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CHAPTER 8

Occurrence and fate of aromaticity driven recalcitrance in anaerobic treatment of wastewater and organic solid wastes

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8.1 Introduction

The actual challenges of industrial wastewater or organic solid waste treatment involve degradation of a wide range of pollutants such as aromatic compounds from the textile and agroindustrial sectors, as well as from urban origin or natural occurrence. Recently, Scientifics have extensively studied the biodegradation of aromatic compounds under aerobic and anaerobic conditions. Nevertheless, anaerobic treatment gets much attention due to several advantages such as its positive energy footprint (Muñoz-Sierra et al., 2019;Yang et al., 2006). Aromatic amines and dyes are commonly present in wastewater from textile industries. Knowing they are carcinogenic in nature, it becomes more significant as to how scientists can mitigate them by tracing their path, their effects, and their mitigation strategies. Similarly, higher lignocellulosic fraction in feedstock may hamper the anaerobic digestion (AD) of agrowastes, especially when they are pretreated by thermal hydrolysis, which generates inhibitory intermediates.

8.1.1 Aromatic amines in wastewater

The development of different human activities has given rise to the production of many harmful substances such as recalcitrant compounds produced by industries in large amounts. Examples of wastewaters with recalcitrant compounds are dye wastewaters from textile industries; lignin-derived effluents from agroindustrial industries, urban wastewater, pharmaceutical wastewaters, etc. These streams are generally treated in wastewater treatment plants (WWTPs) but are only partly treated, causing several environmental problems and risks to human health (Papadimitriou et al., 2007).

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The level of recalcitrance can be explained (in most cases) by the biochemical structure of the compounds. For example, the molecular structure of aromatic amines (AAs) has a crucial effect on their removal (Albahnasawi et al., 2020a, 2020b; Field et al., 1995; Khatiwada et al., 2018; Wang et al., 2012). AAs are derived from the treatment of azo dyes, which are used in the textile industry and are considered carcinogenic and challenging pollutants to be removed from wastewater (Salter-Blanc et al., 2016). The relevance of azo dyes is related to the fact that they represent over 60% of the dye structures used in this industry (Allen, 1971), which make them strong recalcitrant pollutants and significantly present in wastewater.

Dyes are synthetic organic compounds with chromophores (Azo: R1-N=N-R2, carbonyl: -C=O-, methane: -CH) and auxochromes (an electron withdrawing or donating substituents). Azo dyes are the most used in industries, and they have one or more azo groups (Popli and Patel, 2015). They are organic compounds with aromatic components and have one or more azo groups (-N=N-), and they are generally recalcitrant to aerobic biodegradation due to the oxidized -N=N- linkage. Their anaerobic degradation occurs through reductive cleavage of the dye's azo linkages (Albuquerque et al., 2005, Allen, 1971, Guo et al., 2010).

Azo dyes are not easily removed by conventional activated sludge–based WWTPs (Lourenço et al., 2009), and research on their biodegradation reveals the need of a combination between anaerobic and aerobic processes, involving both microbial communities of each process (Assadi et al., 2018, Albuquerque et al., 2005, Bafana et al., 2011, Prasad et al., 2017). However, Bafana et al. (Bafana et al., 2011) reported that a combination of activated sludge and advanced oxidation processes could be a suitable strategy, too.

8.1.2 Recalcitrance in anaerobic digestion of lignocellulosic biomass

AD of lignocellulosic biomass has gained increasing attention from researchers and decision makers being an environmental-friendly process that can utilize various organic wastes and produce biogas. Nevertheless, the recalcitrance of lignocellulosic biomass and the derivatives released after its hydrolysis might be a hindrance for the complete use of this biomass. Therefore the reaction mechanisms of lignocellulose hydrolysate intermediates, under specific operating conditions and at certain concentrations, must be carefully analyzed for a successful process ((Ghasimi et al., 2016; Jönsson et al., 2013; Li et al., 2019a; Taherzadeh and Karimi, 2008). The main derivative compounds released during the hydrolysis of lignocellulosic biomass originate from the degradation of cellulose, hemicellulose, and lignin.

AD is a well-established technology for the treatment of wastewater and organic wastes. Nevertheless, AD of these wastes streams is frequently accompanied by the accumulation of aromatic compounds (Yan et al., 2020; Zheng et al., 2014). Aromatic compounds usually exhibit recalcitrance to biological processes. Thus hazardous AAs from dye degradation or aromatic organics from lignocellulose degradation have been the focus of several investigations (Albuquerque et al., 2005; Bafana et al., 2011; Barakat et al., 2012, 2013; Liu et al., 2019; Quemeneur et al., 2012; Yan et al., 2020). This chapter presents an in-depth analysis of the fate of recalcitrant compounds in the anaerobic treatment of wastewater and biomass. In addition, strategies for the mitigation of these recalcitrant substances are also discussed.

8.2 Anaerobic treatment of recalcitrant rich wastewaters

AD is a promising technology for the treatment of a wide range of industrial effluents (van Lier et al., 2015). Nevertheless, previous studies have shown that for some industrial wastewaters, the anaerobic process is limited since the effluent generated may contain recalcitrant organic substances, such as AAs, which could contaminate the receptive environmental sink (Bafana, 2011; Pandey et al., 2007).

The anaerobic/aerobic sequential biological process has demonstrated its effectiveness for decolorization and even a complete mineralization of AAs from azo dye degradation (Gadow and Li, 2020; van der Zee and Villaverde, 2005; van Lier et al., 2001). In the anaerobic step, the azo bond is reductively cleaved resulting in colorless aromatic amines, which are subsequently metabolized under aerobic conditions (Sandhya, 2010). The rate of color removal in the anaerobic step is related to the type of organic electron donor and the biochemical structure of the dye. The aerobic step was found to be related to the microbial activity, together with the structure of AAs generated (Popli and Patel, 2015). Fig. 8.1 shows an example of azo dye biological reduction in anaerobic conditions and the following aerobic conversion of the produced AAs.

