), observed along with the guaiacyl derived units here studied, revealed less interactions between the species present [37]. While in the previous work several roll-up effects were observed, namely for H, V, S, VO and SO, where SO is being displaced by some other unknown compound, in this work only H roll-up is verified. The complex nature of the tobacco lignin is evident in that work, while less complex lignin, like softwood, is expected to produce simpler adsorption patterns, as is demonstrated in this work. Working with softwood lignins can be beneficial in the

perspective of performing simpler purifications ahead. The added complexity of the presence in the mixture of syringyl derived compounds results in the fractions collected from the chromatographic column posing an additional step of separation between the similar vanillic (VA) and syringic acids (SA), the similar aldehydes syringaldehyde (S), vanillin (V) and *p*-hydroxybenzaldehyde (H) and the similar ketones acetosyringone (SO) and acetovanillone (VO). It should also be noted that in that work, the only membrane applied had a MWCO of 5 kDa, unlike in the present work, where three membranes were used, with one having an average MWCO of 600 Da, before chromatographic separation [37].

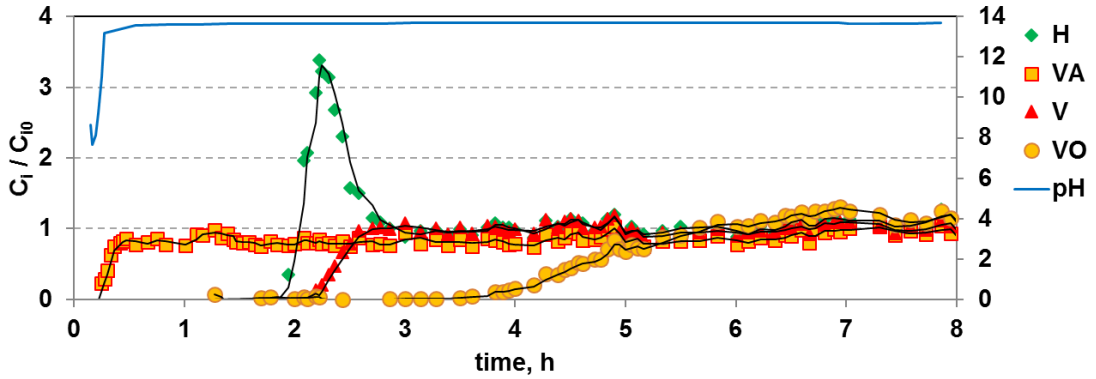

Figure 5-4 – Representation of the normalized concentration histories of the compounds studied in the breakthrough experiment. Feed pH: 13.48. $T = 25$℃, $Q_L = 10\ ml/min$. H – p-hydroxybenzaldehyde, VA – vanillic acid, V – vanillin, VO – acetovanillone. Feed concentrations given in Table 5-5.

The order of elution of the compounds in the breakthrough experiment can be explained in part by the acid dissociation constants pK_a; the pK_a values for the aldehydes V and H, and ketone VO are very close, with the values 7.4, 7.6 and 7.8, respectively. Since the medium is extremely alkaline, these species, V, VO and H, lose the phenolic proton becoming negatively charged. The acid VA possesses two dissociation constants, one very close to the other phenolic compounds, at 8.8 and the other, lower at 4.3 [72-74]. At high pH, the phenolic acid loses the second proton from the phenolic OH group, after having already lost the proton from the carboxylic group with pK_a, having two negative charges. The transformation of the molecules from their neutral form into their ionic forms at high pH values renders them more soluble, but still being able to be adsorbed due to the aromatic-aromatic interactions between the phenolic compounds and the aromatic rings in PS-DVB resin, also called π-π stacking [75, 76]. This is clear for the case of VA, which is a very negatively charged molecule, present at lower concentration but is still adsorbed since the time it takes to elute is higher than the mean liquid residence time of the column. Also, higher pH values are associated with lower adsorption capacities of ionizable phenolic compounds due to the formation of more hydrogen bonds between adsorbate and water leading to higher hydrophilicity and hindering the full extent of adsorption [47, 77, 78]. The aldehydes H and V elute around the same time, although present in very different concentrations, having the intermediate pK_a values of 7.4 and 7.6, respectively. The species eluting last

is the ketone VO, with the highest pK_a value of 7.8. From the compounds followed, VO has the highest pK_a value being the most difficult species to deprotonate, but still negatively charged due to the high pH of the solution.

5.3.3.2 Sequence of adsorption-water desorption-ethanol desorption

In order to perform the fractionation of the most abundant phenolic compounds vanillin (V), vanillic acid (VA), acetovanillone (VO) and no *p*-hydroxybenzaldehyde (H), a previously demonstrated 2-eluent technique was employed [37]. The first cycle of adsorption/desorption is presented in Figure 5-5. First the column is fed for a determined amount of time, 2 h, according to the breakthrough experiment so that the compound of interest, in this case vanillin (V), is still not eluting, but close to the breakthrough time. This feed time can be adjusted so that no *p*-hydroxybenzaldehyde (H) is lost, and this time depends on the solution being fed. Then the aldehydes H and V are desorbed with deionized water in a step that lasts 1 h. Then the phenolic ketone acetovanillone (VO) is desorbed with ethanol (97%, v/v) also in a step that lasts 1 h.

During the feed phase, 2 h, the phenolic acid VA is the only compound, from the most abundant ones, eluting with the same constant concentration, 1.3 g/L, as the feed solution. During this feed period, VA is the predominant compound and can be collected for further purification without having V, H or VO interfering. Also during this feed phase the pH of the solution eluting from the column is constant (above 13.5), its pH being the same as that of the feed solution. Also during this phase, the inorganic charge is eluting, composed mostly of the sodium hydroxide added when preparing the initial solution.

At a time around 2 h of feed, H starts to elute as well. At this point, its concentration in the outlet will increase up to about 12 times the feed concentration (t = 2.31 h); although being 12 times the feed concentration of H, the concentrations is still low (1.31 g/L) compared with the concentration peak of vanillin (V) at around the same time (t = 2.33 h) reaching 30 g/L, which is also about 11 times the feed concentration of V. Also during this water desorption phase the VO reaches a non-pronounced concentration peak of about 2 times the feed concentration (0.58 g/L) which is very low compared to the V concentrations. In this phase also, the pH starts to decrease when V is eluting, reaching a minimum value of 9.55 but then increasing up to around 11. Total solids content analysis to this fraction, collected between 2 h and 3 h, revealed a concentration of total solids content of 45.33(±0.26) g/L, the concentration of only organics solids was 21.96(±0.26) g/L with the difference being dissolved inorganics.

During the ethanol desorption phase, from 3 h to 4 h of the experiment, a peak concentration of VO is reached. Although the peak is about 7 times the feed concentration, it is still very low when compared to V when no normalization is applied to the concentration values (top, Figure 5-5). In this phase, the pH also varies slightly when the VO reaches its peak concentration reaching a new minimum pH of 8.55, and then stabilizing at 9.40. During the whole experiment pH remains in the alkaline range.

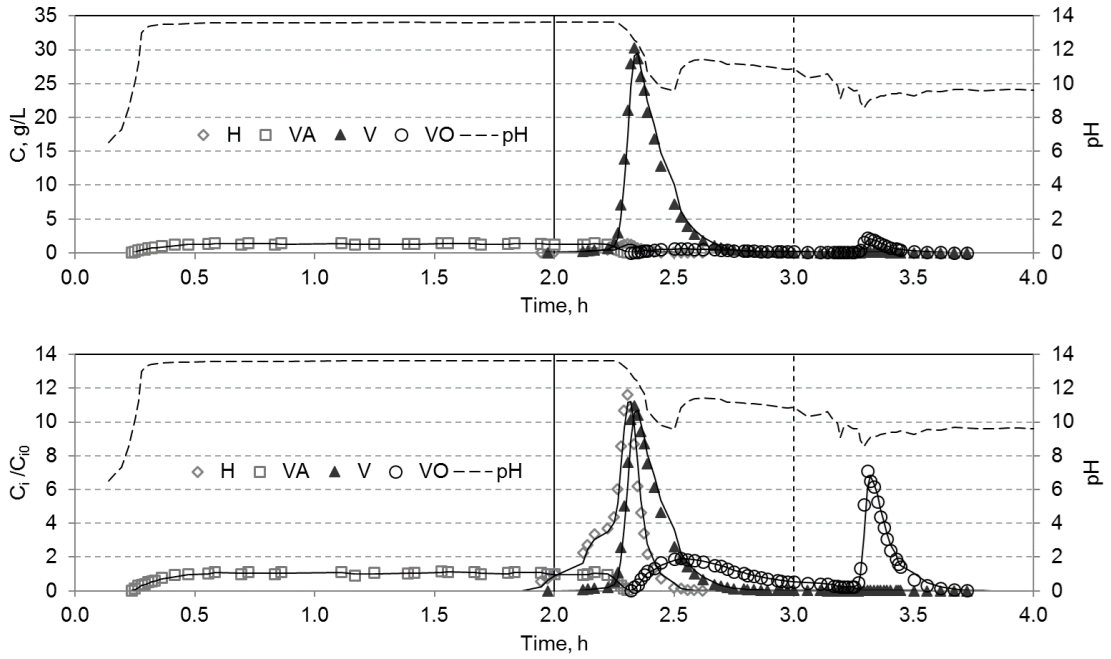

Figure 5-5 – Concentration histories of the adsorption, desorption steps for cycle 1; normalized concentration histories are also represented. Experiment conducted at a feed pH = 13.48. First vertical line marks the beginning of the water desorption; second vertical line marks the beginning of the ethanol desorption step. $T = 25$℃, $Q_L = 10\ ml/min$, column of 446 mm x 26 mm and porosity of 0.37. H – p-hydroxybenzaldehyde, VA – vanillic acid, V – vanillin, VO – acetovanillone. Feed concentrations presented in Table 5-5.

5.3.3.3 Cyclic Operation

In order to study the capacity of the resin to operate in a continuous cyclic manner, four cycles of adsorption/desorption were performed. The comparison of the several cycles performed is presented in Figure 5-6, where the four cycles are overlapped. For the same compound, the concentration histories of the four cycles are very close, and the behavior between each cycle does not change significantly. No regeneration of the resin with methanol was performed between the several cycles performed, except the elution with ethanol, 97% (v/v), which is sufficient. Other authors had success regenerating the stationary phase while in similar applications using lower ethanol composition mixtures 90%, 75% or even 50% which may increase the economy of the process, since ethanol grades below azeotrope can be used [39, 50, 61]. Lower ethanol compositions should be studied.

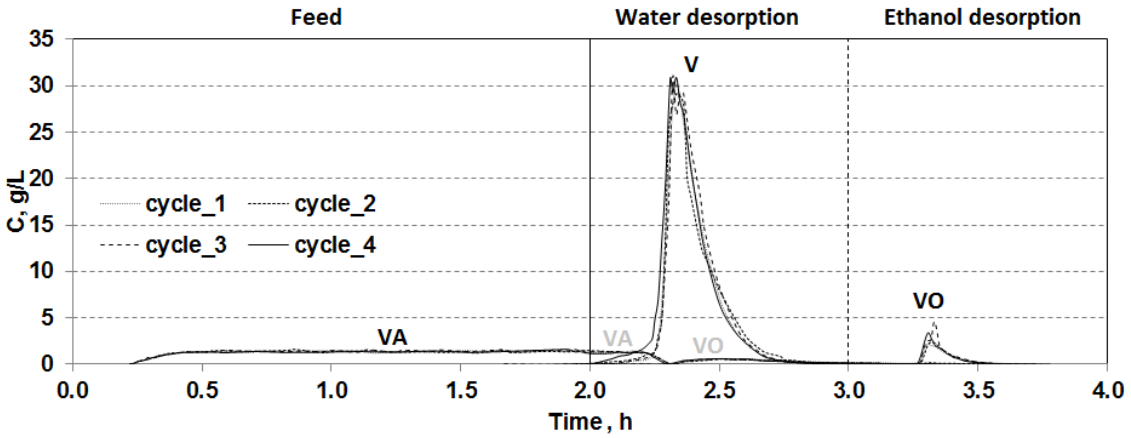

Figure 5-6 – Overlapping of the concentration histories for the four cycles of adsorption and desorption steps. First vertical line marks the beginning of the water desorption; second vertical line marks the beginning of the ethanol desorption step. For simplicity, p-hydroxybenzaldehyde (H) is not presented. Feed concentrations: [V] = 2.916 (±0.345) g/L, [VA] = 1.333 (±0.177) g/L, [VO] = 0.333 (±0.012) g/L.

All the cycles had a very similar behavior, and the mass balances performed confirm this. The amounts fed to the column and simultaneously recovery during the feed phase with comparison with both desorption phases; mass balances presented in Figure 5-7. On average, 1.48(±0.10) g of VA, 3.21(±0.25) g of V and 0.35(±0.04) g of VO were fed to the column in each cycle. From the initial VA average mass fed to the column, 1.39(±0.10) g is recovered during the feed phase and 0.20(±0.01) g during the water desorption phase. No VA was detected during the desorption phase with ethanol. From the V fed initially to the column, 3.21(±0.25) g, during desorption phase with deionized water 3.09(±0.14) g of V were recovered on average; traces of V were detected in the ethanol desorption phase. From the initial 0.35(±0.04) g of VO fed to the column, 0.15(±0.01) g of VO were detected in the water desorption phase and 0.19(±0.02) g of VO in the ethanol desorption phase, on average. Overall, the majority of the mass of the phenolic acid VA is recovered during the feed phase with minimal mass being lost to the water desorption phase; the aldehyde V is recovered almost completely in the water desorption phase along with a small contamination from the phenolic ketone VO, that elutes then to completion in the ethanol desorption phase. In the ethanol desorption phase, VO is the only phenolic compound, among the most abundant ones in the feed, appearing with highest concentration.

From the total mass of each compound fed to the column, it was possible to recover 96.2(±8.7)% of V mass and 43.4(±5.3)% of the VO mass fed in the water desorption step and 52.7(±8.4)% of the remaining VO in the ethanol desorption step. 94.1(±6.9)% of the VA mass fed was recovered in the first eluted fraction. Total average recoveries between the 4 cycles performed were 107.8(±7.0)% for VA, 96.5(±8.7)% for V and 96.3(±9.9)% for VO, across all chromatographic steps (taking into account first eluted fraction and fractions from desorption with water and desorption with ethanol).

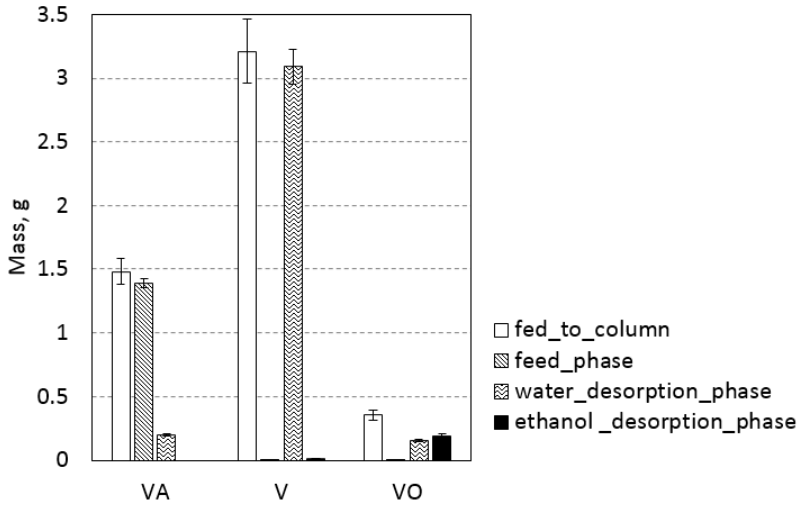

Figure 5-7 – Mass balance to the species fed and recovered at each phase. VA – vanillic acid, V- vanillin, VO – acetovanillone. Average across all cycles, error bars are standard deviations of all cycles.

5.4 Conclusions

In this work, the most abundant low molecular weight phenolic compounds (LMWPC), vanillin (V), vanillic acid (VA) and acetovanillone (VO), obtained in the alkaline oxidation of Indulin AT are fractionated in a chromatographic step that employs water and ethanol as eluents. Prior to the chromatographic step, the feed mixture is submitted to a filtration series of membranes that reduces the organic solids content from the initial 58.8(±0.4) g/L to 31.0(±1.3) g/L before being fed to the chromatographic column.

The resulting fractions form the chromatographic step are significantly different from the feed mixture; the compounds are fractionated by dominant functional groups, phenolic acids, phenolic aldehydes and phenolic ketones. The fraction collected during the feed was composed mostly of phenolic acid VA, the fraction desorbed with water mostly the phenolic aldehyde V, and the fraction desorbed with ethanol the phenolic ketone VO.

Cyclic operation was assessed by performing four cycles of adsorption/desorption without regeneration/cleaning of the column in between the experiments. The cycles performed had very similar behaviors with and the possibility for higher number of cycles performed without cleaning/regeneration. It would be interesting to perform a higher number of cycles.

The two-eluent technique, employing environmentally friendly deionized water and ethanol, is an adequate chromatographic procedure for the fractionation of the phenolic compounds present in kraft softwood lignin oxidation mixtures and it is suitable to be applied in a cyclic mode of operation with high recoveries of the compounds while operating at extreme alkaline pH.

