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4 **Agave bagasse biorefinery: Processing and perspectives**
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Abstract

Agave bagasse is the main solid waste generated by the tequila industry in Mexico, which is an environmental concern due to its considerable volume of production (377 000 Ton in 2016). Agave bagasse is a lignocellulosic biomass that has been considered as a potential feedstock for different industrial uses in the framework of a lignocellulosic biorefinery. The lignocellulosic biomass is a complex structure constituted by cellulose, hemicellulose and lignin. Therefore, for complete waste revalorization, different processing steps would be required. In this work, the scientific advances towards the agave bagasse biorefinery composed by three sequential stages: pretreatment, treatment, and biofuels production are reviewed. Moreover, the byproducts generated during the process could also be recovered and used for the synthesis of value-added products. This integrative approach of agave bagasse in the conceptualized biorefinery generates positive impacts in environment as well as in local and regional economies.

Keywords: *Biorefinery, agave bagasse, lignocellulosic biomass, byproducts, biofuels.*

1. INTRODUCTION

The blue agave (*Agave tequilana* var. Weber) is a perennial arid plant, cultivated and harvested in Mexico to produce distilled alcoholic beverages, such as tequila, since the 17th century (Murillo-Alvarado et al., 2014; Valenzuela, 2011). The agave plant requires from 8 to 12 years to mature while it accumulates fructans in the stem (commonly called “pinecones”). In the production of tequila, the pinecones are cooked into stone or brick ovens to hydrolyze the fructans to fermentable monosaccharides. Afterwards, the cooked pinecones are grinded and pressed to extract the syrup that will be used in the downstream steps of tequila production. As by-product of the syrup extraction, a fibrous residue called agave bagasse (AB) is generated (Barrera et al., 2016). According to Cedeño-Cruz & Alvarez-Jacobs, (1991), the bagasse waste is equivalent to 40% (dry weight) of the initial mass of processed pinecones. Considering the 2016 agave consumption, reported by the Tequila Regulatory Council, the generation of bagasse for that year was equivalent to 377 000 ton (CRT 2016).

The AB is composed by three main fractions, cellulose (31-43 % w/w), hemicellulose (11-22 % w/w) and lignin (11-20 % w/w) (Arreola-Vargas et al., 2015; Corona-González et al., 2016; Iñiguez-Covarrubias et al., 2001; Perez-Pimienta et al., 2016; Saucedo-Luna, Castro-Montoya, Martinez-Pacheco, Sosa-Aguirre, & Campos-Garcia, 2011), embedded in a heterogeneous matrix. Other compounds can also be present in lower concentrations, e.g. calcium oxalate. The cellulose fraction is a glucose polysaccharide that can form two types of microfibrils, amorphous and crystalline, in different ratios (Kestur et al., 2013; Montiel et al., 2016; Perez-Pimienta et al., 2013). The hemicellulose is a heterogeneous group of polysaccharides constituted by hexoses (mannose, galactose and glucose) and pentoses (xylose and arabinose) (Xuebing & Zhang, 2012). Finally, the lignin is an amorphous heteropolymer constituted by phenyl propane monomers (p-coumaryl, coniferyl and sinapyl alcohol).

To avoid environmental problems related to its improper disposal, for instance, leachates, odor generation, and atmospheric pollution, the AB has been used in different applications such as compost, fertilizer, ruminant feed, etc. (Crespo et al., 2013; Velazquez-Jimenez et al., 2013). Another interesting alternative is its incorporation into a biorefinery scheme with the aim to produce biofuels and value-added by-products.

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4 According with the biofuel biorefinery objectives, AB with high proportion of amorphous cellulose and high
5 content of hemicellulose is desirable since these fractions can be readily hydrolysed to soluble carbohydrates
6 that can be used in downstream biological processes, i.e. biofuels production. However, the lignin fraction has
7 been identified as the major barrier to such applications; thus, lignin must be removed (Hendriks and Zeeman
8 2009). Lignin could be separated and recovered to produce chemicals and value added products, achieving an
9 integral revalorization of the lignocellulosic material (Cherubini 2010; Jong and Jungmeier 2015).

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17 In this work, the scientific advances towards the AB biorefinery composed by three sequential stages: I)
18 pretreatment, II) treatment, III) biofuels production (ethanol fermentation, dark fermentation or anaerobic
19 digestion) are reviewed. Moreover, the technological alternatives to revalorize the byproducts generated
20 during this three-stage biorefinery process are discussed. The AB biorefinery concept embraces various steps
21 and byproducts as shown in Fig. 1.
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28 29 2. THE AGAVE BAGASSE BIOREFINERY 30

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32 A biorefinery can be a process, a plant, a facility or a cluster of facilities that integrates upstream, midstream
33 and downstream processing of biomass into a range of valuable products (Jong and Jungmeier 2015).