During combined anaerobic-aerobic treatment of azo dyes, color removal particularly occurs in the anaerobic phase, although additional color removal may occur in the aerobic phase (Pearce et al., 2003, Sosath et al., 1997, Tan et al., 2000). Albahnasi et al. (Albahnasi et al., 2020a) observed that the number of aromatic rings in AAs, together with the existence of an electron-donating group (EDG) or an electron withdrawal group (EWG) influence the removal efficiency of AAs from wastewater by anoxic-aerobic membrane reactors. Authors reported that AAs with one aromatic ring and EDG

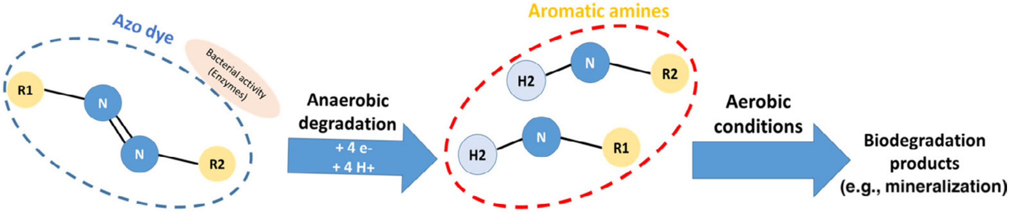

Fig 8.1 Azo dye biological treatment by anaerobic/aerobic process. (From Prasad et al., 2017; dos Santos, 2005; Van der Zee, 2002.)

(e.g., -NH_2, -CH_3) were effectively biodegraded under aerobic conditions, while AAs with two aromatic rings and an EWG (e.g., Cl) have a low biodegradation efficiency. Moreover, authors observed that in addition to the presence of EDG and/or EWG, the number of ring substitutions significantly affects the removal efficiency of AAs. Thus they found that AAs with two or more EDG and no EWG such as 2,4,5-trimethylaniline, 2,6-dimethylaniline, 2,4-dimethylaniline, o-toluidine, and 2,4-diaminoanisole were removed efficiently. Nevertheless, the removal efficiency for AAs containing both EDG and EWG was low, except for the removal of 4,4-diaminodiphenylmethane (with two aromatic rings) of which the two amino groups are considered as a strong EDG. In an early study of Sosath et al. (Sosath et al., 1997), authors studied azo dye removal using a rotating disk bioreactor type for both the anaerobic and aerobic phases and reported a color elimination in the anaerobic step ranging from 90% to 95% and a recovery of 76% of AAs at this stage, which were greatly removed in the subsequent aerobic phase.

Tan et al. (Tan et al., 2000) studied biodegradation of sulfonated azo dyes in a combined sequential anaerobic and aerobic bioreactor. In the anaerobic step, sulfonated azo dyes were reduced into AAs (sulfonated AAs), which were degraded in the following aerobic step. Specifically, the sulfonated azo dye used was Mordant Yellow 10 (MY10), which was anaerobically biotransformed into 5-aminosalicylic acid (5-ASA) and sulfanilic acid (SA). Whereas 5-ASA was easily degraded in the aerobic step, SA was recalcitrant, likely due to the lack of SA-degrading bacterial activity in the bioreactor. Authors enriched the bacterial culture of the bioreactor to reach SA degradation through a bioaugmentation step, which led to SA mineralization. Authors found that anaerobic-aerobic treatment with bioaugmentation strategy in the aerobic phase allowed for 100% of AA removal.

Similarly, Panswad et al. (Panswad et al., 2001) studied bench scale sequencing batch reactors and a combination of anoxic, anaerobic, and aerobic phases during the treatment of synthetic dyes and reached a color removal of around 70% and 8% in anaerobic and aerobic phases, respectively. Authors emphasized that the observed color removal was positively correlated with the duration of the anoxic + anaerobic phase. Recently, Gadow et al. (Gadow et al., 2020) studied an integrated continuous anaerobic-aerobic treatment system for the treatment of recalcitrant textile wastewater containing 2-Naphthol Red dye. Authors found that the combined systems reached 98.4% and 98.9% of color and chemical oxygen demand (COD) removal, respectively, at a hydraulic retention time (HRT) of 6 days, while applying an HRT of 3 days led to a decrease in the upflow anaerobic sludge blanket (UASB) reactor performance.

In the same line, Assadi et al. (Assadi et al., 2018) studied the combined effect of HRT with high concentrations of nitrate ions and salt addition to an anaerobic-aerobic sequencing batch reactor fed with wastewater and azo dyes. Authors found that up to 98% of color removal was achieved during the anaerobic phase and that the HRT increase led to a partial mineralization of COD and intermediates. Moreover, authors highlighted that increasing salt and nitrate ion supplementation to the reactor led to decreased color and COD removal efficiencies. In addition, authors stressed the need for long adaptation periods for the microbial community in the aerobic phase for reaching high removal efficiencies.

Plumb et al. (Plumb et al., 2001) characterized the microbial populations of an anaerobic baffled reactor, successfully treating industrial dye waste from food industry, which contained azo and other dye compounds. They found abundance of *Proteobacteria* and bacteria in the Cytophaga-Flexibacter-Bacteroides phylum, as well as sulfate-reducing bacteria. Moreover, they reported the existence of a methanogenic population dominated by *Methanosaeta* sp. and containing species of *Methanobacterium* and *Methanospirillum*, as well as the scarcely studied methanogen, *Methanomethylovorans hollandica*.

Anthraquinone dyes are the second largest class of dyes after azo dyes. They are generally resistant to degradation in comparison with azo dyes because of their complex and stable structures. Anthraquinone dyes contain anthraquinone chromophore groups, which comprise two carbonyl groups on both sides of a benzene ring (Fig. 8.2).

Lee et al. (Lee et al., 2006) reported that the methanogenic activity was strongly inhibited by increasing organic loading rates (OLRs) when anthraquinone dyes were treated anaerobically, resulting in low color removal. However, depending on the exact biochemical structure, anthraquinone dyes may be mineralized in aerobic, activated sludge-based bioreactors. Authors concluded that the partial color elimination of phthalocyanine and anthraquinone dyes is only feasible by using a combination of anoxic and anaerobic operation conditions.