5.5 References

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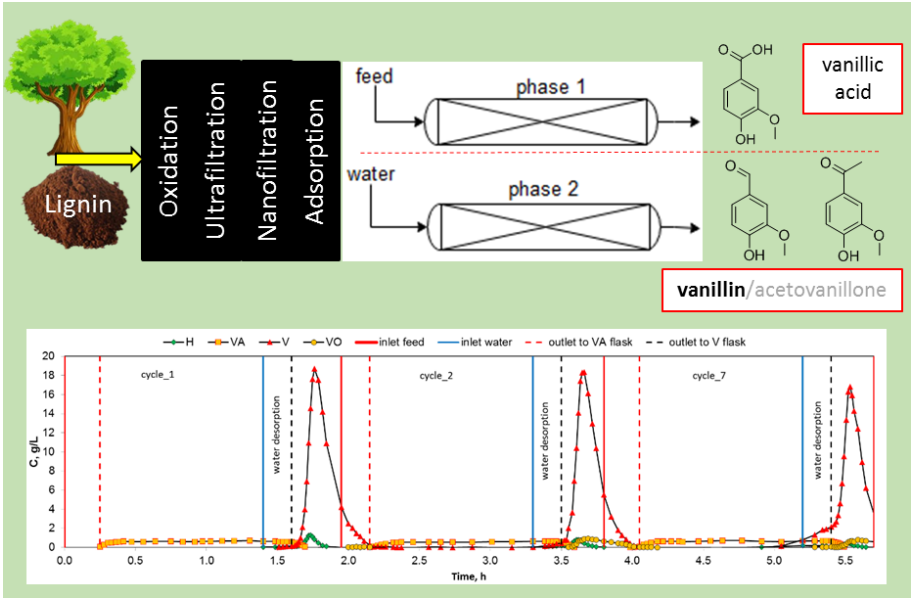
6 Recovery of Vanillin from Kraft lignin depolymerization with water as desorption eluent



In the concept of biorefineries, the production of added-value compounds from Kraft lignin depolymerization is still hindered by the number of separation processes needed to purify the phenolic compounds. In this work, a sequence of oxidation, membrane filtration and chromatographic separation is proposed for the recovery of vanillin. The alkaline wet oxidation achieved a vanillin yield of 4.3% on lignin weight basis. The two-step membrane filtration reduced the initial organic load to 29.5 (±1.8) g/L in the final permeate. The chromatographic step enriches solutions containing vanillin by alternating the feed phase with desorption with deionized water only. In 22 cycles, 71% of the vanillin fed is recovered with at an average 1.5 g/cycle. Recovered fraction has a vanillin concentration of 4.7 g/L, and it can be further concentrated. This presents a major breakthrough for vanillin production from oxidized softwood lignin as it simplifies the purification procedure, reducing costs with desorption solvents and avoiding acid use.

Chapter submitted to *Separation and Purification Technology* as: “Recovery of Vanillin from Kraft lignin depolymerization with water as desorption eluent”.

6.1 Introduction

Lignin holds the potential to become the alternative source for aromatic compounds due to its phenylpropane structure. It is the second most abundant polymer in biomass and the only naturally available source of aromatic ring structures excluding petroleum [1]. The oxidation of lignin produces several phenolic compounds, for instance in the case of softwood lignin, vanillin, vanillic acid, acetovanillone and *p*-hydroxybenzaldehyde (Figure 6-1) among others. Due to their high degree of functionalization, these compounds are valuable for the pharmaceutical industry [2, 3]. The similar structures of these compounds make it difficult to separate them from the lignin rich medium. The main process used to isolate vanillin from lignin still involves reaction steps (reaction with sulfur dioxide) or use of toxic solvents (toluene and benzene) [4]. Separations involving only physical unit operations and safe solvents are lacking.

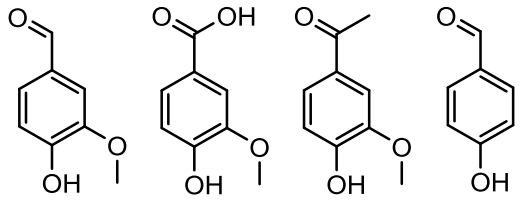


Figure 6-1 – Value added compounds recoverable from lignin oxidation solutions. From left to right: vanillin, vanillic acid, acetovanillone, p hydroxybenzaldehyde.

Lignin is present in side streams of the pulp and paper industry but it requires separation from the black liquors. Currently, the most common way of lignin precipitation is by acidification of the black liquor, either with mineral acids or by acid gas injection, namely carbon dioxide. Commercially available, there are two competing technologies, the LignoBoost and the LignoForce processes [5, 6]. Ultrafiltration and nanofiltration are alternative methods widely studied to remove lignin but they require frequent membrane cleaning or replacement due to reduced permeate fluxes and would benefit from complementary processes like flocculation [7, 8]. Also, the number of membrane filtration steps should be a function of the lignins' average molecular weight [9].

Many works dealing with recovery of phenolic compounds exist (vanillin, vanillic acid and acetovanillone) from standard solutions but few of those studies deal with depolymerized lignin solutions [10]. Forss *et al.* (1986) recovered the phenolic compounds vanillin, acetovanillone, guaiacol, and *p*-hydroxybenzaldehyde from oxidized spent sulfite liquor; strong cation-exchange resin was used with water as eluent, the aim was to fractionate the phenolic compounds from the lignin and excess alkali in solution; vanillic acid is not mentioned [11]. Schmitt *et al.* (2015) performed studies with strong basic anion-exchange resins where the chosen resin was directly contacted with the electrochemical lignin degradation solution; the recoveries are low and desorption is made using a system of ethyl acetate/acetic acid solution [12]. Later, Schmitt *et al.* (2017) proposed a two-eluent system employing

first acidified water/methanol (sulfuric acid) with intermediate regeneration of the resin with 1 M NaOH followed by a second desorption step with acidified methanol (acetic acid); [13, 14]. Wang *et al*. (2010) successfully recovered vanillin and syringaldehyde using non-polar polystyrene divinylbenzene (PS-DVB) resin from spent liquor at acidic pH, resulting in higher adsorption capacities at lower pH. No treatment is performed to remove lignin and regeneration needs to be performed often, probably due to oligomeric lignin adsorbing in the resin [15]; lignin oligomers are known to adsorb in polymeric adsorbents with PS-DVB matrix [16-18]. Works where non-polar resins are used, usually report higher resin capacities for vanillin and/or syringaldehyde than ion-exchange resins [19, 20]. Similarly, other authors Zhao *et al*. (2018) propose the utilization of gel permeation chromatography but this technique is not ideal for large scale recovery of phenolic compounds [21]. Other authors have recognized the need to separate the aldehydes vanillin and syringaldehyde but explore only the issue with standard solutions and do not work with solutions coming from the depolymerization of lignin [22].

In this work, phenolic compounds are obtained by alkaline wet oxidation of Indulin AT. The oxidative depolymerization of this lignin produces several phenolic compounds, by order of abundance: vanillin, vanillic acid, acetovanillone and *p*-hydroxybenzaldehyde. A sequence of physical separation units is used to obtain a final solution concentrated in phenolic compounds namely vanillin. Ultra- and nanofiltration membranes were used, with average molecular weight cut-offs of ~1000 Da and ~600-800 Da, respectively. Then, the alkaline permeate is treated by chromatography in a column packed with SP700 resin. The only desorption eluent during the cycles is deionized water. This decision was made based on the results of our previous work were water and ethanol are used for desorption [23, 24]. No acidifications were performed. To the best of our knowledge, there are no studies where water is employed as the sole desorption eluent to recover/concentrate vanillin from Kraft lignin alkaline depolymerization solutions utilizing a single column cyclic process, preceded by a step of membrane filtration to reduce lignin content.

6.2 Experimental Methods

6.2.1 Lignin Oxidation

A 40 L solution containing 50 g/L of Indulin AT (MeadWestvaco Co.) and 80 g/L of sodium hydroxide (AkzoNobel) was prepared. Lignin concentration is based on previous oxidation experiments and approximate content of lignin in liquors. This solution was oxidized in a structured packed bubble column reactor (SPBCR) [25-27], the setup has one oil bath (Julabo) for the inlet heating and a water bath (POLYSCIENCE, model 462-0228) for the outlet cooling. The experimental setup for the reaction is presented in Figure 6-2. Initially, the reactor is filled with nitrogen at a volumetric flow rate of 1 SLPM; the lignin solution is pumped in until visible in the glass-level meter, this is later used to adjust

the discharge valve. The oxidation was performed at an initial temperature of 140oC and total pressure of 10 bar with a 100% oxygen feed (Air Liquide). The solution was fed at an average liquid volumetric flow rate of 3.6 L/h and the gas (oxygen) was fed to the continuous reactor at a volumetric flow rate of 10 SLPM. The pressure is controlled by adjusting the outlet gas valve. When the temperature reaches 140˚C, the oxygen feed is turned on while the nitrogen is turned off and the oxidation starts. At this point, the gas outlet valve needs to be readjusted due to the increased oxygen volumetric flow rate. Samples were collected before and after oxygen was fed; first in intervals of 5 minutes until 1.5 h of oxidation, then in intervals of 15 min until 2 h and after that, every 30 minutes for a total oxidation time of 9.5 h.

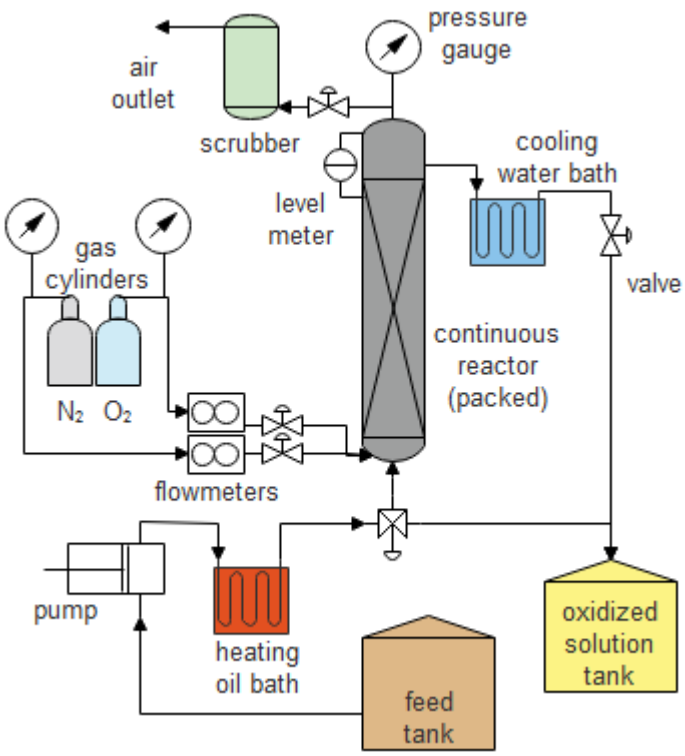


Figure 6-2 – Schematic representation of the oxidation experimental setup. The reactor is a structured packed bubble column reactor (SPBCR). A picture of the jacketed reactor is provided in Figure S 1, in annex.

6.2.2 Ultra- and Nanofiltration

Lignin remaining in solution is partially removed by membrane filtration. Both membrane filtration's steps were performed in a G.E. Osmonics SEPA CF II crossflow filtration system with a useful membrane area of 140 cm^2. This system is coupled to a variable speed diaphragm pump. The pump is set to 460 rpm with an approximate liquid volumetric flow rate of 4.2 L/min, read in an acrylic floating weight flowmeter. Figure 6-3 shows the schematic representation of the membrane filtration system. The retentate is returned to the feed tank until a sufficient volume of permeate is obtained to continue with the process. First, an ultrafiltration membrane with a molecular weight cut-off (MWCO) of

approximately 1000 Da is used (XT, from Synder, bought from Sterlitech, USA); this step was performed at a pressure of 8 bar. In the second membrane filtration step, a nanofiltration membrane with a MWCO of 600 to 800 Da (NFG, from Synder, also bought from Sterlitech, USA) was used; the nanofiltration was performed at 30 bar. Properties for both membranes used listed in Table 6-1. During the membrane filtrations, temperature was maintained between 20 and 26˚C with thermostatic bath (POLYSCIENCE, model 462-0228). The pH was not corrected therefore membrane filtrations were performed always at an approximate pH of 13. The homogenized permeate from the nanofiltration membrane is then fed to the chromatographic column. Permeability tests to the membranes were performed with concentrated NaOH solution (80 g/L) while temperature kept at 25°C by the thermostatic bath. Four measurements of the permeated volume at three working pressures recorded. Before use, membranes were compacted for 60 minutes first with deionized water, then with 80 g/L NaOH solution at pressures slightly above the working pressures.

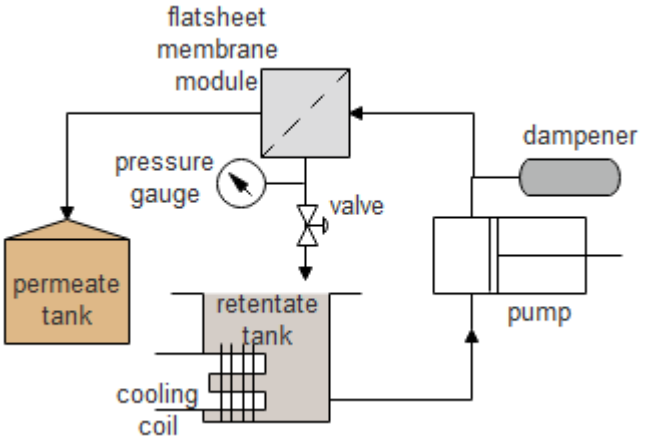


Figure 6-3 – Schematic representation of the membrane filtration experimental setup. System used for both ultra- and nanofiltration operations.

Table 6-1 – Properties of the membranes used in the membrane filtration sequence.

Property	**Membranes**	
	1st	2nd
Type	UF	NF
Manufacturer	Synder	Synder
Model	XT	NFG
Polymer Composition	PES	Polyamide-TFC
Max. Pressure (bar)	8.3	41
Max. Temperature (°C)	55	50
pH range	2 to 11	4 to 10
MWCO (Da)	~1000	~600-800

6.2.3 Chromatographic Installation

The experiments were performed in a simple chromatographic installation (Figure 6-4) composed by a jacketed glass column (Merck-Darmstadt, diameter of 26 mm and length 446 mm). The column is connected to a preparative chromatography pump with maximum volumetric flow rate of 50 mL/min (KNAUER, model Smartline P1000). The column is packed with 151.6 g of non-polar SP700 resin (resin properties in Table 6-2) previously activated as described elsewhere [20]. The column was kept at 25°C by a water thermostatic bath (LAUDA, model RE 206). The column was previously characterized by tracer experiments (chromatographic installation properties detailed in Table 6-3) [23].

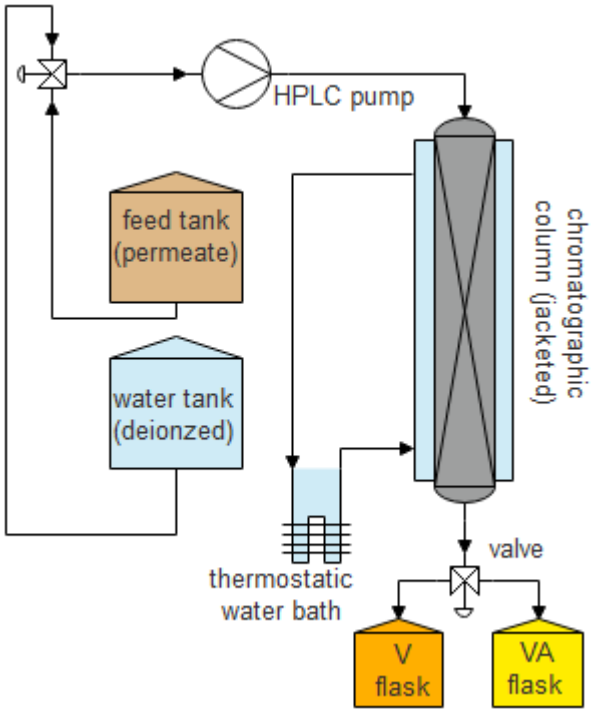


Figure 6-4 – Schematic representation of the chromatographic experimental setup. Column packed with SP700 resin. V – vanillin, VA – vanillic acid.

Table 6-2 – Main properties of the resin used.

Property	Value/Designation
Resin denomination	**SP700**
Manufacturer	Mitsubishi Chem. Corp.
Matrix	PS-DVB*
Surface area (m^2g^{-1})	1200
Density of wet adsorbent (gL^{-1})	1010
Moisture content, %	60-70
Pore Volume (mLg^{-1})	2.1
Average Particle size (μm)	450
Average pore radius (nm)	9

*PS-DVB - Polystyrene divinylbenzene.

Table 6-3 – Properties of the chromatographic installation.

Condition/Property	Value/Designation
Column internal diameter (mm)	26
Column length (mm)	446
Peclet, (-)	555
Mean residence time, (s)	540
Column porosity, (-)	0.37
Volumetric flow rate ($mLmin^{-1}$)	10
Mass of resin, (g)	151.6

6.2.4 Breakthrough Experiment

A breakthrough experiment is performed to assess the breakthrough time of vanillin, since this depends on the phenolic compounds composition of the solution, a consequence of the lignin oxidation's extent and severity. The permeate solution, coming from the last membrane (Synder NFG nanofiltration membrane), is fed to the column continuously at a liquid volumetric flow rate of 10 mL/min. The column temperature is stabilized at 25ºC with a thermostatic water bath (LAUDA, model RE 206). The column is fed with permeate until the outlet concentration is the same as the feed concentration indicating that the column has reached equilibrium; the column was fed over 8 h to ensure that $C_{inlet} = C_{outlet}$. Then the samples are analyzed and the time at which vanillin elutes is set as the maximum time for the feed cycles. The duration of the feed phase for the cycles is then set according to this vanillin breakthrough time.

6.2.5 Fractionation scheme for the cycles

The separation is achieved by collecting the outlet solution in two different flasks at predetermined times, while at the same time at the inlet is being alternated between feed solution (permeate from the nanofiltration membrane) and deionized water. The duration for each phase of the chromatographic separation is presented in Table 6-4. The fractionation scheme was established with the information collected from the breakthrough experiment in conjunction with previous works. A simplistic representation of the cycle's phases is given in Figure 6-5. The cycles have two major phases the feed (adsorption) phase and the water desorption phase; also the outlet is moved to another flask but with a delay of 900 s (0.25h). The desorption is performed in one step, using only deionized water; this was based in our previous works where great potential to enrich vanillin solutions, by using only water, was shown [23, 24]; twenty-two cycles were performed.

Table 6-4 – Fractionation scheme for the 22 cycles performed.