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35 Different processes such as mechanical pretreatments (extraction, fractionation, separation) or chemical
36 pretreatments (acid and alkaline hydrolysis, delignification) and thermochemical (steam explosion), and
37 enzymatic and microbial conversions (enzymatic saccharification, fermentation, and anaerobic digestion) can
38 be included (Sannigrahi et al. 2010; Jong and Jungmeier 2015). It is worth to mention that a well-developed
39 biorefinery system must be economically driven based on innovative and cost-effective use of biomass to
40 produce both biobased products and bioenergy. In addition, it should contribute to the reduction of
41 greenhouse gas emissions and minimize the generation of waste materials (Jong and Jungmeier 2015).
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51 The agave bagasse biorefinery includes the transformation of bagasse organic matter to biofuels along with
52 the recovery of value-added by-products (i.e. lignin derivatives, volatile fatty acids, residual fibers). This
53 review includes the three main steps shown in Fig. 1; the first stage consists in the conditioning and
54 pretreatment of the raw material with the objective to remove lignin and prepare it for the polysaccharide
55 hydrolysis. In the second stage, hydrolysis or saccharification, the solubilization of the cellulose and
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4 hemicellulose with acid or enzymatic treatments take place. The objective of this step is the production of a
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6 liquid fraction rich in fermentable sugars called hydrolysate. In the last step, the hydrolysate can be used in
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8 three main biological processes to produce biofuels: hydrogen by dark fermentation (Contreras-Dávila et al.,
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10 2017), methane by anaerobic digestion (Arreola-Vargas et al., 2016) and ethanol by alcoholic fermentation
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12 (Caspeta et al. 2014). The generated residues (i.e. lignin, residual fibers, and VFA) throughout the bagasse
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14 processing, can be used in different industrial applications that will be reviewed in following sections.
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17 3. CONDITIONING AND PRETREATMENTS

18 3.1 Agave bagasse conditioning

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23 Previous to the use of AB for biofuels production, the AB fibers must be rinsed with water to remove soluble
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25 compounds formed during the cooking process of pinecones (Perez-Pimienta et al. 2015; Corona-González et
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27 al. 2016). Thereafter, the AB fibers must be partially reduced in size (0.5-15 mm) to enhance the pretreatment
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29 efficiency (Perez-Pimienta et al. 2013; Arreola-Vargas et al. 2015b; Corona-González et al. 2016; Montiel et
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31 al. 2016; Velázquez-Valadez et al. 2016). The particle size of lignocellulosic biomass is considered as an
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33 important factor that impacts the process efficiency (the smaller the size particle the better process
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35 efficiency); however, it is important to note that grinding of the material to small sizes is an energy-intensive
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37 process that increases the cost of the process (Li et al. 2015). Therefore, a full economical evaluation of the
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39 pros and constrains of size reduction should be carried out.
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43 3.2 Delignification pretreatment