In a recent study (Cai et al., 2021), authors investigated the effect of resuscitation-promoting factor (Rpf) protein strategy on the biodegradation of anthraquinone dye (Reactive Blue 19 [RB19]) in the AD process. Authors reported an increase in color removal by more than 20% due to stimulating the bacterial growth and recovering the viable but noncultural bacteria due to unfavorable environmental conditions, as well as the enrichment with some dye-degrading species of the family Peptostreptococcaceae such as *Clostridium perfringens* and *C. bifermentans*, due to Rpf addition, in comparison to the control anaerobic digester. Finally, authors deduced that Rpf addition has efficiently influenced the anthraquinone conversion into simple metabolites and led to the dye mineralization. Authors revealed through the GC-MS analysis that there were two main pathways of dye decolorization in the studied anaerobic process. The breakdown of the

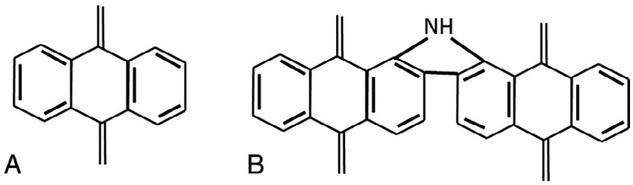

Fig 8.2 Molecular structure of anthraquinone (anthracene-9, 10-dione) (A) and an example of a commercial dye, Vat Yellow 28 (B).

C-N groups of the AAs $C_8H_{11}NO_6S_2$ and the breakdown of the C-S group of this latter into $C_2H_6O_7S_2$ with a lower molecular weight led to a complete mineralization of the anthraquinone dye. Authors emphasized that the combination of Rpf addition and temperature increase from ambient to 35°C significantly improved the AD performance.

Regarding the effect of temperature and the redox mediator on anthraquinone dye degradation, Santos et al. (Santos et al., 2005) found that thermophilic (55°C) AD shows a better performance than the mesophilic one (30°C). Thus higher decolorization rates were observed for themophilic AD of industrial wastewater containing anthraquinone dyes as well as of model compounds. Moreover, kinetics parameters showed that the first order rate constant "k" of RB5 was 11-fold and 6-fold higher at thermophilic temperature for the reactors supplemented of anthraquinone-2-sulfonic acid (AQS) and the control reactors, respectively. Nevertheless, they found that the anthraquinone dye RB19 was strongly toxic to methanogens at both temperature conditions with inhibitory concentrations of 55 mg/L and 45 mg/L for mesophilic and thermophilic conditions, respectively.

In another attempt, Berkessa et al. (Berkessa et al., 2020) used an anaerobic dynamic membrane bioreactor (AnDMBR) for the treatment of anthraquinone dye textile wastewater and reported a soluble COD and color removal of 98.5% and more than 97.5%, respectively. Authors found that the extracellular polymeric substance ending up in the effluent accounted for more than 76.7% of low-molecular-weight fractions, which facilitate their final mineralization. Moreover, authors indicated that the archaeal community was relatively stable, although less diversified, while bacterial community changes throughout the AD process. Finally, authors emphasized that the use of an AnDMBR process is cost effective for anthraquinone dye wastewater treatment due to the rapidly formed decent dynamic layer, enhancing a higher performance.

Costa et al. (Costa et al., 2012) also studied the catalytic effect of the redox mediator (RM) anthraquinone-2,6-disulfonate (AQDS) in the acceleration of the decolorization of both azo dyes and anthraquinone dyes in the AD process. Authors found that AQDS was very effective in enhancing the decolorization of azo dyes but hardly affected the color removal rates of anthraquinone dyes. The large difference in effect was explained by the molecular structure of anthraquinone dyes, which are characterized by a stable structure, hampering efficient electrons transfer.

Other researchers (da Silva et al., 2012) compared the effectiveness of AD removal in one- and two-stage anaerobic systems, with a focus on the impact of AQDS as RM in the process. They found that color removal efficiency was higher in the two-stage anaerobic system (62%) than the one-stage process (33%), both efficiencies without RM supplementation. Moreover, RM addition increased the electron transfer to the dyes and further increased the removal efficiencies in both systems, proportionally. In agreement with other researchers, da Silva et al. (da Silva et al., 2012) also observed a decrease in the efficiency of anaerobic systems due to the increase in the dye concentration in the feedstock.

8.3 Anaerobic digestion of lignocellulosic biomass

Lignocellulosic biomass has been widely regarded as an interesting and promising renewable source for the generation of bioenergy and valuable products (Ahmed et al., 2019; FitzPatrick et al., 2010). Lignin derivatives are components of wastewater streams generated in the chemical processing of wood (Sierra-Alvarez and Lettinga, 1991), fine sieved fraction in raw sewage (Ghasimi et al., 2016), agroindustrial wastes (Aboudi et al., 2016; Kumar et al., 2009; Barakat et al., 2012), sewage sludges (Angelidaki et al., 2000), slaughterhouse wastes (Levén et al., 2012), etc. Lignin derivatives can be either present in wastewater or generated during the hydrolysis of lignocellulosic matter through biological processes and pretreatment techniques (Monlau et al., 2012; Sierra-Alvarez and Lettinga, 1991; Taherzadeh and Karimi, 2008).

8.3.1 Composition of lignocellulosic biomass

Lignocellulosic biomass is a matrix of mainly three organic compounds: cellulose, hemicellulose, and lignin. These compounds are extensively described and studied elsewhere (Hendriks and Zeeman, 2009; Kumar et al., 2009; Olsson and Hahn-Hägerdal, 1996; Sierra-Alvarez and Lettinga, 1991). However, a brief description is provided here.