Cycle count	Duration (h)	Phase (action taken)	
		Inlet	Outlet
cycle 1	0	start feed	-
	0.25	-	to VA flask
	1.4	start water	-
	1.6	-	to V/VO flask
	1.95	start feed	-
	2.15 (end)	-	to VA flask
cycle n	1.15	start water	-
	1.35	-	to V/VO flask
	1.65	start feed	-
	1.9 (end)	-	to VA flask

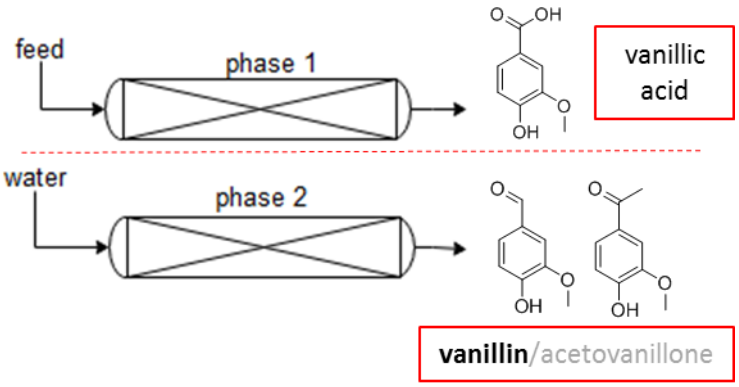


Figure 6-5 – Schematic representation of the main phases of the cycle. Vanillic acid rich solution is produced during the feed phase (phase 1) and vanillin rich solution is produced in the desorption step with water (phase 2).

6.2.6 Sampling and sample preparation

Before analysis by high performance liquid chromatography (HPLC), samples coming from the alkaline wet oxidation step and ultrafiltration are prepared by solid phase extraction (SPE) due to the presence of non-depolymerized lignin. SPE is performed as reported elsewhere [28]. Samples from the chromatographic cyclic separation are not extracted by SPE since SPE is unable to remove the remaining low molecular weight lignin; dilutions from 1:20 to 1:1000 are performed with the acidified HPLC eluent. Each cycle generates over 40 samples to be analyzed by HPLC. Sample collection is based on information from the breakthrough experiment and the first cycle; when important peaks elute, the samples are collected in intervals of 50 seconds.

6.2.7 High Performance Liquid Chromatography

Samples prepared were analyzed by HPLC, using a reverse-phase column ACE 5 with C18-pentafluorophenyl group (250 mm x 3.0 mm, 5 μm particles) in a KNAUER Smartline HPLC setup equipped with a diode array detector (DAD). Quantification wavelength set to 280 nm. The

compositions of the eluents used are water/methanol: 95%/5% (v/v) containing 0.1% (v/v) formic acid and methanol/water: 95%/5% (v/v) containing 0.1% (v/v) formic acid. The development of the HPLC analysis method was reported elsewhere [29].

6.2.8 Total non-volatile solid content

The effectiveness of the membranes at reducing lignin in solution was performed by calcination of the permeate samples. The method for determination of solid content was followed as described elsewhere [24].

6.3 Results

6.3.1 Lignin alkaline wet oxidation

To produce the monomer value-added compounds, the Kraft softwood lignin Indulin AT solution was oxidized in the continuous structured packed bubble column reactor (SPBCR). The oxidation was performed with a gas composition of 100% O_2 with no catalyst. As expected, since Indulin AT is a softwood lignin, during the oxidative depolymerization the compounds produced are mainly from the guaiacyl type with vanillin, vanillic acid and acetovanillone being the most representative, along with low amounts of *p*-hydroxybenzaldehyde. The concentration histories at the reactor outlet for the four compounds monitored are presented in Figure 6-6. For the set of conditions used (presented in Table 6-5), the maximum yields were reached at 2 h of oxidation, the point at which it is considered that the reactor has reached the steady state. The maximum concentration for vanillin was 2.155 (±0.065) g/L, vanillic acid 0.940 (±0.092) g/L, acetovanillone 0.207 (±0.011) g/L and *p*-hydroxybenzaldehyde 0.069 (±0.002) g/L; comparison with initial concentration and steady state average given in supporting information as Figure S 2.

As suggested in the works of Araújo *et al.* [27], the increase in the gas volumetric flow rate and increased oxygen gas composition allowed the production of higher vanillin yields. With this approach, the vanillin concentration, 2.16 g/L, is 3.3 times that of 0.65 g/L obtained previously in the continuous reactor compared to this work. This concentration of vanillin is very close to the maximum peak concentration obtained in the batch reactor for the same type of lignin reported previously, with vanillin concentrations of 2.04 g/L to 2.22 g/L [30]. Comparing the yield obtained here with our latest work, the improvement was also observed, previously, we obtained a maximum vanillin concentration of 1.82 g/L, whereas in this work the value is higher, 2.16 g/L. The benchmark nitrobenzene oxidation of Indulin AT suggests that higher vanillin yields are possible [31, 32], but this would only be achievable with a different type of gas-liquid contacting apparatus; oscillatory flow reactors seem a promising technology for increased oxygen mass transfer [33].

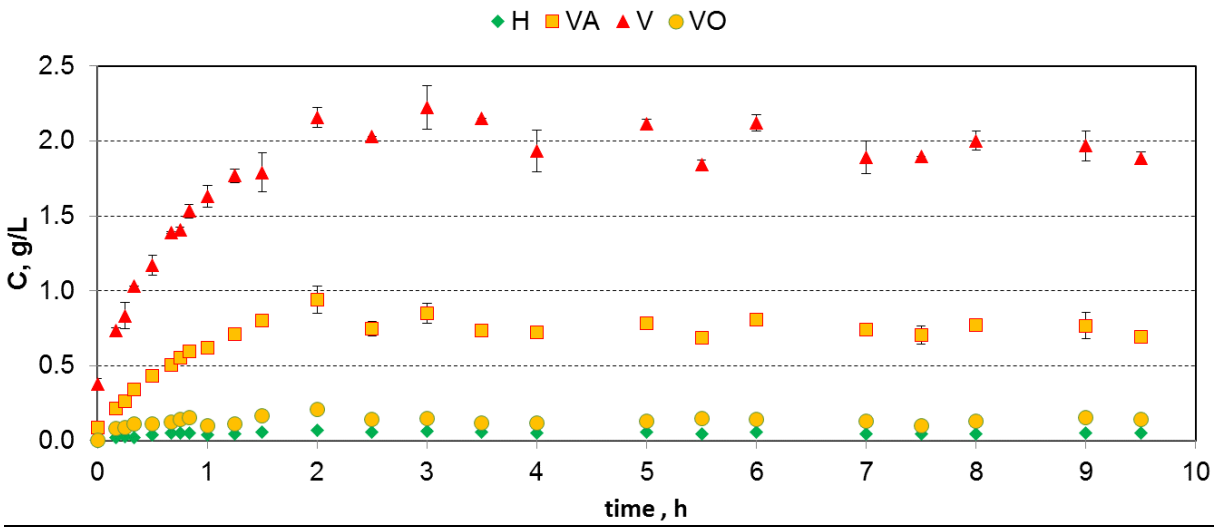


Figure 6-6 – Concentration histories of vanillin (V), vanillic acid (VA), acetovanillone (VO) and p-hydroxybenzaldehyde (H) at the continuous reactor outlet for the oxidation of Indulin AT. [Indulin AT] = 50 g/L; [NaOH] = 80 g/L; $T_{initial}$ = 140˚C, P = 10 bar; average liquid volumetric flow rate = 3.6 L/h; gas volumetric flow rate = 10 SLPM; O_2 gas composition = 100% (v/v). Points represented given as average and standard deviation of three separate SPE sample preparations/analysis.

Table 6-5 – Comparison of yields obtained with previous oxidation experiments in the same continuous reactor.

Reactor	$T_{initial}$, ˚C	p_{total}, bar	Q_G, SLPM	O_2, % (p_{O2}/p_{total})	Q_L, L/h	[Vanillin], g/L	yield, % ($w_{vanillin}/w_{lignin}$)	Ref.
Continuous	140	10	2	50%	2.12	0.65	1.1%	[27]
			5	100%	3.3	1.82	3.0%	[24]
			10	100%	3.6	2.16	4.3%	this work*

*Common to all experiments: [NaOH] = 80 g/L, [Indulin AT] = 60 g/L except this work where [Indulin AT] = 50 g/L. SLPM – standard liter per minute.

6.3.2 Ultra- and Nanofiltration

The permeability of both membranes was tested with NaOH 80g/L solution. Results of the permeability test for each membrane is presented in support information as Figure S 3. The ultrafiltration membrane (XT from Synder) has a permeability to the sodium hydroxide solution of 25.8 L $m^{-2}h^{-1}bar^{-1}$, and the nanofiltration membrane (NFG from Synder) a permeability of 4.0 L $m^{-2}h^{-1}bar^{-1}$; this nanofiltration membrane permeability is over 3 times the permeability of the nanofiltration membrane used in our previous work the NP030, which had a permeability of 1.43 L $m^{-2}h^{-1}bar^{-1}$ [24]. Theoretically, the higher permeability will benefit the treatment of the oxidized lignin stream resulting in shorter filtration times but at the expense of the amount of lignin removed.

The membrane used in the ultrafiltration step was the XT from Synder with a molecular weight cut-off of 1000 Da. The ultrafiltration permeate flux and volume reduction factor (VRF) with this membrane is represented in Figure 6-7. The ultrafiltration started with 33 L of oxidized lignin solution. The ultrafiltration permeate flux had a first initial maximum of 37 L $m^{-2}h^{-1}$ that decreased fast to under 10 L $m^{-2}h^{-1}$ after the first 30 h of operation; the remaining time, the permeate flux stayed below 5 L $m^{-2}h^{-1}$. The last permeate flux measured was 2.8 L $m^{-2}h^{-1}$. Since the permeating flux was too low and the ultrafiltration was already over 280 h of continuous operation, it was interrupted and the 15.36 L of

permeate collected. The ultrafiltration permeate had an organic solids content, depicted in Figure 6-9, of 33.0 (±0.6) g/L. Since the operation was interrupted before the ideal permeate volume was obtained, this resulted in a low VRF of 1.87.

The nanofiltration followed with the solution from the last step, employing the NFG membrane from Synder; this membrane has a smaller average MWCO, between 600 and 800 Da. The evolution of the permeate flux and respective VRF is presented in Figure 6-8. Initially, 15 L of the permeated volume from the ultrafiltration was used in the nanofiltration but since the objective was to obtain high volumes of nanofiltration permeate, 13 L of the remaining solution, from the interrupted ultrafiltration step, was added to the retentate side. The addition of the retentate, in an effort to generate more permeate in this step, was successful and did not affect the evolution of the nanofiltration since no sudden decrease in permeate flux was observed; instead, the same decrease tendency of the flux was observed. The peak permeate flux was 27 L m-2h-1 at an early stage, and slowly decreased to a minimum of 11 L m-2h-1 when nearing the 80 h of operation. At this point 19.7 L of permeate were generated, enough to continue with the separation sequence. The nanofiltration was concluded with a VRF of 3.73; the permeate from the nanofiltration step has an organic solids content of 29.5 (±1.8) g/L (Figure 6-9).

The solid content analysis (Figure 6-9) revealed that the processing of the oxidized solution with the NFG (nanofiltration) membrane, after XT (ultrafiltration) membrane, showed small improvement (decrease) of the organic solid content; an organic solid content of 33.0 (±0.6) g/L before nanofiltration and 29.5 (±1.8) g/L after was obtained; this suggests that this nanofiltration would be enough to reduce the lignin content in the solution. The small reduction in the lignin content could be explained by the close average of both membranes MWCO, 1000 Da and 600-800 Da. Besides, the content of inorganic solids is not affected by the membrane filtration steps, with both permeates, remaining close to the initial NaOH concentration with 77.1 (±1.2) g/L and 78.4 (±1.6) g/L, respectively.

In the ultrafiltration step, the temperature remained at ambient temperature, 20.9 ˚C. Due to the higher working pressure, the nanofiltration step required cooling to keep the retentate at an average 25.3˚C.

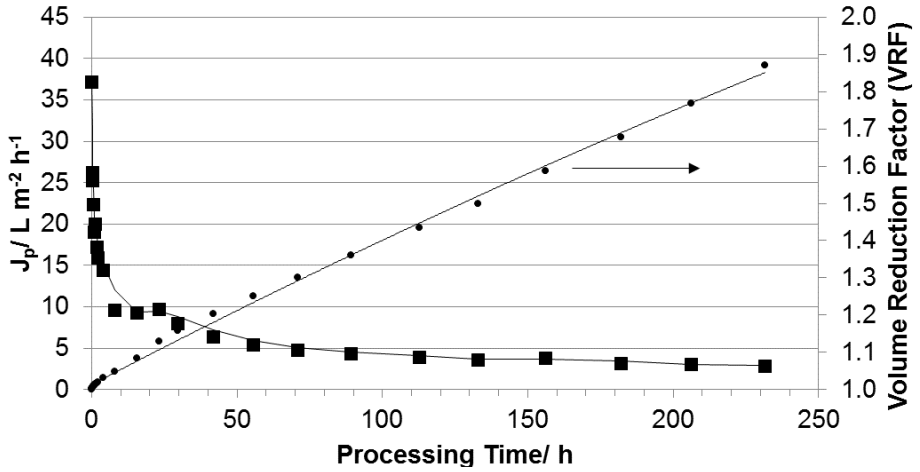


Figure 6-7 – Permeate flux of the ultrafiltration step with membrane XT from Synder (MWCO = 1000 Da). Total processing time ≈231h.

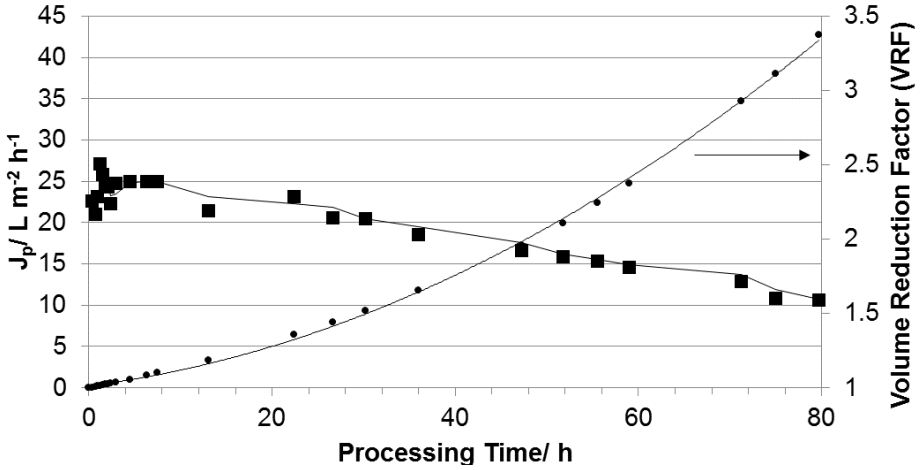


Figure 6-8 – Permeate flux of the nanofiltration step with NFG from Synder (MWCO = 600-800 Da). Total processing time ≈80h. 30 bar.

Table 6-6 – Summary of the experimental results for the ultrafiltration and nanofiltration steps.

Parameter	**Membrane steps**	
	1st	2nd
Average Operation Pressure (bar)	8	30
Average Operation Temperature (ºC)	20.9	25.3
Maximum Permeability (L h^{-1} bar^{-1} m^{-2})	4.650	0.903
Minimum Permeability (L h^{-1} bar^{-1} m^{-2})	0.354	0.353
Final Volume Reduction Factor (VRF)	1.87	3.37
Experiment Duration, (h)	≈231	≈80
Permeated Volume, (L)	15.36	19.7

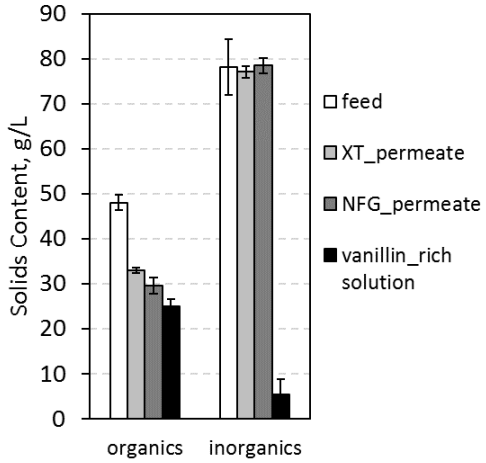


Figure 6-9 – Results of the non-volatile solid content analysis. (Vanillin rich solution refers to the solution obtained from the chromatographic column.) Values represented as average and standard deviation of analysis performed in triplicate.

6.3.3 Breakthrough experiment

Chromatography is used with the objective of fractionate the monomer phenolic compounds in the filtered oxidized lignin solution as achieved in previous works [23, 24]. The breakthrough must be performed to study the interaction of the phenolic compound rich solution with the resin until equilibrium is reached. This is necessary since the behavior of the chromatographic step depends on the concentration of the monomer phenolic compounds, known and unknown, and oligomer structures that can permeate the nanofiltration membrane. The membrane has a molecular weight cut-off in the range of 600 to 800 Da and trimers or tetramer model compounds can easily reach these molecular weights. The utilization of membrane separation is an attempt to reduce the amount of lignin and lignin oligomers in the oxidized lignin solution since they are known to adsorb in polymeric adsorbents with PS-DVB matrix, as is the case with SP700 resin [16-18].

The permeated solution from the nanofiltration step was fed directly to the chromatographic column without any acidification (Figure 6-10), having a pH of 13.33. Followed phenolic compounds are vanillin, vanillic acid, acetovanillone and *p*-hydroxybenzaldehyde. The concentrations of the monitored phenolic compounds are the following: by order of abundance, vanillin 2.620 (±0.208) g/L, vanillic acid 0.548 (±0.044) g/L, acetovanillone 0.224 (±0.014) g/L and *p*-hydroxybenzaldehyde 0.101 (±0.009) g/L; an HPLC chromatogram from the permeate solution is presented in supporting information as Figure S 4. From followed compounds, vanillic acid is the first one to elute, at 0.25 h and at approximately 0.44 h it reaches its feed concentration. The vanillic acid profile remains unchanged until the end of the experiment.