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46 The lignocellulosic biomass is a highly recalcitrant material, i.e. biomass is hardly biodegradable by
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48 microorganisms and/or enzymes mainly due to the presence of lignin; thus, a pretreatment for its removal will
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50 be generally required (Zhu et al. 2008; Perez-Pimienta et al. 2013; Li et al. 2016). In the delignification
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52 pretreatment, the biomass is swelled and the lignin structure gets disrupted and solubilized (Zhu et al. 2008).
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54 As a result, the hemicellulose and the cellulose microfibrils become more accessible to enzymes or
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56 microorganisms in subsequent stages (Saini et al. 2016).
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4 A universal pretreatment is difficult to envision given the diverse nature of lignocellulosic residues. In this
5 regard, different delignification pretreatments have been suggested during the last years (Table 1). These can
6 be classified into biological, chemical and physico-chemical pretreatments (Alvira et al. 2010).
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11 In the case of AB, mainly physical, chemical, and physico-chemical pretreatments have been applied,
12 including autohydrolysis, thermo-mechanic-chemical process, ammonia fiber expansion (AFEX™), ionic
13 liquids, ozonolysis, and acid hydrolysis (Perez-Pimienta et al. 2013, 2016; Ávila-Lara et al. 2015; Barrera et
14 al. 2016; Montiel et al. 2016; Rios-González et al. 2017). In these pretreatments, hemicellulose is
15 depolymerized and solubilized, while a small fraction of the lignin is dissolved (Saucedo-Luna et al. 2011;
16 Perez-Pimienta et al. 2016). However, in the AB biorefinery, the aim is to apply a selective pretreatment to
17 remove lignin with minimal effects in the hemicellulose fraction. In this sense, organosolv, alkaline and
18 oxidative-alkaline pretreatments have advantages that have already been evaluated in AB.
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29 The organosolv pretreatment consists in the extraction of lignin with organic solvents including methanol,
30 ethanol, ethylene glycol, glycerol, etc. (Taherzadeh and Karimi 2008; Zhao et al. 2009). Pérez-Pimienta et al.
31 (2017) used organosolv to remove lignin for ethanol production using 25 g bagasse with 500 mL of solution
32 (74.5% water, 25% ethanol and 0.5% H₂SO₄). The experiment was carried out into a high-pressure chemical
33 reactor (160 °C and 138 psi) for 10 min. In this work, delignification yields of 45% with a loss of xylan of
34 86% were reported (Pérez-Pimienta et al. 2017). The organosolv pretreatment has the advantage of allowing
35 solvent recovery to be re-used, which makes it a cost-effective process (Carvajal et al. 2016); however, the
36 delignification yields are generally low (Pérez-Pimienta et al. 2017).
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46 Alkaline pretreatment has also been used for the delignification of bagasse of *Agave atrovirens*, another
47 species of the genus *Agave* (Hernández-Salas et al. 2009). In this hydrolytic pretreatment, the authors used an
48 alkali solution of NaOH (2% w/v) at 121 °C by autoclaving at 1.1 kg/cm² for 4 h, although the delignification
49 yields were not reported. With rice straw, other authors reported delignification yields of 28.4% using an
50 alkali solution of NaOH (6% w/v) (He et al. 2008). Moreover, He et al. (2008) argued that, in such
51 pretreatment, the ester bonds between lignin and carbohydrate complexes are broken, releasing cellulose and
52 hemicellulose to further utilization.
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4 The alkaline-oxidative pretreatment is another process that has been used to treat AB (Velázquez-Valadez et
5 al. 2016). This pretreatment consisted of two sequential steps. The first one was an alkaline pretreatment
6 employing a 6% (w/v) NaOH solution in a ratio of 1:5 with respect to the bagasse solids. This mixture was
7 autoclaved at 120° C and 2 atm for 1 h. Subsequently, an oxidative pretreatment was performed by adding 6%
8 (w/v) H₂O₂ to the aforementioned mixture, maintaining the initial solid-liquid ratio, at 30 °C for 24 h. In this
9 pretreatment, the H₂O₂ decomposes into more active radicals such as hydroperoxyl (\bullet OOH), hydroxyl (\bullet OH)
10 and superoxide (O₂ \bullet) (Sun et al. 2002; Wilkinson et al. 2014). These radicals disrupt the ether and ester
11 bonds between the subunits of lignin and hemicellulose, which causes the lignin solubilization. The
12 delignification yield with this method was 82.6%, while only 3.8% of the structural carbohydrates were
13 released (Velázquez-Valadez et al. 2016). Important constrains of this process is the use of high amounts of
14 reagents and energy, which make it a relatively expensive process. In this regard, Su et al. (2015) improved
15 the oxidative process by using only 2% (w/v) H₂O₂ at 50 °C and pH 11.5, for 1.5 h. Under such conditions,
16 these researchers reported a lignin removal efficiency of 74% from corncob. This study makes evident that the
17 oxidative pretreatment can be optimized to use less reagents, while keeping good delignification yields. In
18 addition, it is worth to note that the waste generated in this process is considered environmentally friendly
19 (e.g. use of less hazardous reagents). Therefore, the alkaline-oxidative pretreatment seems to be an excellent
20 option to implement in a biorefinery of AB from the efficiency point of view, although further studies are
21 needed to optimize this process.
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41 3.3 Lignin revalorization