Cellulose is a linear polymer composed of D-glucose subunits linked by β-(1→4) glycosidic bonds. Each unit formed through this linkage is called cellobiose. Cellulose polymers are linked together by hydrogen and van der Waals bonds ((Kumar et al., 2009; Olsson and Hahn-Hägerdal, 1996). Crystallinity is the main characteristic of cellulose, and a small proportion of unorganized cellulose chains forms amorphous cellulose. Therefore cellulose is more likely to be degraded by enzymes in its amorphous form (Kumar et al., 2009). The breakage of β-(1→4) glycosidic bonds by acid or enzymatic activity leads to the production of fermentable D-glucose (Kumar et al., 2009).

Hemicellulose is composed of linear polymeric branches with different sugars, including xylose, rhamnose, and arabinose from C5; glucose, mannose, and galactose from C6; and uronic acids such as D-glucuronic and D-galactouronic acids. The sugars contained in hemicellulose depend on the origin of the lignocellulosic biomass, and the hemicellulose structure is not crystalline and hence is easier to hydrolyze than cellulose (Taherzadeh and Karimit, 2008). Lignin is amorphous with an irregular three-dimensional structure and is composed of cross-linked phenolic polymers. Lignin is responsible for giving the lignocellulosic biomass its resistance to microbial activity, impermeability, and structural support. The chemical structure is highly complex in comparison to cellulose and hemicellulose. Lignin is formed by three phenyl propionic alcohols units, which are guaiacyl (G), p-hydroxyphenyl (H), and syringyl (S). These phenolic monomers are linked together by alkyl-aryl, alkyl-alkyl, and aryl-aryl ether bonds (Huang et al., 2019; Palmqvist and Hahn-Hägerdal, 2000; Rasmussen et al., 2014; Roy et al., 2020).

8.3.2 Fate of recalcitrant compounds in anaerobic digestion of lignocellulosic biomass

The hydrolysis and breakdown of lignocellulosic biomass depends on several factors such as the origin and inherent properties of this biomass as well as the hydrolysis conditions such as temperature, pH, redox potential, and pretreatment conditions (Barakat et al., 2013; Du et al., 2010; Huang et al., 2019; Larsson et al., 1999; Rasmussen et al., 2014).

There has been growing interest among researchers in the AD of lignocellulosic biomass (Aboudi et al., 2016; Barakat et al., 2012; Monlau et al., 2014; Wang et al., 2020). Hydrolysis of macromolecules is the first and the rate-limiting step in AD of slurries and solid waste (Ghasimi et al., 2016; Millati et al., 2020; Wang et al., 2020), which can be enhanced applying physicochemical or biochemical pretreatment (Kumar et al., 2009; Mirmohamadsadeghi et al., 2021; Sun et al., 2020; Taherzadeh and Karimi, 2008). However, pretreatment can also lead to the release of intermediates coming from the lignocellulosic matrix, which, at certain concentrations and operating conditions, could inhibit the anaerobic process (Ahmed et al., 2019; Palmqvist and Hahn-Hägerdal, 2000). The type and concentration of released intermediates during pretreatment depend on the origin of the lignocellulosic biomass, the applied pretreatment techniques, and the prevailing operational conditions (Alvira et al., 2010; Du et al., 2010; Rasmussen et al., 2014). During acidogenesis of the hydrolyzed monomers, the intermediates hydrogen and volatile fatty acids (VFA) are produced. However, also acidogenesis can be affected by the release of lignocellulosic derivatives in the medium (Hu et al., 2019; Kaparaju et al., 2009; Quemeneur et al., 2012).

Intermediates derived from lignocellulosic biomass include phenolic compounds, furans, and aliphatic acids (Barakat et al., 2012; Gonçalves et al., 2020; Hu et al., 2019; Palmqvist and Hahn-Hägerdal, 2000). Pentose (C5) degradation leads to the formation of furfural, while hexose (C6) degradation gives HMF. HMF can be further degraded into levulinic and formic acids. Rasmussen et al. (Rasmussen et al., 2014) reported that furfural formation from xylose depends on the site of protonation (i.e., whether this is on the hydroxyl group of xylose or a direct protonation of the pyranose oxygen). Moreover, they indicated that at least four pathways for the formation of HMF from glucose and three pathways for furfural formation from xylose are possible. Rasmussen et al. (Rasmussen et al., 2014) also reported the formation of humins from HMF, meaning that biomass monosaccharides may react further at elevated temperatures to form pseudolignin and humins. Yoon et al. (Yoon et al., 2014) found that the high reaction temperature caused the severe degradation of formed monosaccharides at the range of 100°C to 120°C. Sipponen et al. (Sipponen et al., 2014) found that pseudolignin formation from wheat straw occurs within the temperature range of 170°C to 200°C. Kumagai et al. (Kumagai et al., 2016) reported the formation of pseudolignin and humins at 200°C during hot compressed water pretreatment of Hinoki Cypress.

The degradation of lignin leads to the generation of phenolic compounds, specifically lignin with guaiacyl, and syringyl units lead to the production of vanillin and syringaldehydes as intermediates, respectively.

Humic substances (HS) are generally derived from the decomposition and transformation of organic matter of plant and animal residues, microorganisms, sludge, and compost (Azman et al., 2015; Liu et al., 2019). They are also used in some industries (e.g., food industry) (Liu et al., 2019). On the basis on their solubility, HS have been classified in three categories: humic acid (HA), fulvic acid (FA), and humins. Besides, it has been reported that humins could be generated from glucose under thermal hydrolysis conditions, mainly from the reaction of HMF with glucose and/or the reaction of HMF with 2,5-dioxo-6-hydroxy-hexanal (hydrated HMF) and subsequent polymerization (Dee et al., 2011; Patil and Lund, 2011; Rasmussen et al., 2014), or from phenolic compounds (Fernandes et al., 2015). HS are characterized by the presence of several active functional groups, such as carbonyl and carboxyl groups, through which they may interfere in physicochemical reactions (Liu et al., 2019; Liu et al., 2015; Li et al., 2019; Lipczynska-Kochany, 2018). It has been reported that during AD, HS affect the biomass degradation pathways, mainly by the inhibition of hydrolysis and methanogenesis (Azman et al., 2015, 2017; Brons et al., 1985; Li et al., 2019; Liu et al., 2015, 2019). Fernandes et al. (Fernandes, 2015) suggested that the presence of HS may negatively impact hydrolysis due to potential binding of hydrolytic enzymes or impacting hydrolytic bacterial groups. Azman et al. (Azman et al., 2017) and Li et al. (Li et al., 2019) suggested that the competition for electrons would negatively impact methanogenesis.