The aldehydes, vanillin and *p*-hydroxybenzaldehyde elute almost at the same time, with *p*-hydroxybenzaldehyde eluting first before 1.47 h and vanillin at 1.53 h. *p*-hydroxybenzaldehyde reaches a peak concentration at 2 h; the roll-up phenomenon happens when there is competitive

adsorption, in this case, between both aldehydes, vanillin and *p*-hydroxybenzaldehyde. The breakthrough time of vanillin will be a reference for the set duration of the feed period in the cycles performed next.

Acetovanillone starts to elute at 3.06 h. Acetovanillone takes over 2 h to reach the feed concentration. Acetovanillone is present in low concentration and this is why it takes longer to reach the inlet concentration; but so is *p*-hydroxybenzaldehyde, but for the case of *p*-hydroxybenzaldehyde, it is being displaced by vanillin, the competing aldehyde present in higher concentrations. Also, the slight increase above inlet concentration for vanillin while acetovanillone is still adsorbing, followed by normalization after 5.28 h, suggest that vanillin is being displaced by acetovanillone. This agrees with the fact that acetovanillone is more strongly adsorbed as observed in previous works where to desorb acetovanillone completely, it was necessary to use ethanol as desorption eluent [24].

For adsorption of ionizable organic compounds, the acid dissociation constant, pK_a, plays an important role. Since vanillic acid can lose two protons, it has two acid dissociation constants, the first pK_a at 4.3 and the second at 8.8; the aldehydes vanillin and *p*-hydroxybenzaldehyde and the ketone acetovanillone can only lose one proton, their single pK_a is 7.4 and 7.6 and 7.8 respectively [34]. The operation at pH above 13 keeps all the known compounds ionized rendering them more soluble while operation at a pH lower than the pK_a of a particular species would increase the extent of the adsorption [18].

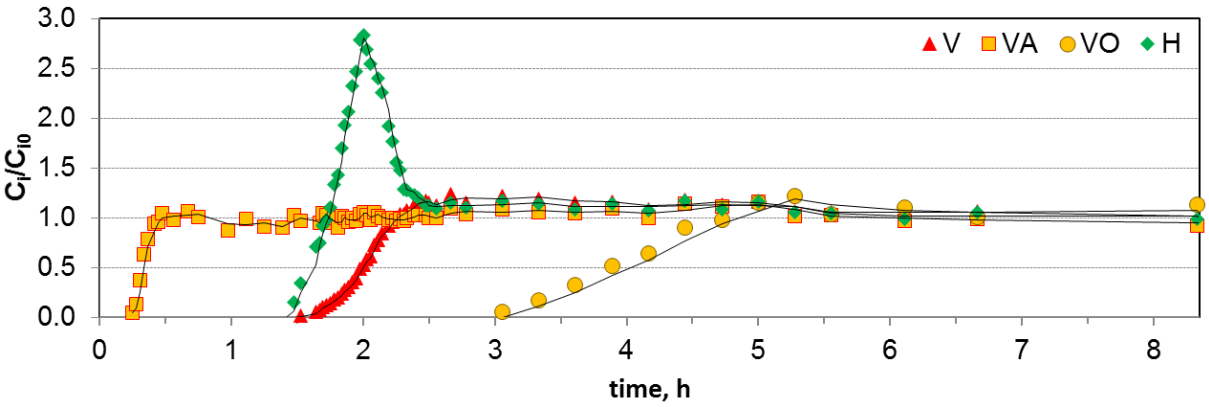


Figure 6-10 – Normalized concentration histories of the compounds studied in the adsorption breakthrough experiment. Feed pH: 13.33, $T = 25℃$, $Q_L = 10\ ml/min$. H – p-hydroxybenzaldehyde, VA – vanillic acid, V – vanillin, VO – acetovanillone. Feed concentrations: [V]=2.620 (±0.208) g/L, [VA]=0.548 (±0.044) g/L, [VO]=0.224 (±0.014) g/L, [H]=0.101 (±0.009) g/L, values given as averages and standard deviation of three separate feed sample preparation and analysis. Remaining conditions presented in Table 6-3.

6.3.4 Cyclic Operation

The recovery of phenolic compounds must be performed in a cyclic way and so, 22 cycles were performed. Only the cycles 1, 2, 7, 13, 19 and 22 were sampled for HPLC analysis. All the cycles are presented in Figure 5-5, next to each other. The cycles have 2 major phases the feed phase and the water desorption phase; also the outlet is moved to another flask but with a delay of 0.25 h (900 s)

and the fractionation scheme presented in Table 6-4 was followed; it was based on the elution times of the several species obtained in the breakthrough experiment.

In Figure 5-5, the vanillin peaks reach concentrations up to 18.7 g/L for all cycles analyzed, the vanillin maximum concentration peak remains over 4 times the feed concentration but with a tailing appearing very noticeably in cycle 7. Vanillic acid is eluting during the feed phase of the cycle at the same concentration of the feed. It keeps eluting during also 0.25 h (900 s) of the water desorption phase, at this point the vanillin collection starts. Acetovanillone is also desorbing with around 4 times its feed concentration. Its maximum concentration analyzed was 1.15 g/L during cycle 19. Acetovanillone is not present in the first desorption cycle phase but it is present in all remaining cycles. In the first cycles H, reaches a concentration peak 13.4 times the feed concentration, and in the second cycle, the peak reached 8.4 times the feed concentration. For the remaining cycles analyzed, the maximum concentration reached remained at 4 times that of the feed. The decrease of the peak maximum is accompanied by an earlier elution. *p*-hydroxybenzaldehyde is present in all desorption cycle phases eluting at the same time as vanillin during the desorption phase.

The fractionation scheme using only water as eluent could be useful in the pre-treatment of vanillin containing solutions leading to smaller volumes with higher vanillin concentrations. Also, the proposed solution could serve to remove the vanillic acid from the vanillin/acetovanillone solution.

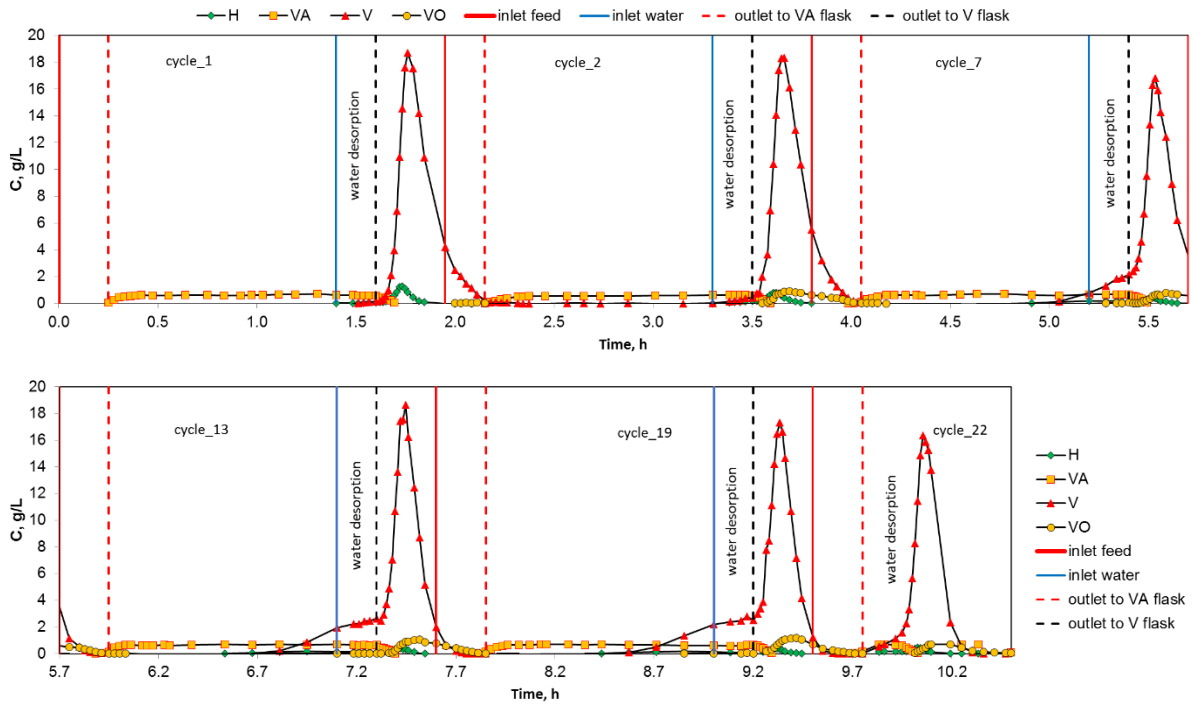


Figure 6-11 – Concentration histories for the selected sampled cycles 1, 2, 7, 13, 19 and 22. Experiment conducted at a feed pH = 13.33. H – p-hydroxybenzaldehyde, VA – vanillic acid, V – vanillin, VO – acetovanillone. Feed concentrations: [V] = 2.638 (±0.202) g/L, [VA] = 0.696 (±0.035) g/L, [VO] = 0.231 (±0.017) g/L, [H] = 0.095 (±0.014) g/L. $T = 25$°C, $Q_L = 10\ ml/min$, column of 446 mm x 26 mm and 0.37 porosity. Remaining conditions presented in Table 6-3.

The concentration histories for vanillin during cycles 1, 2, 7, 13 and 19 are isolated from the other compounds and are presented in Figure 5-6. For the first and second cycles, the behavior is very similar with no significant tailing taking place before the collection of the vanillin fraction starts (first vertical dashed line); only at cycle, it is possible to see the vanillin starting to elute sooner. Given that vanillin is the compound with more relevance in this work, the same information is provided for vanillic acid, *p*-hydroxybenzaldehyde and acetovanillone as supporting information in the annex. The vanillin concentration peaks obtained during the cycles are presented in Figure 5-6. The general tendency, after 22 cycles, is that the vanillin will start to elute sooner with each cycle, due to column saturation with other compounds present in lower amounts in the nanofiltration permeate, namely acetovanillone. Deep regeneration of the column is necessary after a determined number of cycles since the elution of vanillin happens earlier with each cycle; a compromise between vanillin lost to the vanillic acid flask and the number of cycles before undergoing regeneration should be made. Shaded area in Figure 5-6 represents a possible fractionation scheme for the collection of the solution coming out of the chromatographic columns; the volume corresponding to the shaded area would also be discarded and the collection window for vanillin would be tighter, resulting in less dilution and a solution with higher vanillin concentration. The process could be further improved by acidifying the feed solution resulting in increased resin capacity.

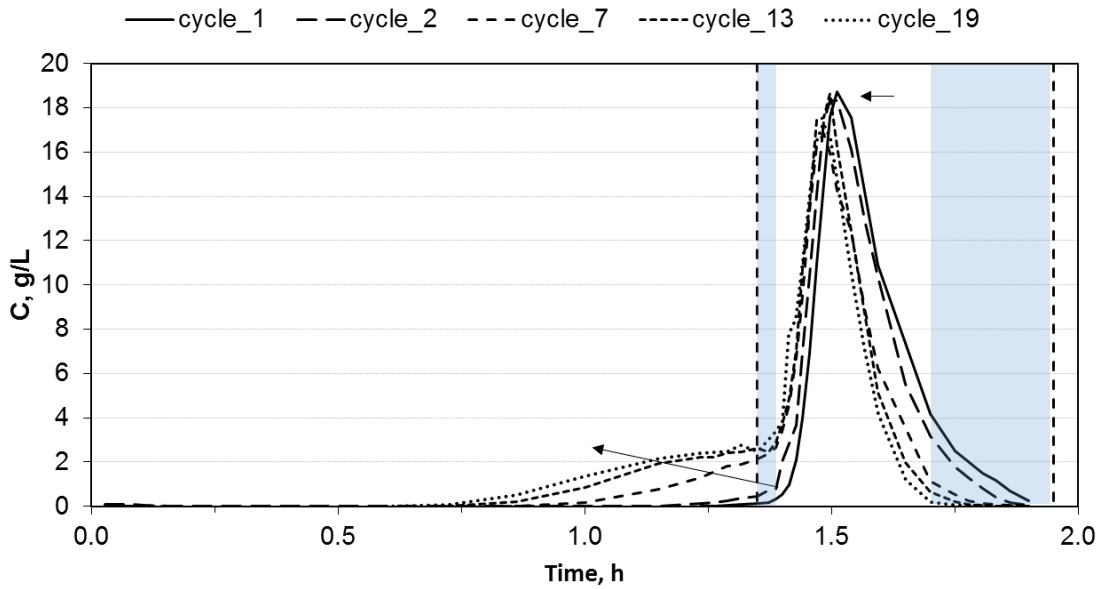


Figure 6-12 – Vanillin concentration histories for the selected analyzed cycles: 1, 2, 7, 13 and 19, overlapped. First vertical dashed line marks the beginning of collection to the second flask (1.35 h); second vertical line marks the beginning of the collection to the first flask (1.9 h). Cycle 1 adjusted to match the start of the vanillin recovery on the remaining cycles. Last cycle, 22, not represented. Vanillin feed concentration = 2.638 (±0.202) g/L. Cycle time = 1.9 h. Arrows represent the observed tendency of the vanillin peak to elute earlier as the cycles continue.

The mass balance for the recovery of vanillin along with the other compounds is presented in Figure 6-13. 70.7% of the vanillin is recovered in the final solution, with average 34.1 g being recovered. Acetovanillone is also recovered with 84.5% of the mass being recovered in the same solution totaling

3.54 g. Although present in small quantities, 47.5% of the *p*-hydroxybenzaldehyde, 0.83 g, is recovered in the same solution. 9.4% of the initial vanillic acid mass is present in the final solution. The vanillic acid in solution is being left in the solution that is collected during the feed; it is carried along with the majority of the alkali charge (inorganic) as the solids content analysis shows for the vanillin rich solution (Figure 6-9). The vanillin-rich solution has an inorganic solid charge of 5.4 (±3.3) g/L.

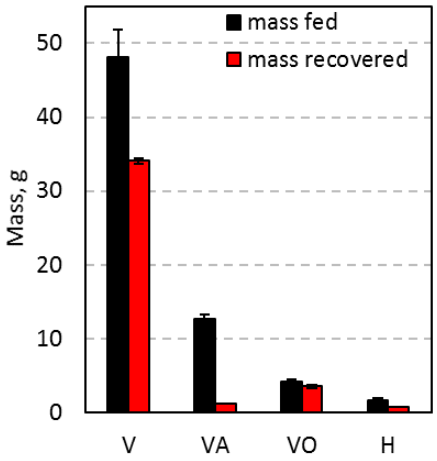


Figure 6-13 - Mass balance of the phenolic compounds fed during the 22 cycles and recovered in the final solution (7.26 L). VA – vanillic acid, V – vanillin, VO – acetovanillone, H – p-hydroxybenzaldehyde.

6.4 Conclusions

Vanillin, vanillic acid and acetovanillone are produced in the alkaline wet depolymerization of Indulin AT. Lignin content is reduced by membrane filtration and, vanillin is concentrated by adsorption. During chromatography, only water is used to generate high concentration peaks of vanillin reaching 18 g/L. Resin was successfully used in 22 cycles of feed - water desorption resulting in a final solution were vanillin concentration is 4.7 g/L. 71% of the vanillin fed is recovered, 34.1 (±0.4) g, resulting on an average vanillin recovery of 1.5 g/cycle. Reduced amounts of vanillic acid are present in the final solution. 84% of the acetovanillone fed is also recovered in the same solution.

6.5 References

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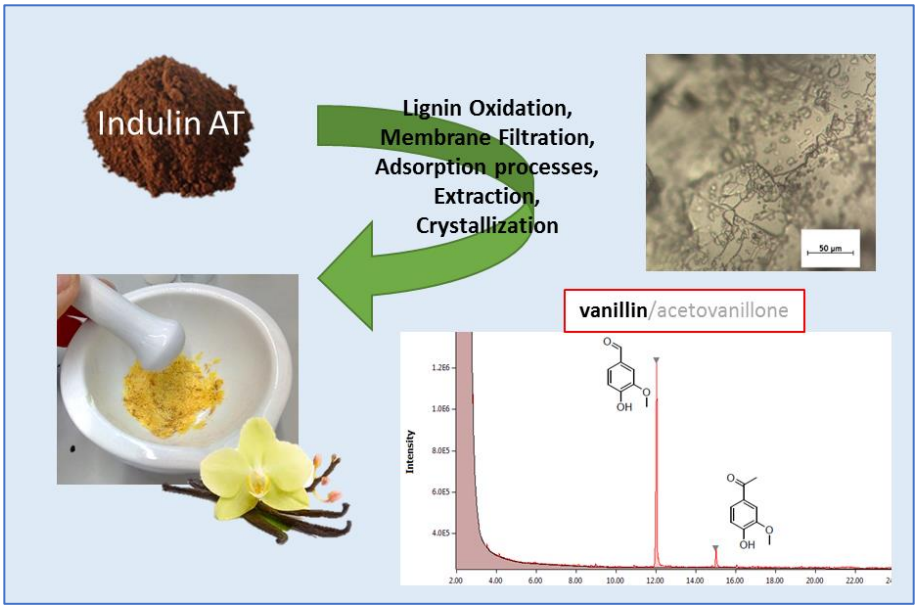
7 Crystallization of vanillin produced from kraft lignin Indulin AT



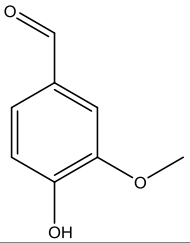
Vanillin is a highly functionalized phenolic compound that can be obtained from alkaline wet depolymerization of lignin. The natural lignin derived vanillin is still not widely available due to the laborious purification process. In this work, vanillin rich solution is produced through preparative chromatography, this solution is then extracted with ethyl acetate and vanillin is then crystallized from aqueous solution. Vanillin needle crystals were obtained from oxidation of kraft lignin Indulin AT by crystallization at room temperature. The pH of extraction plays an important role on the success of the crystallization. Purity is assessed by HPLC, melting point determination and GC-MS. The melting point of the product crystallized was in the range of 77-79ºC. Vanillin with purities up to 96% were obtained.