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45 The solubilized lignin obtained in the pretreatment of AB can be concentrated to obtain lignin powder. Its
46 properties will depend on the origin and the type of the pretreatment process. For instance, as result of
47 oxidative pretreatment, lignin can be fragmented into monophenolic compounds (Ouyang et al. 2014). The
48 lignin precipitation from the aqueous phase can be done by concentrating it (previously diluted with ethanol
49 to remove impurities) and adjusting its pH to 1.5 (Su et al. 2015). Lignin possesses structural features that can
50 make it a promising starting material that enables its further revalorization into value-added products (Stewart
51 2008).
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4 According with the results reviewed previously, in the oxidative pretreatment, up to 112 kg of lignin/ton
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6 bagasse can be obtained. However, despite the enormous research efforts, the feasibility of the conversion of
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8 lignin to value-added products has yet to be established (Zhou et al. 2016). In fact, vanillin is currently the
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10 only molecular phenolic compound manufactured at industrial scale from softwood lignin (Fache et al. 2016).
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12 Nevertheless, the formation of vanillin and other compounds is strictly linked to the available percentage of
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14 its precursor in the lignin structure (Silva et al. 2009). Lignin from softwoods (gymnosperms) is
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16 predominantly based on structural units derived from the coniferyl alcohol (guaiacyl units), which are the
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18 precursors for vanillin. However, in herbaceous (angiosperm) plants, such as Agave, lignin is constituted not
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20 only by guaiacyl units, but also by sinapyl alcohol (syringyl units) and p-coumaryl alcohol (4-hydroxyphenyl
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22 units) (Zhou et al. 2016). Therefore, applications of herbaceous lignins for vanillin production may be limited.
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26 Kalliola et al. (2015) reported that oxidized lignin have a potential as a renewable plasticizer in cement-
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28 containing products, such as concrete, because it has the ability to endure under alkaline conditions, and it
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30 does not introduce air in concrete. Oxidized lignins may provide a sustainable and techno-economically
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32 feasible option for future plasticizer technology. Lignin recovered from the oxidative pretreatment of AB
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34 could be used in this developing application.

35 36 37 4. TREATMENTS 38

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40 The treatment of agave bagasse to obtain fermentable sugars is an essential step in the biorefinery since it can
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42 substantially increase the biological availability of the substrate and reduces the processing time. In this
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44 regard, saccharification of AB with chemical (acid hydrolysis) and biological (enzymatic hydrolysis)
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46 treatment has been evaluated.

47 48 4.1 Acid hydrolysis 49

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51 The acid hydrolysis consists in the conversion of lignocellulosic biomass into monosaccharides and
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53 oligosaccharides; however, the saccharification efficiency depends on the severity of the hydrolysis
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55 conditions (temperature, reaction time and acid concentration). In general, there are two types of acid
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57 hydrolysis, diluted and concentrated, being the first one the most implemented due to its highly efficient
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59 hemicellulose depolymerization (mainly xylan) and low cost (Jiang et al. 2016). However, at high
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4 temperatures and pressures, the carbohydrates can be degraded into furfural and hydroxymethylfurfural
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6 (HMF) (Mussatto and Roberto 2004); which may affect the microbial metabolism in the fermentation step
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8 (Saha 2004). Thus, this aspect has to be carefully considered in a biorefinery scheme.
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11 Using AB, acid hydrolysis treatment has been tested and optimized by Saucedo Luna et al. (2010) in two
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13 sequential batch stages. In the first stage, the optimal conditions were 151 °C, 2% of sulphuric acid for 10 min
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15 of reaction. In second stage, the optimal experimental conditions were 175 °C, 2% of sulphuric acid and 30
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17 min of reaction. The total fermentable sugars yield from the overall process was 326 g/kg dry matter, which
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19 represented 48.5% of the theoretical value. In recent years, the acid hydrolysate of AB has been used for
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21 hydrogen and methane production. In these cases, HCl has been used instead of H₂SO₄ to avoid the sulphate-
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23 reduction processes (Arreola-Vargas et al. 2015b). Arreola-Vargas et al. (2015) reported a total sugar
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25 concentration of 27.9 g/L and HMF concentration up to 1,2 g/L using 2.7% of HCl at 123.6 °C for 1.3 h of
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27 reaction.
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30 4.2 Enzymatic hydrolysis