Fig. 8.3 shows the main intermediates resulting from lignocellulose degradation.

Alvira et al. (Alvira et al., 2010) reported that pretreatment is necessary for altering the structural characteristics of lignocellulosic biomass to increase the glucan and xylan accessibility for enzymatic attack. Similarly, Bali et al. (Bali et al., 2015) reported that pretreatment techniques modify the polysaccharides and lignin content, thus enhancing the accessibility for cellulase enzymes. Rai et al. (Rai et al., 2019) reported that efficient degradation of lignocellulosic biomass requires the presence and synergy between several enzymes, including modular and nonmodular glycosyl hydrolases (endoglucanase, exoglucanase, β-glucosidase, endoxylanase, β-xylosidase, α-arabinofuranosidase, α-glucuronidase, α-galactosidases, and β-mannosidases), carbohydrate esterases (combinatorial extension "CE" proteins), and other auxiliary enzymes (lytic polysaccharide monooxygenases "LPMOs", cellobiose dehydrogenase "CDH", and laccases).

The possible inhibitory effect of intermediates is dependent on their chemical structure, concentration, and operational conditions (Barakat et al., 2012; Ghasimi et al., 2016; Jönsson and Martín, 2016; Rasmussen et al., 2014). In addition, the inhibitory level depends on the type of microorganisms involved in the process as well as their metabolism and the ratio of microorganisms/substrate in the medium (Akobi et al., 2016; Monlau et al., 2014).

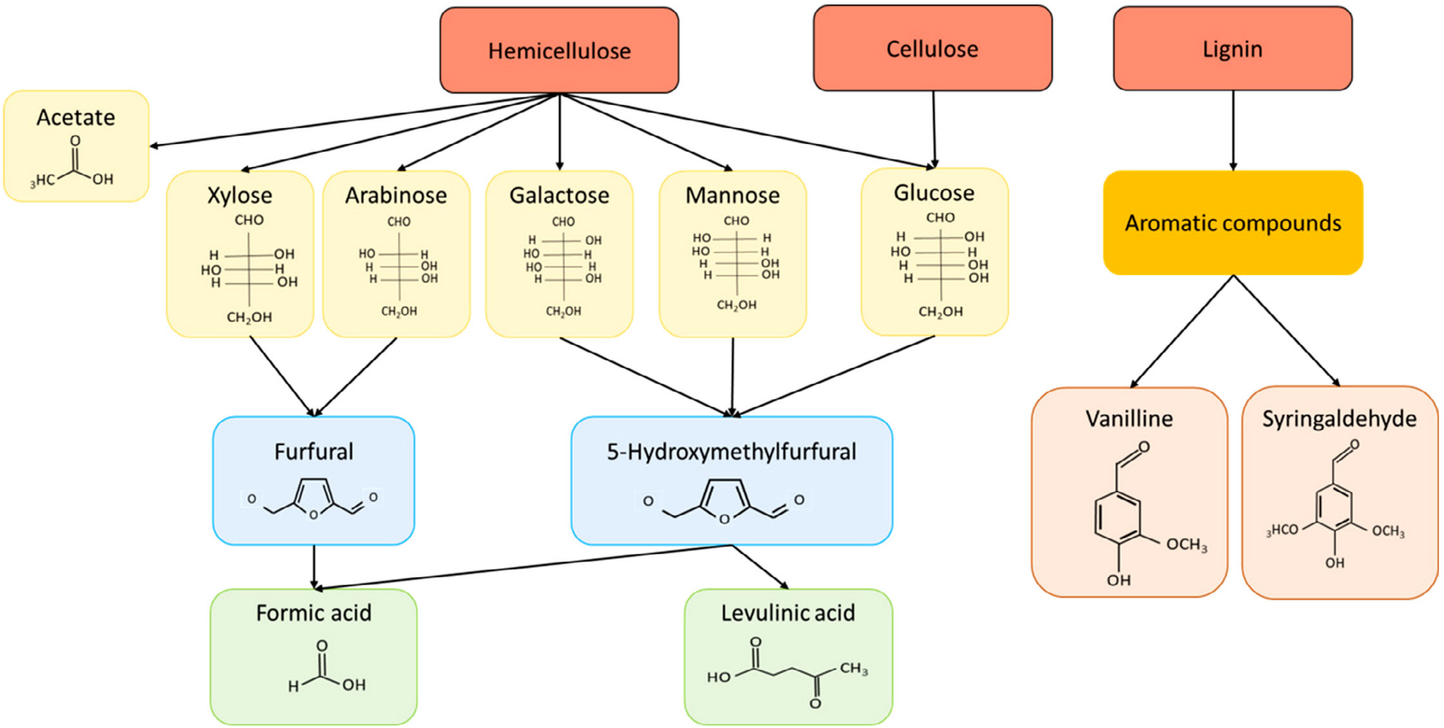

Fig. 8.3 The main lignocellulose-derivative compounds after degradation and hydrolysis of lignocellulosic biomass. (From Jönsson and Martín, 2016; Jönsson et al., 2013; Palmqvist and Hahn-Hägerdal, 2000; Taherzadeh and Karimi, 2008.)

Other authors (Sierra-Alvarez and Lettinga, 1991) reported that the toxicity of lignin intermediates is related to the molecular weight, as well as the presence of specific functional groups on the aromatic ring. Results showed that phenolic compounds with low molecular weight were found to be more toxic to microorganisms than the compounds with high molecular weight (Palmqvist and Hahn-Hägerdal, 2000; Rasmussen et al., 2014; Sierra-Alvarez and Lettinga, 1991). The mechanisms of inhibition by phenolic compounds rely on damaging the microbial cells by a selective alteration of the membrane permeability and therefore producing a leakage of intracellular components and inactivation of essential enzymatic systems (Campos et al., 2009; Monlau et al., 2014; Palmqvist and Hahn-Hägerdal, 2000; Zhao et al., 2012).