Chapter to be submitted as article.

7.1 Introduction

Vanillin (properties in Table 7-1) is an important phenolic aldehyde that can be obtained from the cured pods of *Vanilla planifolia* and, alternatively, from the depolymerization of Kraft lignins [1]. Biotechnological production is still a niche market although available as well [2]. Borregaard is the only company nowadays producing vanillin from lignin. Vanillin is used as a major flavoring and fragrance agent as well as a key organic intermediate in the synthesis of important active pharmaceutical ingredients (APIs) like papaverine, L-dopa or trimethoprim [3] and biobased polymers [4]. Therefore, there is interest in producing vanillin from a readily available product like lignin. The vanillin yield of the lignin derived process is still low compared to the present vanillin petroleum based route which accounts for over 90% of the vanillin produced. Also there is an additional difficulty, the separation efficiency following the lignin oxidation step is still low when trying to obtain a food grade vanillin product due to the presence of vanillin homologue molecules [5]. Obtaining the natural compound from the lignin oxidation mixtures in the purest form possible is desirable in order to be able to compete with the present vanillin producing technology, the guaiacol route [3].

Table 7-1 - Vanillin properties.

Common name:	**Vanillin**
IUPAC Name:	4-hydroxy-3-methoxybenzaldehyde
CAS Number:	121-33-5
Chemical formula:	$C_8H_8O_3$
Representation:	
Molecular weight:	152.147 g/mol
Melting point:	81˚C
pKa*:	7.4
Number of Hydrogen Bond Acceptors/Donors:	3/1
Polar Surface Area†:	46.53 Å^2

* reference [6]; †calculated in Chem3D 18.1.

Crystallization is one of the most important separation and purification processes in chemical engineering industries, especially in pharmaceutical industries where purity is extremely important but also because crystallization can control the properties of the product being crystallized (e.g. particle size distribution, and polymorph form) which affects the bioavailability of the drugs [7-11]. Crystallization is the main unit operation used in the pharmaceutical industry for the purification of APIs since it also allows easy separation from the enriched broths. Batch crystallization has been the most frequently used technique for many years. However, batch crystallization has some drawbacks,

such as variation of product quality, low capacity, high requirements of human intervention and high facility cost [12]. Continuous crystallization process provides many advantages, such as constant product quality, improved yield and capacity, and lower facility cost and space requirements [13]. While many pharmaceutical production processes use multipurpose equipment to manufacture several drugs in partial-year campaigns, very high volume drugs are sometimes produced on dedicated equipment [13]. Crystallization or recrystallization is one of the most widely purification methods applied in the last stages when obtaining/purifying a drug but this knowledge is somehow only developed on the job [14]. Favorable conditions for crystallization to occur can be achieved by three main different ways: evaporation, cooling, anti-solvent and by means of a reaction; sometimes two of these techniques can be combined for enhanced results [15-18]. In this work, cooling and evaporative crystallization were explored. Cooling crystallization is the most common form of crystallization employed. Both techniques reach supersaturation of the solution in different ways; in cooling crystallization, the solubility of the solute is decreased by cooling the solution so that the solubility line and meta-stability zone are crossed, reaching the supersaturation zone (horizontal arrow in Figure 7-1), while in evaporative crystallization, supersaturation is reached by evaporating the solvent by keeping the solution at a relatively high temperature, or letting it evaporate slowly at room temperature; the solvent evaporates increasing the concentration of the solution, until it reaches the supersaturation zone (vertical arrow in Figure 7-1). Operation above the solubility line will increase the likelihood of nucleation to occur.

Alternative methods for the removal of vanillin from broths containing other similar phenolic compounds with ketone groups, namely acetovanillone, *p*-hydroxybenzaldehyde and syringaldehyde, are the bisulfitation reaction of the ketone groups followed by precipitation, reaction (reverse) followed by crystallization; an others use toluene or benzene as extraction solvents; several patents are available employing that process [19].

Vanillin is known to form oil as a secondary liquid phase during crystallization as previous authors have reported [20, 21]. Oiling out is a phenomenon that occurs when the solvent molecule appears as a secondary liquid phase, it is also called liquid-liquid phase separation (LLPS) and it is undesirable when it happens before crystallization, but little is reported in literature. The oiling out should be accounted for when dealing with saturated solutions since it can decrease purity of the final crystallized product. Oiling out can be expected for organic molecules with low solubilities in the solvent and low melting points [22]. Some authors proposed protocols to avoid/minimize the occurrence of oiling out when crystallizing phenolic/organic compounds. Hussain et al. advise not to heat vanillin solutions to temperatures above 51^{o}C [23].

The solubility of vanillin in water and in some other solvents is published in the literature. A compilation of the solubility of vanillin in water is presented in Figure 7-1. The values collected ranged

from 4 to 80°C and a curve was fitted to this data, resulting in the following equation: $S = 0.0066T^2 + 0.1392T + 2.6530$, $R^2 = 0.977$, where S is in g/L, T in °C. The availability of vanillin solubility data in pure water is important as reference for crystallization experiments even though in this work, the presence of impurities in solution can affect the solubility deviating from vanillin solubility in pure water, namely salts and other organic molecules [24], and other parameters like pH [25].

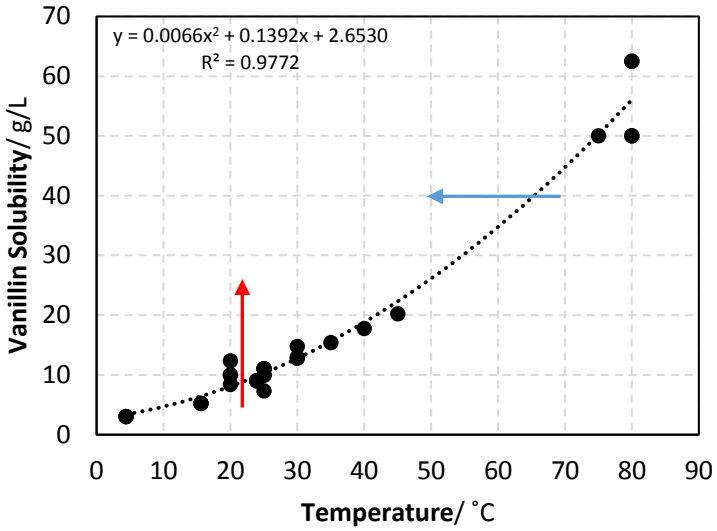


Figure 7-1 – Vanillin solubility in pure water. Curve fitted from compiled data in literature [26-29]. Vertical arrow denotes the working principle behind evaporative crystallization, and horizontal blue arrow denotes the operating principle of cooling crystallization.

In this work, vanillin solution produced by the oxidative depolymerization of kraft lignin Indulin AT, enriched in a previous preparative chromatographic step, is extracted and purified by crystallization. The lignin-derived vanillin, was successfully obtained and to the best of the author's knowledge, it is the first time that solution enriched in vanillin through a chromatographic step is used to obtain vanillin by crystallization.

7.2 Experimental Methods

7.2.1 Production of the Vanillin rich solution

Lignin Depolymerization – A solution with concentration of 50 g/L of Indulin AT (MeadWestvaco Corporation) and 80g/L sodium hydroxide (99.8%, AkzoNobel) was prepared and oxidized in a bubble column continuous reactor previously built [30]. Oxidation was performed at an average temperature of 140°C and total pressure of 10 bar of oxygen (100% O_2). The liquid mixture was fed at a volumetric flow rate of 3.6 L/h and the gas at a rate of 10 standard liter per minute (SLPM).

Removal of non-oxidized Lignin – The oxidized mixture is filtered utilizing an ultrafiltration membrane with a molecular weight cut-off (MWCO) of 1 kDa (XT from Synder Filtration) and nanofiltration with MWCO of 600-800 Da (NFG from Synder Filtration) in a GE Osmonics SEPA CF II

filtration system. Both filtrations were performed in recirculation mode. Ultrafiltration was performed at a pressure of 8 bar on the retentate side and temperature of 25°C and the nanofiltration was performed at a pressure of 30 bar on the retentate side and temperature of 25°C.

Chromatographic Installation – The resin (SP700 from Mitsubishi Corp., resin properties in *Table 4-1*) was activated following a procedure described in section 4.2.2, similar to Mota *et al.* [31]. Experiments were carried out in a jacketed glass column (Merck–Darmstadt) with internal diameter of 26 mm and 446 mm of total height, packed with 151.6 g of resin. A mean residence time of 540 s, Peclet number of 555 and a bed porosity of 0.37, were determined by tracer experiments with blue dextran as described elsewhere [32]. The preparative chromatographic column was kept at 25°C by a water thermostatic bath. The HPLC type pump is a Smartline P1000 from KNAUER; all experiments performed at liquid volumetric flow rate of 10 mL/min. A Smartline UV 2500 detector from KNAUER set at a detection wavelength set at 280 nm is used for monitoring resin cleaning/regeneration.

Chromatographic Separation – The solution fed to the column is the homogenized nanofiltration permeate which is strongly alkaline. The vanillin rich solution was produced following a similar procedure as published in previous works where water is used to desorb the vanillin rich fraction [33, 34]. It is always necessary to perform a breakthrough experiment to determine the concentration histories at the column outlet and to determine the time at which vanillin elutes. The time at which the aldehydes start to elute in the breakthrough experiment (approximately 2 h) is used as indication for the maximum feed time for the cyclic operation (1.9 h). The desorption step has a duration of 0.5 h and is performed with ultrapure (UP) water (Millipore), then a new cycle begins with alkaline solution as feed. The solution resulting from the water desorption step is homogenized and later used in the extraction and crystallization steps. Since the solutions are derived from lignin, they contain many unknown phenolic compounds from lignin depolymerization and not exclusively the four main representative compounds followed by high performance liquid chromatography (HPLC).

7.2.2 *Extraction*

In a second phase of attempt to choose an extraction solvent, volumes of 20 mL of homogenized solution containing the vanillin from the depolymerization of Kraft lignin is acidified to pH 4 and transferred to a 50 mL extraction flask to be extracted with the same amount of solvent (1:1) several times until the vanillin in the original solution is completely extracted. Solvents used then for this extraction solvent selection study were ethyl acetate (99.8%, Fischer Scientific), diethyl ether (99.9%, Fischer Scientific), isopropyl acetate (99.5%, Fischer Scientific) and benzene (99.5%, PanReac). After selection of the solvent, the solution extraction step follows an acidification step with sulfuric

acid (96%, PanReac) diluted with water in a volumetric ratio of 1:1 (v/v). During the acidification, the pH and temperature is monitored with a pH meter (pH 110 model, VWR). pH used for extraction was initially 4 and then 6. The solvent selected for extraction (ethyl acetate) was evaporated and recycled in each step of extraction. Extractions were performed with a 1:1 solvent / solution volumetric ratio.

From the solvents selected, the nontoxic solvent with the best extraction performance (determined by the lowest concentration of vanillin achieved with just one step of extraction), ethyl acetate, is then used to extract the vanillin rich solution in two 250 mL extractions (totaling 500 mL) for each trial. All of the trial experiments have the same vanillin solution as starting point. The concentration of the vanillin in the starting solution is 3.76 g/L. A scheme for the general extraction procedure is presented in Figure 7-2.

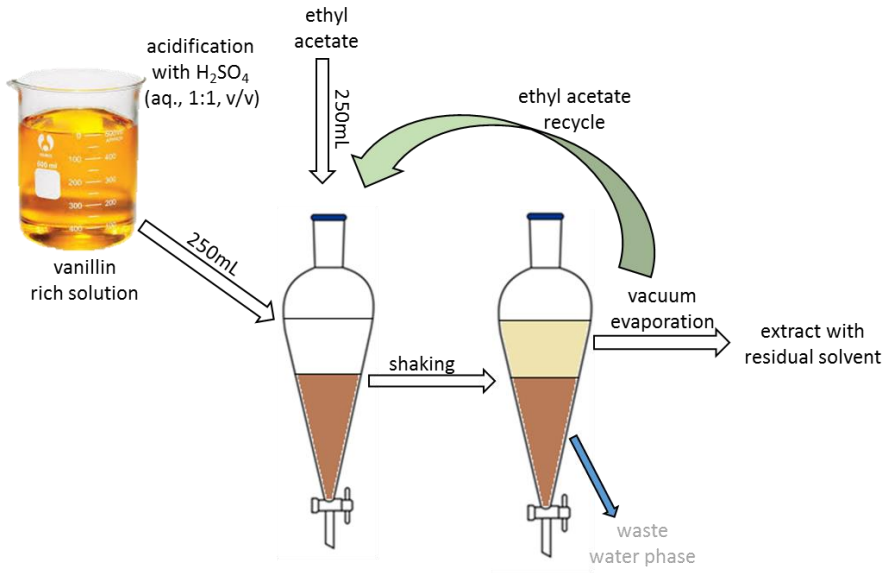


Figure 7-2 – Scheme of the followed extraction procedure.

7.2.3 Crystallization

Extract volume coming from the ethyl acetate extraction step is gradually evaporated under vacuum at a maximum temperature of 40°C in a rotary evaporator (RE100, BIBBY). Vanillin saturated solutions were prepared for the crystallization by taking the evaporated/concentrated extract and then dissolving it in up to 200 mL of UP water, which will be the crystallization solvent. Dissolution of the extracts was made with the help of a sonication bath with temperature control (HSC300TH, VWR). The scheme of the crystallization attempts was summarize in Figure 7-3.

Whenever the crystallization was hard to initiate, classical procedures were performed in order to force it: seeding of the saturated solution, cooling in a water bath at 0°C (for more than 48h), flash cooling at -24°C, and vacuum partial evaporation of the water for further concentration in the rotary evaporator. At times, for induction of crystallization or for the growing of the crystals, it is

necessary to cool down the crystallizing solutions; for this purpose, a thermostatic bath is used, a VWR recirculating bath with capacity of cooling of 500 W capable of reaching -40°C provided that water-polyethylene glycol mix is used. A programmed cooling step/ramp was used in some of the crystallization attempts; the following program was used: 20°C to 15°C with a cooling rate of 0.01(6)°C/min and held for 10h, then from 15 to 10°C at the same cooling rate and held for 10h again and the type of step is repeated 2 more times until final 5°C. When nothing is stated, the crystallization was performed at room temperature. Although mixing rate is a factor with great importance [35] it was not studied and experiments were made in static conditions.

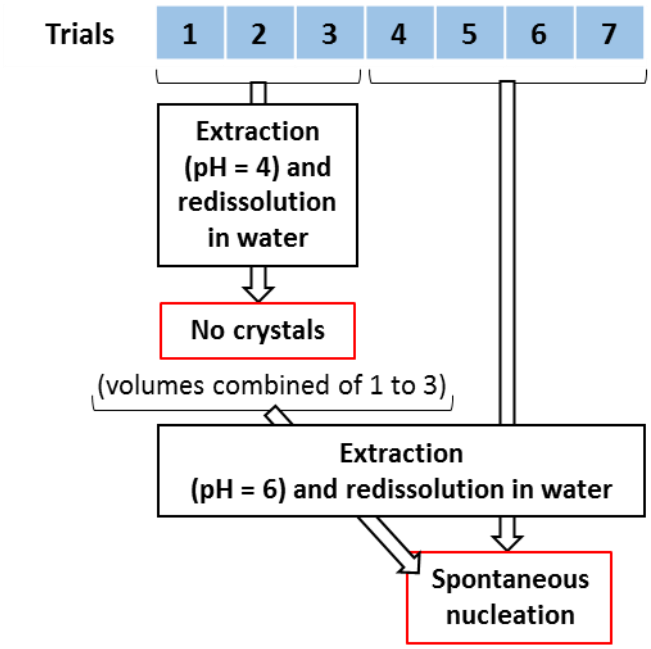


Figure 7-3 – Scheme followed during the crystallization experiments.

7.2.4 High Performance Liquid Chromatography (HPLC)

Standards of *p*-hydroxybenzaldehyde (98%, Aldrich), vanillic acid (97%, Aldrich), vanillin (99% Aldrich) and acetovanillone (98% Fluka) were used in the HPLC calibration curves. The analytical column is a reversed phase ACE 5 C18-pentafluorophenyl group (250 mm x 3 mm, 5 μm particle diameter). The column is kept at 30°C and a constant volumetric flow rate of 0.6 mL/min is used. The eluents used in the gradient are composed of methanol (99.9%, Fisher Scientific) and UP water with eluent A composition of water/methanol 95%/5% (v/v) and eluent B composed of methanol/water 95%/5% (v/v) both acidified with 0.1% (v/v) of pure formic acid (99-100%, Sigma-Aldrich). The gradient used is the following: 90%A/10%B for 3.3 min, then a ramp to 80%A /20% B until 6.7 min, then remain until 20 min with the same composition, then a ramp until 40 min and composition of 40%A/60%B, then a ramp to 100%B until 43.3 min, then keep same composition until 46.70 min, then ramp until 50 min until composition of 90%A/10%B is reached and keep this composition for 15 min. Total run

time is 65 min [36]. Samples generated in the nanofiltration step are filtered by 0.2 μm syringe filter and diluted in the first eluent (water/methanol 95%/5% (v/v) acidified with 0.1% of pure formic acid before analysis by HPLC.

7.2.5 *Gas Chromatography – Mass Spectrometry (GC-MS)*

The presence of contaminants and purity of the final dry product was assessed by gas chromatography – mass spectrometry using a SHIMADZU GC-MS (model TQ8040) equipped with an automatic split/splitless injector. The desiccated product was dissolved in pyridine (99%, Sigma-Aldrich). The column used is a RESTEK Rxi-5Sil MS (30 m x 0.25 mm x 0.25 µm film thickness). The GC oven temperature was programmed from 100°C (2 min) to 180°C at 3°C/min, then to 200°C (5 min) at 3°C/min and then to 250°C (3 min) at 15°C/min. The temperatures of injector and detector were 250˚C and 280˚C, respectively. The carrier gas was helium (Air Liquide) at a column volumetric flow of 1.32 ml/min. Sample is diluted in pyridine and an injection volume of 1 µL is used. The identification was performed with NIST Standard Reference Database 147. Chromatograms were processed using the software OpenChrom Lablicate Edition (Lawrence) 1.4.0.

7.2.6 Melting point determination

The melting point of the products obtained is determined by fusion of the material in a Stuart Scientific SMP3 apparatus depicted in Figure 7-4. The crystal product is crushed for homogenization and obtention of a powder material; then a capillary is filled with about 3 mm of powder material; the temperature was then set to 70°C, approximately 10°C below the melting temperature of the pure vanillin standard, then the heating is started at a constant rate of 2°C/min. The assessment is made visually and two temperatures are recorded: when the first droplet of melt forms and then when the material is completely melted giving an interval of melting temperature.

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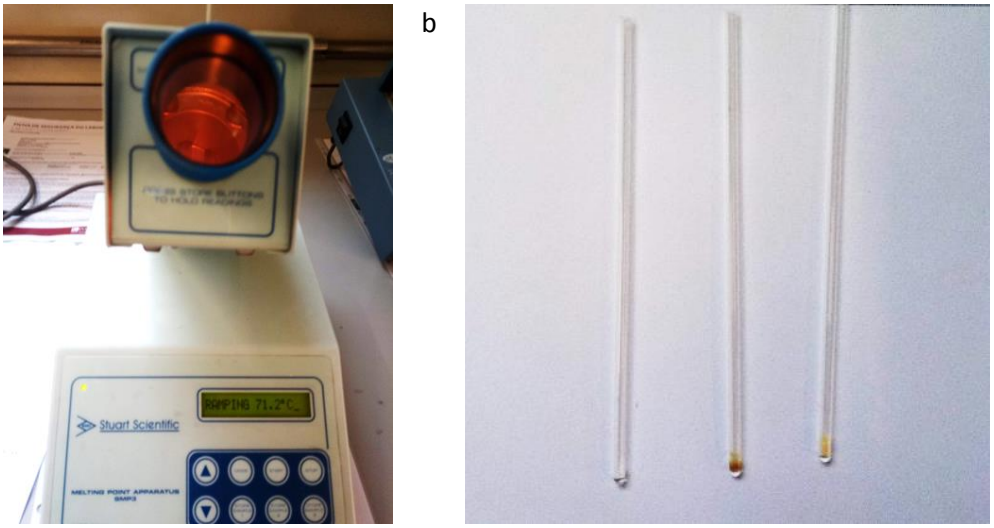

Figure 7-4 - Melting point determination apparatus (Stuart Scientific SMP3) (a), and (b) example of capillaries used in the determinations.

7.2.7 Optical Microscopy

The morphology of the crystals obtained was observed through optical microscopy using two microscopes: a Leica DM 2000 optical microscope (Leica Application Suite Interactive Measurement Imaging software) and a Carl Zeiss – Axiotech optical microscope equipped with an Axiocam 105 (Zeizz Zen 2.4 Blue Edition Imaging Software). Magnifications of 10x up to 200x were used.

7.3 Results

7.3.1 Solvent selection

Three solvents ethyl acetate, diethyl ether and isopropyl acetate were selected as candidates from literature as having low toxicity and also low boiling points [37, 38]. Benzene was used as reference and in initial exploratory experiments since it was widely reported [19] for being a good extraction solvent and still used for vanillin extraction. The solution used for test had a vanillin concentration of 2.16 g/L, vanillic acid of 0.48 g/L, acetovanillone of 0.12 g/L and *p*-hydroxybenzaldehyde of 0.09 g/L. The solution was acidified to pH of 4 before extractions with the four different solvents. The concentrations before and after extraction with each solvent are presented in Figure 7-5. After only one step of extraction, ethyl acetate reduced the concentration of vanillin in 97% (final concentration of 0.07 g/L) and 100% after a second extraction step. With all the remaining solvents diethyl ether, isopropyl acetate and benzene, a third extraction was needed to remove all vanillin in solution. Benzene was the solvent with the worst performance among the solvents tested. The compounds being extracted, namely vanillin are slightly polar so the application of a slightly polar solvent is also beneficial; ethyl acetate is able to establish hydrogen bonding whit vanillin. The solvent

that performed better was ethyl acetate that was selected as extraction solvent for the remaining experiments.

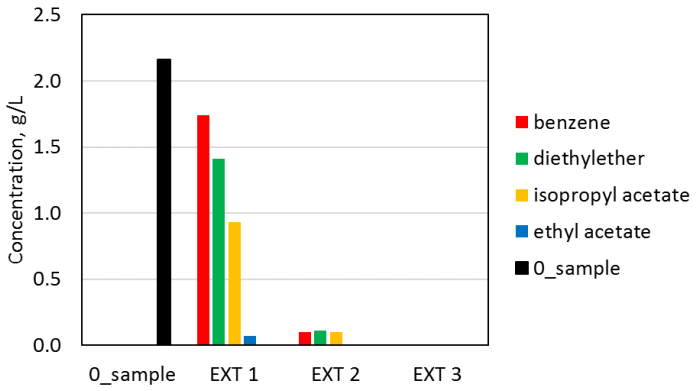

Figure 7-5 – Concentration of vanillin rich solution after extractions with selected solvents. Conditions: pH = 4, room temperature, initial vanillin concentration = 2.16 g/L. Black bar is the initial concentration without any extraction (0_sample).

7.3.2 Ethyl acetate extraction and effect of extraction pH (4 and 6)

The products obtained in the extraction are then dissolved in volumes from 75 mL to 200 mL of ultra pure water in an attempt to generate different vanillin concentrations so that the crystallization takes place in the region over the metastable zone, the supersolubility zone. Flasks 1 to 3 extracted at pH 4 develop a "cloudy" (that can be seen in flask 1) deposit but do not develop needles as expected (Figure 7-6, top). Flasks 4 to 7 extracted at pH 6 start by developing an opaque solution after hot dissolution and cooling (Figure 7-6, bottom) that eventually starts to grow crystals and end up with a clear solution with the crystals formed.

Figure 7-6 - Appearance of the vanillin rich solutions redissolved in water after extraction with ethyl acetate at pH 4 (top, flasks 1 to 3) and at pH 6 (bottom, flasks 4 to 7).

Since extraction at pH 4 was not performing as intended, a pH value of 6 was selected for further study. This value was chosen taking into account the acid dissociation constant (pK_a) of vanillic

acid and vanillin, 4.5 and 7.4, respectively [6]. In order to better understand why a lower pH for extraction was not performing as intended, a comparison between mother solutions after extraction at the two pH values used was performed (Figure 7-7). HPLC analysis and comparison of these depleted mother solutions, after extraction with ethyl acetate, revealed that the higher pH of 6 enabled the elimination of the majority of the vanillic acid from the extract. The vanillic acid stays in the mother solution with only 13% reduction in concentration. In comparison, the vanillin loss is only increased from 4% to 7% due to more vanillin being solubilized at higher pH. 14% of the acetovanillone is also left in the mother solution when extracted at pH 6, when compared to 6% at pH 4. The other known aldehyde present, *p*-hydroxybenzaldehyde, is also extracted along with vanillin. Although it is clear from the figure that the presence of vanillic acid was significantly reduced after extraction at pH 6, it is speculated that other phenolic compounds present might be interfering as well, along with vanillic acid, since vanillin, in both pH values, is almost completely in neutral form (non-ionized). For ionizable compounds, the Henderson–Hasselbalch equation (1) can be used to predict the amount of the chemical compound that is ionized [39, 40],

$$pH = pK_a + \log\left(\frac{[V^-]}{[V]}\right) \qquad (1)$$

where as example for vanillin, V represents the number of moles of vanillin in solution in its neutral form and V^- represents the number of vanillin moles in solution in its ionized state (this can be also used for the other species like vanillic acid, acetovanillone and p-hydroxybenzaldehyde). According to the equations (1), at pH 4, 30% of the vanillic acid is ionized, approximately, while at pH 6, 98% is ionized while the same increase in extraction pH only contributes for a 3% ionization of the vanillin in solution. Other non-aromatic carboxylic acids are known for being present in alkaline wet depolymerization broths, like the most abundant, formic, succinic and acetic acids [41]. These could be present in the extract since their pK_a values are higher than 4 (4.7 succinic and 4.21 acetic), except for formic acid which is 3.75 and is partially in ionic form. In fact, ethyl acetate can be used for the extraction of acetic acid from aqueous solutions [42, 43] and also succinic acid [44]. Raising the pH guarantees that, if present, the majority of these acids will remain in the spent solution since they are ionized and thus are more soluble or selective for water. After this step of extraction, the crystallization of vanillin from aqueous solution has less interference from foreign molecules. With this information, the success in earlier experiments with benzene as extraction solvent can be explained by the fact that the benzene is apolar and polar molecules like acetic and succinic acids are extracted to a less extent even though they are at lower pH, thus interfering less in the crystallization step.

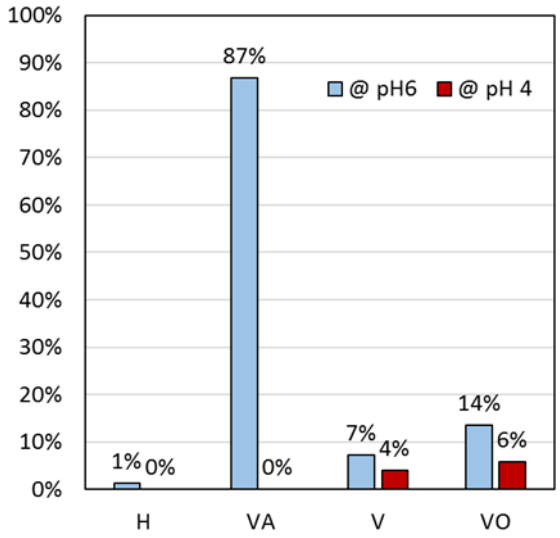

Figure 7-7 - Composition of mother solution after vanillin being extracted at pH 4 and at pH 6 in terms of the known compounds H, VA, V, and VO. (H – p-hydroxybenzaldehyde, VA – vanillic acid, V – vanillin, VO – acetovanillone).

The extraction of the vanillin rich solution at a higher pH followed by dissolution in water allowed for the production of the characteristic "milky" vanillin solution. This is indicative of the presence of homogeneous nucleation due to supersaturation of the solution.

7.3.3 Oiling out of Vanillin

Oiling out was observed during several attempts of crystallization of the vanillin from the rich solution. When the solution is in supersaturation zone, the vanillin has tendency to form a secondary liquid phase. It was observed that small oil droplets formed when cooling of a supersaturated solution and then coalesce into larger oil bodies; crystallization can start from this vanillin oil medium which is problematic. This medium is rich in the compound of interest but also a source of impurities in the dry/crystallized final product [45]. This oiling out phenomenon delays the formation of vanillin crystals; it also traps contaminants and crystals formed can get wetted by these drops that carry impurities. The oiling out can be reversed if the material is dissolved again. In several attempts of crystallization from water, drops of liquid formed and coated the wall of the flasks and then got deposited in the bottom of the flask (Figure 7-8). The fast cooling of the saturated solution can increase this behavior. The vanillin molecules that otherwise would deposit in the crystals are more easily accommodated in a liquid phase. It is attributed to the diffusion limitation of the vanillin in solution. Some authors propose a seeding before crystallization before cooling with a specific seed crystal area (approximate combined area of the crystals added based on their size distribution/amount). Svärd *et al.* (2007) claim that oiling out will happen if the water-vanillin solution is heated above 51°C [20].

Vanillin oiling out was reported also for 1-propanol water mixtures; the authors concluded that it only happens for the named system if vanillin concentration exceeds a certain value and that it can be controlled [21]. On the perspective of reducing induction times, when performing crystallization,

sonication could be applied while cooling is done at the same time. It has been shown to be effective in reducing the oiling out [46].

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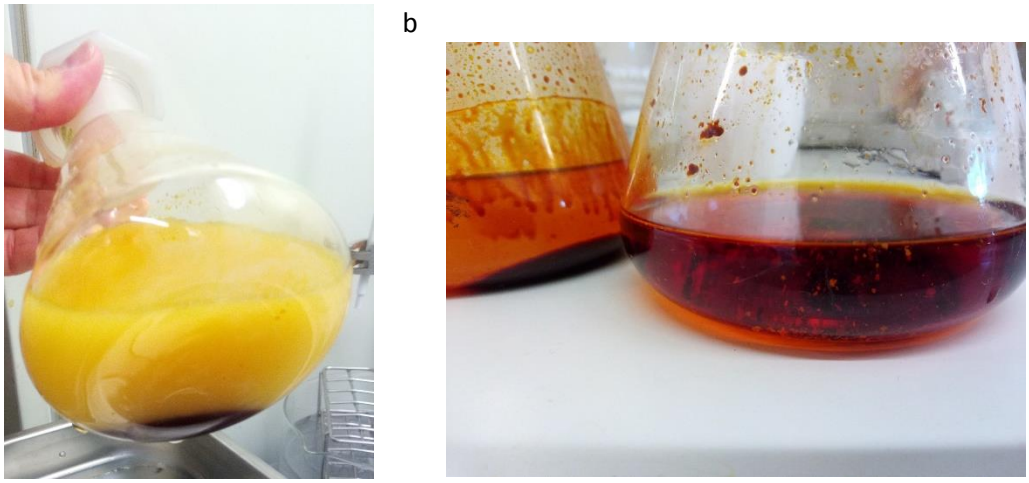

Figure 7-8 – Formation of a liquid-liquid phase separation (oiling out) in a vanillin rich solution. (a) - Coalescence of oil drops facilitated by sonication and (b) oil formed coating the flask walls.

7.3.4 Morphology of the crystallized material

Vanillin is reported to crystallize in several polymorphs; previous authors reported a maximum number of 4 polymorphs with 2 of them appearing only as product of melt crystallization (crystallization after fusion instead of crystallization from solution) [47]. The slow evaporation of the surface layer of liquid will result in that solutions at the layer to become saturated while the bulk solution will be in the metastable zone [48]. This effect dominates in crystallization by evaporation at constant temperature. The needles formed can be observed in the Figure 7-9, which formed at the surface of the crystallization solution without interference of the LLPS. Also observable are the needle-clusters that seem to grow outwards from a common nucleation point. This gives rise to a shape that is commonly known as spherulite [47]. The crystal material formed here seems like a single block but in reality it is a bundle of needles.

In Figure 7-10, the prismatic crystal obtained is presented. By comparison with previous works, it is believed that this is the form II. It was observed that, almost at every attempt, polymorph form I was obtained from water systems, which is in agreement with literature where it is stated that vanillin needle-like crystals are more easily obtained [23, 47].

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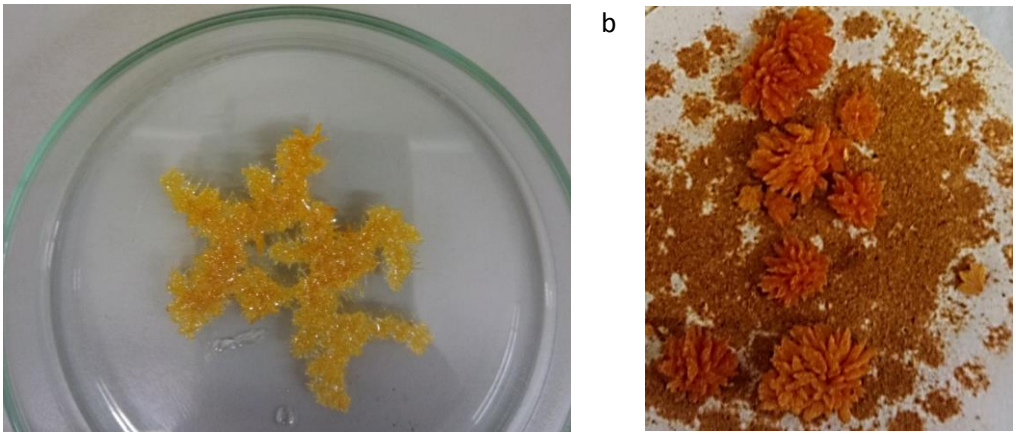

Figure 7-9 – Vanillin form I: (a) Cluster of vanillin crystals that grew from the top layer of mother solution in a petri dish evaporating at room temperature (crystals removed from original petri dish and placed in a clean one). (b) – the spherulite nature of the vanillin crystals.

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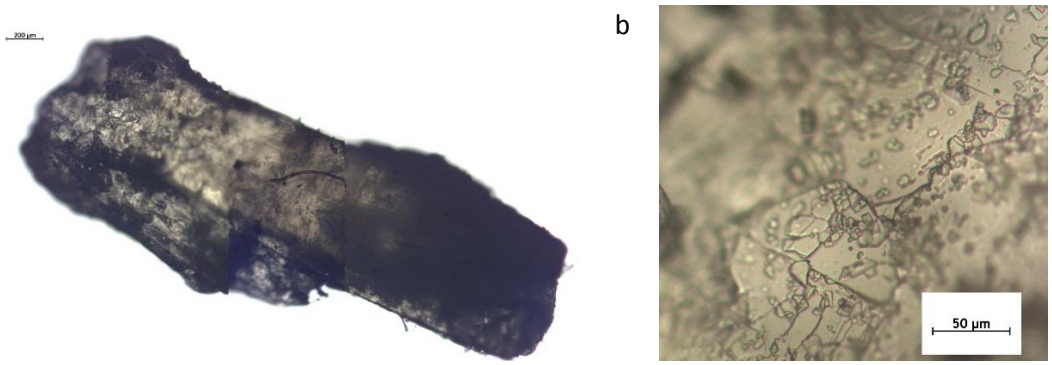

Figure 7-10 – Vanillin form II: (a) crystal with approximate dimensions of 2.8 x 0.9 x 0.8 mm3; image reconstructed from 3 different microscopic pictures of the same crystal (200 µm bar scale); (b) detail of the plates in the crystal surface (50 µm bar scale).