Moreover, intermediates with aldehyde groups or apolar substituents were more toxic than those with carboxylic groups. Derivative compounds with aldehyde groups have shown to inhibit methanogens (Barakat et al., 2012). Similar is the case for furan-type of compounds, for which furfural has shown to be more inhibitory than HMF due to its low molecular weight in comparison to HMF (Barakat et al., 2012; Quenemeur et al., 2012). Palmqvist and Hahn-Hägerdal (Palmqvist and Hahn-Hägerdal, 2000) reported that in the AD process of lignocellulosic biomass, the conversion rate of HMF is lower than that of furfural, leading to a longer lag-phase period and lower methane yields.

Furthermore, the mechanisms of inhibition by furans are related to cell growth inhibition by damaging their DNA and altering the activity of several enzymes involved in the glycolysis pathway (Almeida et al., 2007; Palmqvist and Hahn-Hägerdal, 2000). In addition, derivative compounds with high hydrophobicity might affect the integrity of microbial cell membranes (Monlau et al., 2014). Lin et al. (Lin et al., 2015) reported that phenolic compounds exhibited stronger inhibition on hydrogen production from glucose than furan derivatives. Thus vanillin showed longer lag-phase periods for hydrogen production and glucose consumption compared to furfural, which likely can be attributed to the consumption of reducing equivalents leading to the reduction of R-CHO to RCH_2OH. In this line, Levén et al. (Levén et al., 2012) analyzed the AD of organic wastes in the presence of phenolic compounds and they reported that the mineralization of phenols in methanogenic consortia follows different pathways and requires a consortium of various microorganisms.

Liu et al. (Liu et al., 2019) studied the mesophilic digestion of activated sludge containing HS, with emphasis on the content and structure of HA and fulvic acid FA. The dynamic changes in HA and FA structural compositions were related to the loss of aliphatic moieties and the enrichment in aromatic components. Thus it was concluded that the changes in the structural composition of HS led to an increase in the humification degree, aromaticity, and amounts of oxygen-containing functional groups. Moreover, the comparison of HS in liquid or solid phase has shown different patterns of inhibition of the anaerobic process. Hence the concentrations of HA and FA in the solid phase decreased gradually but constantly with digestion time by 6.7% and 40.9%, respectively, whereas the concentrations of HA and FA gradually increased by 48.3% and 43.9% in the liquid phase, respectively. The difference was attributed to the higher number of oxygen-containing groups in HA and FA in the liquid phase in comparison to the solid phase. Furthermore, it has been found that FA is more easily humified than the HA due to the simpler structure and low molecular size.

In the same way, Li et al. (Li et al., 2019a) studied the impact of HA on a mesophilic semicontinuous anaerobic digester treating excess sludge, with a focus on the impact of HA on the associated enzyme and microorganism dynamic. It was observed that increasing the HA:VSS ratio leads to an increase in the inhibitory effect of HA by lowering the α-amylase and proteinase involved in hydrolytic activities and the F420 coenzymes involved in methanogenesis. This inhibition was likely related to electrostatic forces, covalent bonding, and sweep flocculation between HA and the associated enzymes, which corroborates their results from a previous similar study (Li et al., 2019b). An interesting observation was an increase in the acidogenic enzyme activity, mainly the pyruvate-ferredoxin oxidoreductase, acetate kinase, phosphotransacetylase, butyrate kinase, and phosphotransbutyrylase enzymes, proportionally to the increase in HA concentrations. These finding were also corroborated from other studies (Azman et al., 2015; Li et al., 2019b). Therefore, for dark fermentation processes for the production of hydrogen and VFA as valuable chemicals, HA addition at certain concentrations may favor and enhance the product yield. A possible explanation is that HA acts as a terminal electron acceptor to accept the "surplus electrons" from long-chain fatty acid oxidation to acetate leading to more favorable thermodynamic conditions and making the fermentative processes independent of partial hydrogen pressure (Li et al., 2019a; Liu et al., 2015). The microbial analysis of the study carried out by Li et al. (Li et al., 2019a) demonstrates a decrease in acidogenic and methanogenic microorganisms as HA concentrations were increased in digesters, indicating that in addition to effect of HA on the bioconversion of substrates, HA also affected the microorganism's growth. Comparison of results obtained in semicontinuous digester and batch assays (Li et al., 2019b) at the same HA dosages indicated that the semicontinuous strategy did not result in a higher efficiency as was expected and that under the specific operating conditions adaptation of microorganisms did not occur.

In one study (Ghasimi et al., 2016), authors investigated the effect of HA on the AD process under both thermophilic and mesophilic conditions and reported that HA dosages below 2 g/L did not inhibit methanogenesis at both operating temperatures. Moreover, the AD of primary sludge and HA of 0.8 g/L concentration, passed through fine sieve, has resulted in a higher methane yield under thermophilic conditions, while under mesophilic conditions a similar production as that of the control was observed. Nevertheless, when sodium acetate at a concentration of 0.5 g/L was used as the substrate, HA slightly affected the rate of methanogenesis and led to higher

CH_4 production than obtained from the control. These findings indicate that at lower concentrations, HA could favor the digestion process. Earlier studies have shown that only the hydrolytic stage of AD of some substrates is inhibited by HS humins (Brons et al., 1985), whereas recent research demonstrated that methanogenesis could also be affected depending on the substrate used, the HA concentrations, and operating conditions (Azman et al., 2015; Ghasimi et al., 2016; Li et al., 2019a, 2019b; Liu et al., 2015; Zhou et al., 2014).