It was observed that room temperature crystallization produced good results and cooling seldom was used to grow the vanillin crystal product since induction times were still high, in the order of days. It was also observed that vanillin crystals started to form around the edges of the glass flasks. Nucleation of saturated solution seems to start on the contact line between the frosted glass of the flask and the solution; this has been reported for other compounds including paracetamol [49] and glycine [50]. Also depending on the nature of the surface, according to literature, may serve as packing template for the crystal; as examples tin (Sn) and polytetrafluoroethylene (PTFE) surfaces have been used to decrease induction times and control the type of polymorph obtained [51].

7.3.5 Purity

The dry products from the successful crystallization attempts were analyzed for the determination of melting points and are represented in Table 7-2. The reported temperature for the pure vanillin melting point can range from 81 to 83°C. The product from the flask 6 that was seeded had a melting point range of 73.8 to 78.4°C. The crystals obtained from flask 7 were seeded as well but

a glass rod was rubbed against the walls of the flask; these crystals had a higher melting point ranging from 77.6 to 79.3°C meaning that they were purer. Although the products obtained are not pure, the melting points are close to the pure vanillin melting point. The presence of small amounts of impurities can have a great effect on the decreasing of the melting point as the crystal formed during crystallization is not so tightly packed and thus less energy is required for melting. Acetovanillone present as contaminant has a melting temperature around 115°C [52]. Moreover, the existence of different polymorphs of vanillin can explain the variability of the melting points in literature [53].

Table 7-2 - Melting points and purity of the dry vanillin rich products obtained.

Experiment	Melting point range,°C	Purity, %*
literature†	81 – 83	-
standard	82.2 - 83.3	-
Flask_6	73.8 - 78.4	91.3
Flask_7	**77.6 - 79.3**	**81.3**
Flasks_1_to_3	**-**	**96.0**

†-values from reference [53]. *-determined by HPLC.

From the desiccated product obtained from flask 6 and flask 7, the purity was assessed by HPLC as well. 48.9 mg of dry product from flask 6 and 48.3 mg of flask 7 were diluted in 10 mL of HPLC eluent (95% methanol/ 5%water +0.1%formic acid) and then diluted to 500x. Flask 6 product reached a purity of 91.3% and flask 7 product a purity of 81.33%; this is in accordance with the wide range of melting point obtained, revealing still the presence of impurities.

The product from the flasks 1 to 3 from the unsuccessful attempts was again extracted but at pH of 6 resulting in successful extraction. This product was then diluted in 450 mL of water and placed in a crystallizing dish at ambient temperature where it was left to crystallize. The starting solution had an approximate vanillin concentration of 12.5 g/L from the combined volume of the 3 flasks thus above the solubility line in water. The crystal product obtained from this crystallization was then left to dry in a desiccator and HPLC analysis of the dry product followed, 12.5 mg diluted in 1 mL eluent (95% methanol/ 5%water +0.1%formic acid) against a standard of vanillin, 12.1 mg diluted in the same eluent; a dilution of 1000x was necessary. The HPLC assessment revealed a purity of 96.0% with vanillin as the main contaminant along with traces of p-hydroxybenzaldehyde.

Also, a qualitative GC-MS analysis was performed with the powdered crystallized material obtained and the resulting chromatogram is presented in Figure 7-11. The analysis revealed the presence of vanillin as expected and acetovanillone. The compounds were identified based on their characteristic fragmentation patterns obtained given in Figure 7-12). With the used temperature program, the retention time of vanillin was 12.050 min and for acetovanillone 15.030 min. Acetovanillone is more apolar than vanillin, due to methyl group, so it elutes later. Silylation was not

necessary since the column used is very apolar and the compounds of interest resolve well. The relative abundances for the peaks are 91.8% and 8.2% for vanillin and acetovanillone, respectively. The solvent peak is due to pyridine. Acetovanillone is present since it is produced in the alkaline depolymerization of softwood lignin Indulin AT. Acetovanillone is also present in natural cured vanilla beans along with other vanillin related phenolic compounds like guaiacol, 4-methylguaiacol and vanillyl alcohol [54].

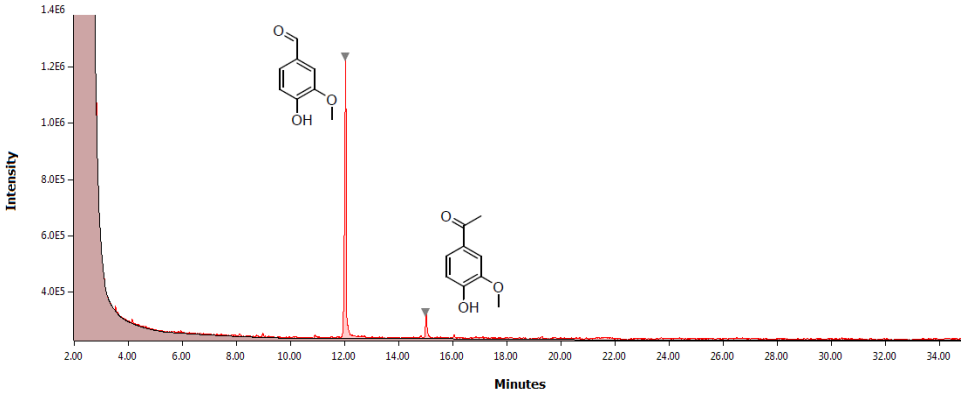

Figure 7-11 – GC-MS chromatogram of the crystallized material dissolved in pyridine (4.0 mg in 1 mL pyridine and diluted 100x). Retention times for vanillin and acetovanillone are 12.050 min and 15.030 min, respectively. Analysis method described in experimental section.

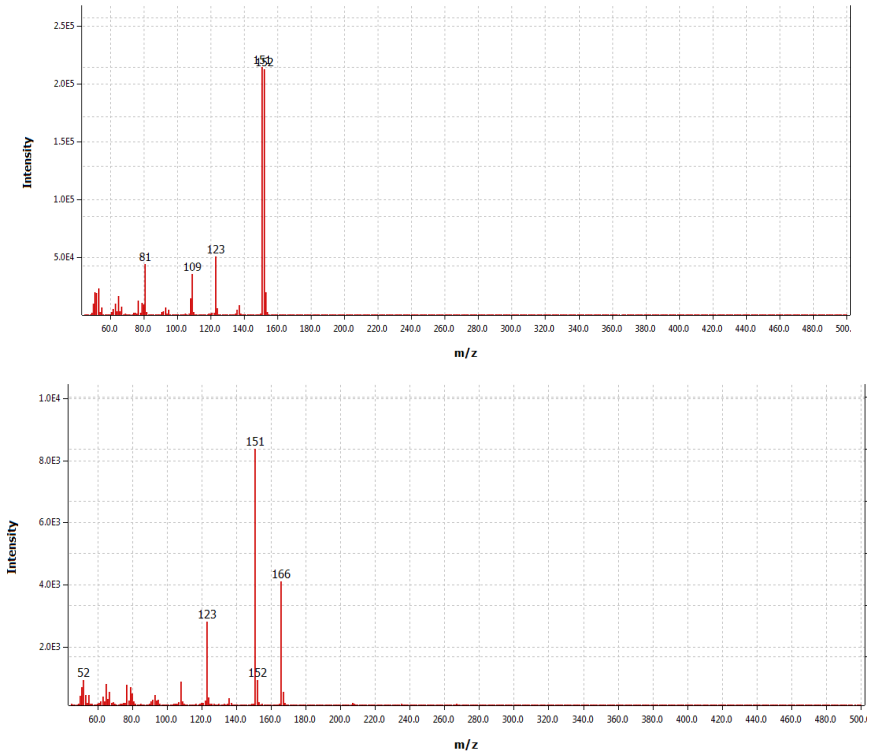

Figure 7-12 – Fragmentation spectra of vanillin (top) and acetovanillone (bottom) obtained by GC-MS. Relative abundances for vanillin fragments, m/z 151 (22%, M-H), 152 (21%, M+), and 123 (5%, M-COH) and for acetovanillone fragments m/z 151 (9%, M-CH_3); 166 (5%, M+) and 123 (3%, M-COH-CH_3).

7.4 Conclusions

Vanillin produced in the alkaline wet oxidation of lignin Indulin AT was recovered by crystallization. The original solution containing several lignin byproducts like p-hydroxybenzaldehyde, vanillic acid, vanillin and acetovanillone was enriched in vanillin by a chromatographic process that uses water as desorbent for vanillin.

Ethyl acetate was selected as extraction solvent due to its low toxicity and high extraction yields. The pH of the vanillin rich solution also plays an important role in the crystallization of the final product; extraction at pH 6 resulted in facilitated crystallization.

Vanillin crystallized preferably in needle-like shape from saturated water solutions but it is also possible to obtain the prismatic form if the solution is in the metastable zone.

The solutions discarded in this work are rich in vanillic acid, acetovanillone, and *p*-hydroxybenzaldehyde. These solutions can be further processed for the production of a more diverse range of lignin derived phenolic compounds advancing the biorefinery concept where all biomass should be valorized.

7.5 References

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8 Conclusions and Future Work

8.1 Conclusions

The main contributions of this work are:

Lignin depolymerization by alkaline wet oxidation

a) the yield of vanillin, and other monomer compounds from lignin oxidation, can be increased with the improvement of the mass transfer of oxygen; a maximum difference of about 2 orders of magnitude was obtained when comparing the volumetric mass transfer coefficient, k_La, in the continuous column reactor against the batch BÜCHI reactor;
b) for the purpose of producing vanillin, Indulin AT showed the best results from the lignins tested;
c) the modification of the gas feed (flow rate and oxygen composition) led to increased yields; with maximum vanillin yield of 4.31 % (lignin weight basis);

Phenolic compounds fractionation by preparative chromatography

a) alkaline lignin oxidation mixture was successfully fractionated by adsorption with SP700 resin into families of compounds namely the phenolic acids (vanillic and syringic), phenolic aldehydes (vanillin and syringaldehyde) and phenolic ketones (acetovanillone and acetosyringone) based on a two-eluent technique with water and ethanol.
b) the experiments performed at different pH values (9 to 12) can be used to adjust the adsorption process according to the phenolic compound of interest; a pH of 10 was found to give the best compromise between maximizing phenolic aldehydes in the water desorption step while minimizing phenolic ketones; the higher the pH, the less phenolic aldehydes (vanillin and syringaldehyde) are lost to the ethanol desorption step; phenolic ketones (acetovanillone and acetosyringone) decrease in the ethanol desorption step as the pH increases while the ketones content increases in the water desorption step. Phenolic acids (vanillic and syringic) leave during the feed phase and are completely absent from the ethanol desorption step;
c) in the treatment of the alkaline oxidation of Indulin AT, vanillin, vanillic acid and acetovanillone can be successfully fractionated in a chromatographic step employing water and ethanol as eluents resulting in fractions significantly different from the feed mixture. The fraction collected during the feed phase was composed mostly of vanillic acid, the fraction desorbed with water, mostly vanillin, and the fraction desorbed with ethanol, acetovanillone;

d) cyclic operation was assessed by performing 4 cycles of adsorption/desorption without regeneration/cleaning of the column in between the experiment; the two-eluent technique, employing water and ethanol, proved to be suitable to be applied in a cyclic mode of operation with high recoveries of the compounds while operating at alkaline pH.
e) water was successfully used, as the only desorption eluent, to generate high vanillin concentration peaks, over 18g/L. A maximum of 22 cycles of feed-water desorption were performed revealing that water can be efficiently used in a vanillin pre-concentration step while still working at extremely alkaline pH;
f) 71% of the vanillin fed was recovered in the cyclic operation resulting in a productivity of 1.5g of vanillin per cycle, in average. Acetovanillone is the second major phenolic compound present.

Phenolic compounds purification by crystallization

a) the vanillin rich fractions obtained in the chromatographic experiments were efficiently extracted with ethyl acetate for further purification; the utilization of a pH of 6 in the extraction resulted in a eased crystallization;
b) vanillin crystals were obtained with melting points ranging from 73.8 to 79.3; the purity of the final product was determined to be up to 96% (HPLC) and GC-MS revealed that acetovanillone was, in fact, the main contaminant in the final vanillin product.

8.2 Future work

Lignin depolymerization by alkaline wet oxidation

The current yields in the alkaline wet oxidation of lignin in the continuous reactor already allow to continue with the separation sequence on the route to obtaining the purified phenolic compounds. Continuous improvement is necessary and this should be revisited in the future. The oxygen mass transfer limitations must be overcome, either by installation of an increased gas volumetric flow rate system or a newly designed reactor based on a more efficient method of delivering oxygen to the reaction medium (e.g. impinging jets, cavitation). After this, catalyst can be employed to further drive the conversion yields of lignin into its depolymerized phenolic products. Another type of reactor, coupled with ceramic membrane is proposed. The reactor discharges through a ceramic membrane (due to continuous feed of lignin, the solids content in the reactor must be analyzed periodically to keep organic (lignin) solids below a certain amount), the liquid permeate flux of the membrane is the same as the inlet of the fed solution flow rate. If operating with an industrial black liquor stream, a pre-treatment with membrane filtration with high molecular weight cut-off can benefit the oxidation

process. On the other hand, working with commercial lignin can avoid the excess of process chemicals like inorganics and sugars (from hemicellulose and cellulose).

Lignin Removal by filtration processes

The step of lignin fractionation by membranes is essential for the reduction of the organic solids contents, which hinders the ensuing separation steps. The objective is to obtain an oxidized solution with reduced lignin oligomers as low as possible by utilizing the lowest number of membrane at the same time; this, when combined with reasonable permeate fluxes is a demanding and time-consuming task. A one-step nanofiltration membrane separation should be attempted in the future; The membrane tested NP030 seems suitable for this task, which if successful, will allow for a simplified process. Acidification of the lignin oxidized broth can be an alternative for lignin precipitation/removal but should be well weighted against membrane filtration. When considering an integrated biorefinery concept, this would imply that the chemical ratios of returning streams would be changed; membrane filtration does not interfere with chemical ratios.

Phenolic compounds fractionation by preparative chromatography

The contribution of the chromatographic processes is essential for the obtention of purified compounds. Without this step, complicate derivatizations procedures and extraction are necessary. We successfully separated the added-value compounds by employing solely nontoxic physical separations. Significant progresses were made regarding the separation with chromatographic processes namely the separation of vanillic acid vanillin and acetovanillone. The utilization of hardwood Kraft lignins in the near future will increase the complexity of the separation since vanillin will elute at the same time with syringaldehyde, vanillic acid will elute with syringic acid and acetovanillone will elute with acetosyringone. A chromatographic method for the separation of these two very similar aldehydes is to be developed also. With this development, similar procedures will be employed for separating vanillic acid from syringic acid and acetovanillone from acetosyringone. Vanillic acid is present in the first fraction produced during the feed of the oxidized mixture, an ion-exchange step could be employed to further enrich this fraction or simply acidification before another adsorption step in the same type of column. The development of an adjustable feed time for the cyclic procedure developed in chapter 6, where some vanillin was lost due to accumulation of strongly adsorbed compounds in the resin from previous cycles since water only desorbs the less strongly adsorbed species. Based on the cyclic process studied a reduction in feed time can be calculated for each cycle reducing the vanillin lost. After a while a regeneration with a strong eluent like ethanol can be performed and the cyclic with only water desorption only can be repeated.

Phenolic compounds purification by crystallization

Successful crystallization of vanillin was performed. It is equally essential to perform the crystallization of syringaldehyde from syringaldehyde/vanillin rich media from hardwood lignin depolymerization since it is one of the main depolymerization products. The attention should also be directed towards the crystallization of the phenolic acids like vanillic and syringic which are obtained in a vanillin depleted solution during chromatographic separation. Acetovanillone can also be recovered in significant amounts so a study could be performed in acetovanillone rich solutions. Basic studies of the saturation limits of solutions of the compounds of interest should be performed. The availability of solubility data is important for crystallization studies of the several depolymerization phenolic compounds. This information is scarcely available. The crystal products should be further characterized by using techniques like X-ray diffraction. Also, the crystallization under different mixing conditions should be performed. Application of sonication should also be tested for decreased induction times during cooling crystallization. Recrystallization and decolorizing of the vanillin product obtained should be performed in order to increase purity and appearance of the final product. The solutions discarded in this work are rich in vanillic acid, acetovanillone, and *p*-hydroxybenzaldehyde. In the concept of biorefinery, all steams must be valorized for the so these solutions should be further processed for the production of a more diverse range of lignin derived phenolic compounds.

The technical and economical analysis of the process here outlined is to be performed based on the data collected from the experiments.

Current Publications and Communications

Publications:

Article under review – Elson D. Gomes, Alírio E. Rodrigues – Crystallization of vanillin from kraft lignin oxidation.

Article in revision– Elson D. Gomes, Alírio E. Rodrigues – Recovery of vanillin from lignin depolymerization with water as desorption eluent. Separation and Purification Technology, **2020**.

Article – Elson D. Gomes, Alírio E. Rodrigues – *Lignin Biorefinery: Separation of Vanillin, Vanillic Acid and Acetovanillone by Adsorption*. Separation and Purification Technology, **2019**.

Article – Elson D. Gomes, Maria I. Mota, Alírio E. Rodrigues – *Fractionation of acids, ketones and aldehydes from alkaline lignin oxidation solution with SP700 resin*, Separation and Purification Technology, Volume 194, **2018**,

Communications:

Poster Presentation – **3rd LignoCOST** meeting - Peso da Régua, Portugal, November 12-14, **2019**. – Elson D. Gomes, Alírio E. Rodrigues *Separation of Phenolic Acids, Aldehydes and Ketones from Lignin Oxidation Mixture by Adsorption in SP700 Resin.*

Oral Presentation – **4th ANQUE-ICCE-CIBIQ 2019** – Santander, Spain, June 19-21, **2019** – Elson D. Gomes, Alírio E. Rodrigues – *Separation of Vanillin/Acetovanillone from Vanillic Acid in Oxidized Lignin Solution with Water as Desorption Eluent*. #864676

Oral Presentation – **4th CIAB** – Jaen, Spain, October 24-26, **2018** – Elson D. Gomes, Maria I. Mota, Alírio E. Rodrigues – *Separation of Acids, Aldehydes and ketones from Lignin Oxidation Mixture by Adsorption*. #55

Oral Presentation/Poster Presentation – **1st PAPTAC Lignin Conference** - Alberta, Canada, September 18-20, **2018** – Elson D. Gomes, Maria I. Mota, Alírio E. Rodrigues – *Separation of Phenolic Acids, Aldehydes and Ketones from Lignin Oxidation Mixture by Adsorption in SP700 Resin*. Session #9

Oral Presentation – **DCE17** – Porto, Portugal, June 8, **2017** – Elson D. Gomes, Maria I. Mota, Alírio E. Rodrigues – *Fractionation of acids, ketones and aldehydes from lignin oxidation broth with SP700 resin*. #191

Oral Presentation – **TECNICELPA XXIII** – Porto, Portugal, October 12, **2016** – Elson D. Gomes, Paula C. R. Pinto, Alírio E. Rodrigues – *Lignin Valorization: Oxidation of Eucalyptus Kraft Black Liquor and Kraft Lignin and Oxidation Products Concentration*.