Regarding the effect of operating conditions on the release of lignocellulose derivatives in the medium, it has been reported that at low pH values, furfural and 5-HMF are released, while at high pH values phenolic compounds are generated (Alvira et al., 2010; Monlau et al., 2014; Taherzadeh and Karimi, 2008). Ghasimi et al. (Ghasimi et al., 2016) compared mesophilic and thermophilic temperature conditions in AD of lignocellulosic biomass (fine sieve fraction of raw sewage) and reported that temperature plays an important role in the tolerance of microbial community to lignocellulose derivatives. Thermophilic microorganisms were more sensitive to increased concentrations of furfural and HMF than the mesophiles. Thus lower methane yields and longer lag-phase periods were observed at high temperature conditions. Nevertheless, for the specific methanogenic activity assays (SMA), the high concentration of furfural and HMF intermediates (2 g/L) led to low methane production rates for both temperatures. Interestingly, the authors observed the existence of a synergistic effect of the added intermediates and the used lignocellulosic substrate under mesophilic conditions. It resulted in higher methane productions than the control for all derivative compounds (furfural, HMF, vanillin, and HA). The synergistic effect was only observed for HA addition under thermophilic temperature conditions.

Researchers (Levén and Schnürer, 2005) examined the effect of temperature on the degradation efficiency of different aromatic compounds by a mesophilic or a thermophilic anaerobic microbial community. At mesophilic temperature, a successful mineralization of benzoic acid, phthalic acid, methylphthalate, phenol, m-cresol, and p-cresol occurred. Benzoic acid was the sole aromatic compound mineralized under thermophilic conditions due to the blockage of the channelling reactions to the central intermediate benzoyl-CoA in the thermophilic microbial community. Hence they concluded that the degradation efficiency of aromatic compounds by the mesophilic and thermophilic microbial communities in the anaerobic digesters could be related to the differences caused by selective pressure on the composition of the microbial communities or by a direct effect of temperature on the enzyme activity.

The effect of lignocellulose derivatives on biological processes depends on the presence of a single compound or several derivatives together in the medium. Hu et al. (Hu et al., 2019) studied the individual and synergistic effects of different lignocellulose-derived compounds on biohydrogen production, using the pure culture of *Thermosaccharolyticum MJ1*. It was reported that ferulic and p-coumaric acids showed stronger inhibition than other intermediates. Hence the growth of *MJ1* was completely inhibited when concentrations of ferulic and p-coumaric acids were higher than 1.5 g/L and 0.5 g/L, respectively. Moreover, the increase in furfural and HMF concentrations slightly affected the growth in comparison to the control, without a remarkable inhibitory effect even at concentrations of 2 g/L. However, concerning biohydrogen yields, the inhibitory effect of vanillin and syringaldehyde was much stronger than for the *MJ1* growth. Authors deduced that vanillin and syringaldehyde inhibited the biohydrogen production mainly due to the redirection of metabolic flux, while the rest of intermediates inhibited the biohydrogen production by altering the growth of the microorganism. Interestingly, syringaldehyde at a low concentration of 0.25 g/L even had a positive effect on *MJ1* growth, while higher concentrations led to a slight decrease without a remarkable inhibitory effect. Generally, authors observed that for all the studied derivative compounds, biohydrogen production gradually decreased with increasing inhibitor concentrations and that the *MJ1* strain was very sensitive to p-coumaric acid addition leading to low biohydrogen production. It has been reported that the effect of lignocellulosic-derivative compounds on a biological process depends on the strain involved in the process as well.

In contrast to the findings of Hu et al. (Hu et al., 2019) Cao et al (Cao et al., 2010) previously observed that the growth of *T. thermosaccharolyticum W16* was completely stopped at concentrations of 1.5 g/L of HMF, 2.0 g/L of furfural, and 1.5 g/L of syringaldehyde. Nevertheless, they found that the vanillin addition had no severe inhibition on growth even at the concentration of 2 g/L. In the same line, Giraldeli et al. (Giraldeli et al., 2018) reported that *C. acetobutylicum ATCC 824* metabolized HMF more efficiently than *C. beijerinckii Br21,* revealing that *ATCC 824* is a robust strain for H_2 production from biomass hydrolysates in comparison to *Br21.* They also observed that *C. beijerinckii Br21* could convert organic acids effectively, which makes this a more robust strain when dealing with the presence of organic acids in combination with HMF. In addition, they found that levulinic and formic acids have a stimulatory effect on H_2 production by *C. beijerinckii Br21,* whereas their combination with HMF induced an inhibitory effect. Hence they deduced that the mixture of HMF with levulinic and formic acids has shown to have an inhibitory effect on the lactate and ethanol pathways, while levulinic and formic acids mainly affected the H_2 production by *C. acetobutylicum ATCC 824* through the acetate pathway (the most) and the butyrate pathway (the least).

The substrate concentration in presence of lignocellulose derivative compounds also seems to play an important role in the methane production rates. Ghasimi et al. (Ghasimi et al., 2016) observed that for SMA assays, the initial substrate concentration (i.e., a concentration of 1 g/L sodium acetate) did not affect the methane production rates while at lower substrate concentration (0.5 g/L), methane production rates were affected with different patterns for each intermediate. Vice versa, the carbon source used in the biological process also affects lignocellulose derivative compound degradation.

Other researchers (Gonçalves et al., 2020) studied the removal of syringaldehyde as the sole carbon source and with the addition of two mixed cosubstrates (i.e., glucose and sodium acetate) in thermophilic batch AD assays. They reported that syringaldehyde was hardly removed when used as a sole carbon source, while in codigestion with glucose and sodium acetate it was degraded efficiently, reaching a COD removal of 92%.

Based on these results, it can be concluded that the effect of lignocellulose derivatives on biological processes could be either due to one specific intermediate or to the synergy in a mixture of two or more derivative compounds (Ghasimi et al., 2016; Giraldeli et al., 2018; Hu et al., 2019). Furthermore, it has been deduced that the concentration of lignocellulose derivative compounds has a crucial effect on microorganisms, which generally have more tolerance to lower concentrations with even a stimulatory effect than for high concentrations.

8.3.3 Mitigation of inhibitory effect of lignocellulosic biomass derivatives

Several strategies and approaches have been suggested to mitigate the inhibitory effect of lignocellulose derivatives in biological processes (Cantarella et al., 2004; Jönsson et al., 2013; Palmqvist and Hahn-Hägerdal, 2000; Qui et al., 2011). A posttreatment as a detoxification or conditioning method is proposed for eliminating by-products from the lignocellulose hydrolysates. Possible successful application depends on the type of the lignocellulosic biomass together with the nature or concentration of derivatives in hydrolysates (Shilpa, 2014). Alternative detoxification methods include physical treatment (heating and evaporation, steam stripping, electrochemical), chemical (chemicals addition), or biological (enzymatic and microbial). For preventing accumulation of intermediates, high inoculum to substrate ratios are commonly adopted. However, at industrial scale this strategy also has been shown to be less effective (Chung and Lee, 1985; Issah et al., 2020; Wingren et al., 2003). Nonetheless, the approach is quite effective in those processes where active digestate recirculation is feasible. For instance, the DRANCO process, with recirculation of a large proportion of the outgoing digestate to inoculate the raw material input, leads to a good mixing and has shown to be effective for dry and semidry AD of municipal solid wastes and lignocellulosic biomass (De Baere, 2010; De Baere and Mattheeuws, 2012; Ghasimi, 2016).

Ghasimi et al. (Ghasimi et al., 2016) investigated different inoculum to substrate ratios for the AD of lignocellulosic biomass fine sieve fraction (FSF) and demonstrated that using batch or horizontal plug flow reactors with recirculation of the digestate waste is of interest for the AD of the lignocellulosic biomass (FSF) with a significant biogas production potential and low capital costs. In that case, the estimated minimal recirculation rate for the thermophilic digestion of FSF would be approximately 50% with contact time of 7.5 days (amount of recirculated inoculum [QR] of 3.47 m^3/d and recirculation factor [R] of 1). These authors also found that the use of higher recirculation rates could result in incomplete substrate digestion, leading to a lower methane production per kg of substrate.

Azman et al. (Azman et al., 2015) studied the impact of multivalent cation addition to counteract the inhibitory effects of humic compounds in anaerobic digesters treating cellulosic biomass and reported that the addition of magnesium, calcium, and iron salts significantly mitigated the inhibitory effects of HA, reaching higher hydrolysis efficiencies of 75%, 65%, and 72%, respectively, whereas potassium and sodium salt addition did not mitigate the inhibition showing lower hydrolysis efficiencies. Besides, the positive effect of the multivalent cations was also clear from the higher observed methane yields. The mitigation of the HA inhibitory effects by multivalent salt addition was due to inactivation and reduction of active sites of HA. Moreover, the affinity of each cation to HA was different for each salt. The dosage of the used salts was said to be crucial for the mitigation effect. In another study, Azman et al. (Azman et al., 2017) reported that the addition of hydrolytic enzymes contributed to reverse the negative effects of HA in anaerobic digesters. Cheng et al. (Cheng et al., 2015) used activated carbon adsorbent to remove lignocellulose derivatives from the hydrolysates obtained by microwave-assisted dilute H_2SO_4 and cellulose pretreatments of hyacinth plant. Removal efficiencies of 84.8%, 45.4%, and 39.5% for vanillin, HMF, and furfural, respectively, were obtained. Other researchers (Hu et al., 2018) used laccase, $NaBH_4$, and washing as a detoxification of hydrolysates from pretreated sugarcane bagasse prior to the dark fermentation process. It was observed that the best studied strategy was the washing, which led to 10 times higher hydrogen production in comparison to the nondetoxified hydrolysate and that detoxification by laccase or $NaBH_4$ were not suitable for biohydrogen fermentation by the studied bacterial strain (*Thermoanaerobacterium thermosacchar-olyticum*).

Another strategy for mitigating the inhibitory effect of lignocellulose derivatives on bioconversion consists of bioabatement, which involves microorganisms such as fungus or bacteria, which have the ability of metabolizing and removing the inhibitors from the hydrolysate selectively. This being chemical free does not generate toxic or nonbiodegradable waste, and is scalable (Nichols et al., 2008, 2010; Ran et al., 2014; Singh et al., 2017,2019). Singh et al. (Singh et al., 2017) studied the use of the isolated bacterial strain *Bordetella* sp. *BTIITR* for the degradation of lignocellulose derivatives and reported a significant removal of HMF and furfural from lignocellulosic hydrolysate liquor without significant consumption of sugars. However, it was reported that the use of free cells requires an additional downstream step of cell separation from the process stream, which led to a longer and expensive process. In a recent study, Singh et al. (Singh et al., 2019) suggested the alternative of immobilizing the cells of the studied strain within chitosan beads, and the beads were utilized to detoxify hydrolysate liquors. A degradation of 86% HMF + 100% furfural from sugarcane bagasse hydrolysate liquor was obtained. The use of immobilized cells has the advantages of an easy cell recovery, prevention of cell washout, cell recycling in repeated batch operations, and the flexibility of bioreactor designing and improved operational stability. Therefore it was concluded that immobilized cells could detoxify lignocellulosic hydrolysate liquor at a broader range of operating pH, temperature, and inhibitor concentration as compared to the free cells.

In a recent study, Nichols et al. (Nichols et al., 2020) investigated the use of biological conditioning of spent fermentation liquor using fungal strain *C. ligniaria NRRL 30616*, which has been demonstrated to be a specific strain for soluble lignin-based and furan-based inhibitor metabolization (Nichols et al., 2008). The bioabatement reduced the concentrations of furfural, HMF, and phenolic compounds in hydrolysate by 96%, 50.3%, and 66.6%, respectively. Moreover, authors reported that the conditioned hydrolysate by the strain could be reused several times at laboratory scale for the same purpose, which gives a sustainable solution to water use for bioethanol production. Ali et al. (Ali et al., 2020) used two constructed microbial consortia (*CS-5* and *BC-4*), which were developed by the enrichment culture technique, on lignocellulosic biomass degradation and chlorophenol detoxification concomitantly with CH_4 production, reaching chlorophenol degradation efficiencies of 89% and 95 % by each strain, respectively. In addition, the use of both stains simultaneously led to a synergistic action enhancing yields over 64% in comparison to the control assay.

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