Oral Presentation – **ICOSCAR5** – Donostia - San Sebastian, Spain, June 22-24, **2016** – Elson D. Gomes, Cátia Oliveira, Paula C. R. Pinto, Alírio E. Rodrigues – *Experiments of oxidation of hardwood pulping liquor and lignin on co-current gas–liquid flow structured packed reactor.*

Nomenclature

Symbols

C_i	concentration of the *i*-th component in at the column outlet,	[g L^{-1}]
C_{i0}	concentration of the *i*-th component in the feed reference mixture,	[g L^{-1}]
C_i/C_{i0}	dimensionless concentration of the *i*-th component,	[-]
C_L	concentration of oxygen in the liquid	[g L^{-1}]
$C_{L,0}$	concentration of oxygen in the liquid at the instant t=0	[g L^{-1}]
C_L^*	concentration of oxygen in the liquid in equilibrium with the gas phase	[g L^{-1}]
Da	Dalton	[g mol^{-1}]
$-\Delta_{sol}H$	dissolution enthalpy	[J mol^{-1}]
H_{O_2}	Henry constant for molecular oxygen	[atm L mol^{-1}]
H^{ref}	reference enthalpy, known at determined temperature	[J mol^{-1}]
k_La	volumetric mass transfer coefficient	[s^{-1}]
M_{O_2}	molecular weight of oxygen	[g mol^{-1}]
P	total pressure	[atm] or [bar]
Pe	Peclet number,	[-]
pK_a	logarithm of the dissociation constant,	[-]
Q_L	liquid volumetric flow rate,	[mL min^{-1}]
V_{bed}	bed volume	[mL]
T^{ref}	reference temperature at which H^{ref} is known	[K]
$T_{initial}$	initial temperature (of oxidation)	[˚C]
$\bar{t}_r$	mean liquid residence time,	[h]
y_{O_2}	oxygen molar fraction in the gas phase	[-]

Acronyms

DAD	diode array detector
DO	dissolved oxygen
EA	ethyl acetate
EtOH	ethanol
GC-MS	gas chromatography – mass spectrometry
H	*p*-hydroxybenzaldehyde
HPLC	high performance liquid chromatography
KBL	Kraft black liquor
LLPS	liquid-liquid phase separation
LMWPC	low molecular weight phenolic compounds
MeOH	methanol
MWCO	molecular weight cut-off, [g mol^{-1}]
NaOH	sodium hydroxide
NF	nanofiltration
ONCB	*o*-nitrochlorobenzene
PS-DVB	polystyrene-divinylbenzene
PTFE	polytetrafluoroethylene
SLPM	standard liter per minute, [L min^{-1}]
V	vanillin
V^{--}	vanillin in its ionized form (phenolic proton lost)
VA	vanillic acid
VO	acetovanillone
VRF	volume reduction factor
S	syringaldehyde

SA	syringic acid	
SPBCR	structured packed bubble column reactor	
SPE	solid phase extraction	
SO	acetosyringone	
TMP	transmembrane pressure,	[bar]
UF	ultrafiltration	
UP	ultrapure (water)	

Greek letters

ε	bed porosity, [-]
θ	dimensionless time, [-]

Annex

Detail of the reactor

Figure S 1 – Picture of the structured packed bubble column reactor, a Mellapack Y50 packing element is visible (left) and with the heating oil tubes and thermal insulation jacket mounted and the middle thermocouple is also visible.

Low molecular weight phenolic compounds concentration before and after oxidation

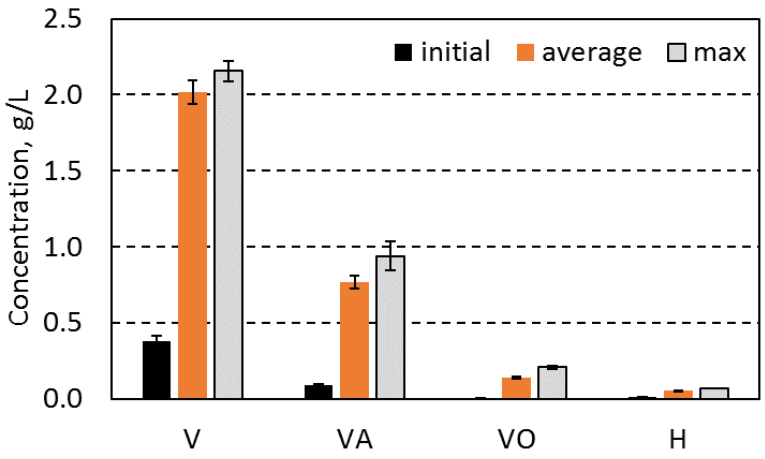

Figure S 2 – Comparison of the concentrations before and after oxidation of the most abundant low molecular weight phenolic compounds. Vanillin (V), vanillic acid (VA), acetovanillone (VO) and p-hydroxybenzaldehyde (H). Values represented as averages and standard deviation of three separate sample preparation and analysis.

Data from the membrane permeability tests.

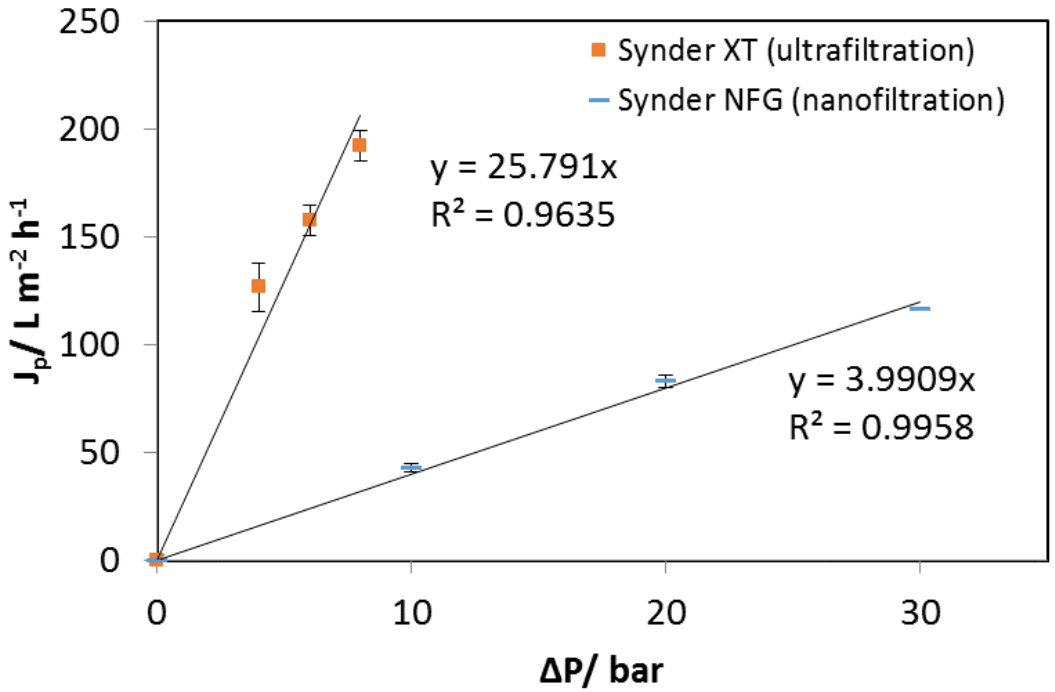

Figure S 3 – Permeability tests to the membranes used. Test performed at 25˚C.with [NaOH] = 80 g/L. Pressures tested for XT membrane: 4, 6, and 8 bar; pressures tested for NFG membrane: 10, 20 and 30 bar. Values represented as averages and standard deviation of at least four separate liquid volumetric flow rate measurements.

HPLC Chromatogram

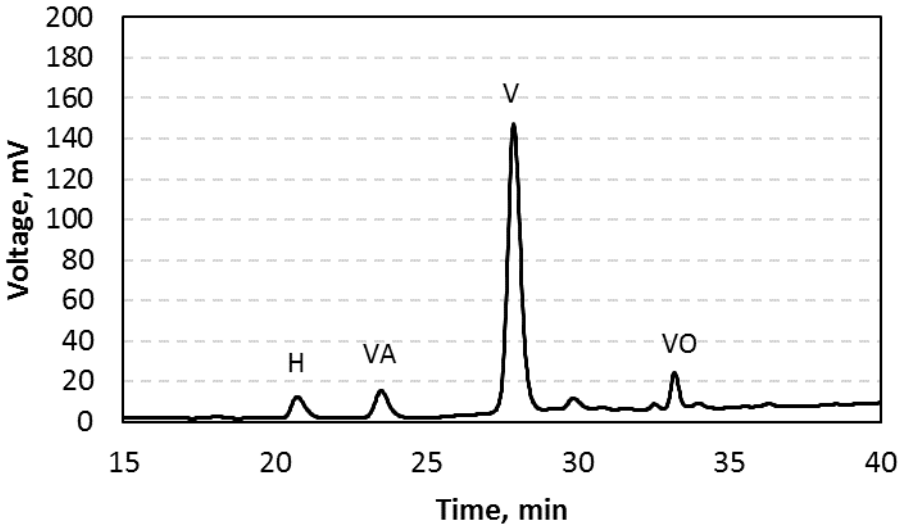

Figure S 4 – Chromatogram of the permeate from the NFG membrane permeate diluted 200x. H – p-hydroxybenzaldehyde, VA – vanillic acid, V – vanillin and VO – acetovanillone. Details of the analysis is provided I section 2.4 of the article.

Concentration histories for vanillic acid *p*-hydroxybenzaldehyde and acetovanillone

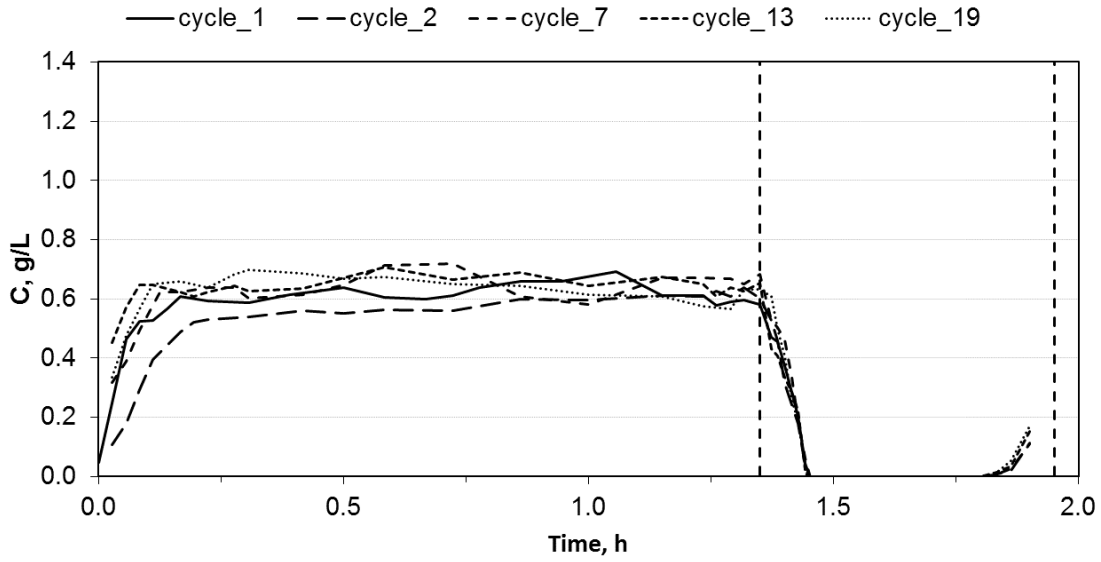

Figure S 5 – Vanillic acid concentration histories for the selected analyzed cycles: 1, 2, 7, 13 and 19, overlapped. First vertical dashed line marks the beginning of collection to the second flask (1.35 h); second vertical line marks the beginning of the collection to the first flask. Cycle 1 adjusted to match the remaining cycles' collection times. Last cycle, 22, not represented due to its different nature. Vanillic acid feed concentration = 0.696 (±0.035) g/L. Total cycle time = 1.9 h.

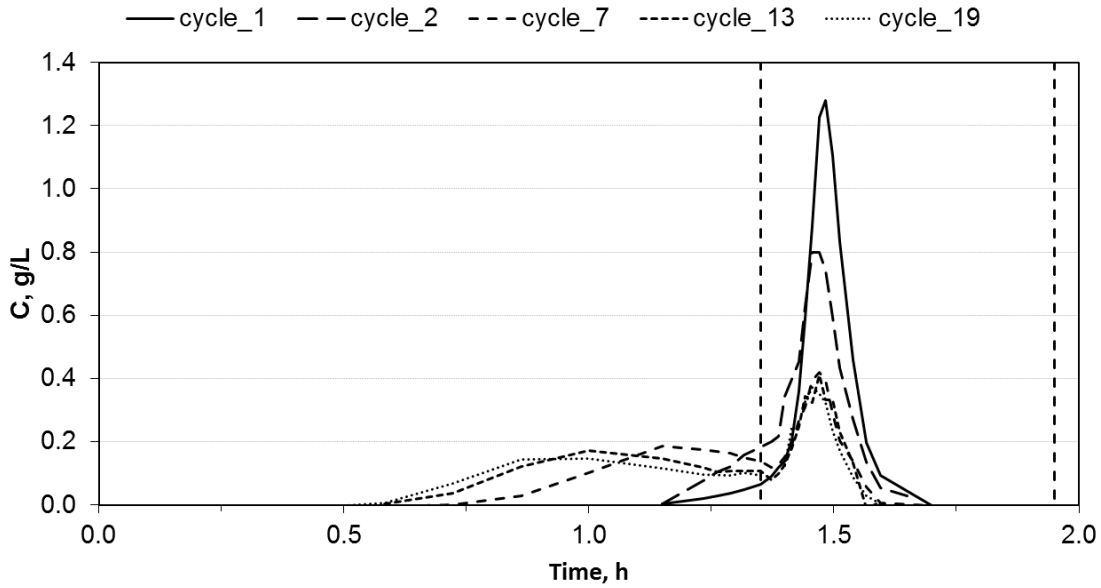

Figure S 6 – p-hydroxybenzaldehyde concentration histories for the selected analyzed cycles: 1, 2, 7, 13 and 19, overlapped. First vertical dashed line marks the beginning of collection to the second flask (1.35 h); second vertical line marks the beginning of the collection to the first flask (1.9 h). Cycle 1 adjusted to match the remaining cycles' collection times. Last cycle, 22, not represented due to its different nature. p-hydroxybenzaldehyde feed concentration = 0.095 (±0.014) g/L. Total cycle time = 1.9 h.

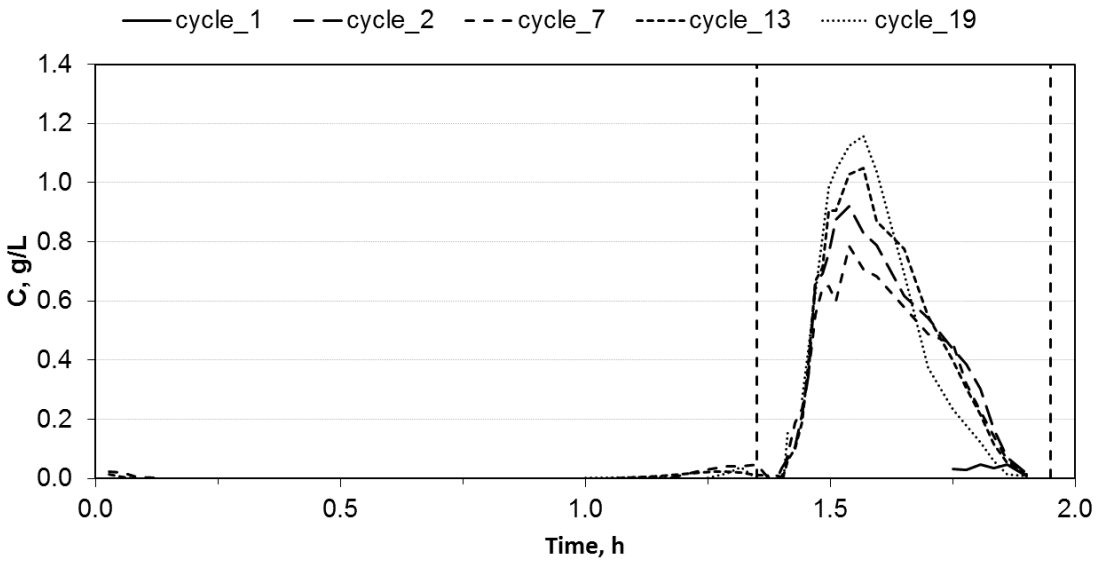

Figure S 7 – Acetovanillone concentration histories for the selected analyzed cycles: 1, 2, 7, 13 and 19, overlapped. First vertical dashed line marks the beginning of collection to the second flask (1.35 h); second vertical line marks the beginning of the collection to the first flask (1.9 h). Cycle 1 adjusted to match the remaining cycles' collection times. Last cycle, 22, not represented due to its different nature. Acetovanillone feed concentration = 0.231 (±0.017) g/L. Total cycle time = 1.9 h.