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32 Enzymatic hydrolysis of lignocellulosic substrates requires several enzyme types working in synergy, such as
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34 cellobiohydrolases (exo-glucanases), endo-glucanases, β-glucosidases, endo-xylanases, etc. In contrast to
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36 other treatment processes, the enzymatic process has the main advantage of high specificity; consequently, it
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38 does not produce by-products. Nevertheless, nowadays it is not economically viable because of the high
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40 enzymatic cost, slow time of reaction, and high quantities of enzyme required. Nonetheless, some studies
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42 have explored different solutions to cope these constraints (Montiel et al. 2016).
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46 The enzymatic hydrolysis of AB has been used after pre-treatment as well as without pre-treatment. Table 2
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48 shows some results of enzymatic hydrolysis of agave bagasse after pre-treatment reported from the literature.
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50 Regarding to enzymatic hydrolysis without any pre-treatment, Contreras-Davila et al. (2017) reported
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52 hydrolysis using an enzymatic preparation (Celluclast 1.5 L) at pH 4.5, 100 rpm and 45 °C for 10 h obtaining
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54 about 12.5 g total sugars/L. Arreola-Vargas et al. (2016) studied the enzymatic hydrolysis using Celluclast
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56 1.5 L at pH 4.5 and 45 °C for 10 h, and 8.9 g total sugar/L, 328.7 mg phenols/L were obtained. In accordance
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58 with literature, the enzymatic hydrolysis has a promising potential towards the revalorization of AB residues,
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60 though optimization of enzymatic cocktails has to be further studied.
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4.3 Fibre uses

The residual fibre of the different pre-treatments has been studied for several applications, for example, absorbent of contaminants such as Cd(II), Pb(II) and Zn(II) ions from water (Velazquez-Jimenez et al. 2013), compostable and biodegradable composites (Kestur G. et al. 2013; Torres-Tello et al. 2017), and to elaborate cellulose hydrogel films (Tovar-Carrillo et al. 2013, 2014). All of these alternatives could improve the energy and economical balance of the integrated process of AB biorefinery.

5. BIOFUELS

The hydrolysate of AB is considered as an ideal feedstock for biofuels production since they are generally composed by a mixture of glucose and xylose (Arreola-Vargas et al. 2016a; Pérez-Pimienta et al. 2017). Anaerobic microorganisms, some of which can readily convert it into a wide range of energy sources, easily metabolize these sugars. Due to their economic potential, level of research, and technology status, this review is focused on the alcoholic fermentation (AF), dark fermentation (DF) and anaerobic digestion (AD).

Among these bioprocesses, the AF is the partial oxidation of carbohydrates that leads to the ethanol production and is commonly carried out by yeasts, mainly *Saccharomyces cerevisiae*. AF is the most mature and industrialized technology for biofuel production (bioethanol) in comparison with DF and AD. The DF is the partial oxidation of carbohydrates to volatile fatty acids (VFA), mainly acetate and butyrate, with the concomitant production of molecular hydrogen (H₂). This process is carried out by acidogenic microorganisms (e. g. *Clostridiaceae* and *Enterobacteriaceae* families). Finally the AD continues with the consumption of H₂ and acetate by hydrogenotrophic and acetoclastic methanogens, respectively. The metabolic pathways for these biotransformations are summarized in Fig. 2.

5.1 Ethanol from agave bagasse

AF of food crops (e.g. sugarcane or corn) has established as the main and one of the most developed alternatives for biofuels production worldwide. In 2015, the global production of fuel ethanol was above 97.2 x 10⁶ m³, being the USA (57%) and Brazil (27%) the leading producers (RFA 2017). Thus, the AF infrastructure is well known and it is relatively easy to adapt for second-generation ethanol production from

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4 lignocellulosic hydrolysates.
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7 Conversion of glucose to ethanol can be performed by *S. cerevisiae* with high efficiency of conversion.
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9 However, when non-model substrates such as AB hydrolysates are used as carbon source, the fuel production
10 and energy yields can be affected (Table 3). Caspeta et al. (2014) used enzymatic hydrolysates of organosolv
11 pretreated AB (12.4 g_{Glucose}/L) for ethanol production with *S. cerevisiae* and reached a maximum glucose
12 conversion into bioethanol of 96%. The 64 g/L of ethanol obtained from the saccharification at 20% w/w
13 organosolv pretreated solids of AB is the highest ethanol concentration reported for this lignocellulosic
14 material. Overall the potential conversion of AB to fuel ethanol was 0.25 g/g, which is 85% of the maximum
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24 The main limitation of ethanol production processes is the presence of pentoses in lignocellulosic
25 hydrolysates (e.g. xylose) since *S. cerevisiae* is not capable to transform them to ethanol; for this reason,
26 researchers have explored the potential of other microorganisms. For example, Saucedo-Luna et al. (2011)
27 used the native yeast *Pichia caribbica* UM-5 to ferment sugars (hexoses and pentoses) produced from acid
28 and enzymatic hydrolysates. The final optimized process generated 8.99 g ethanol/50 g of AB, corresponding
29 to an overall 56.75% of theoretical ethanol (w/w) (Saucedo-Luna et al. 2011).
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38 In the same way, Pérez-Pimienta et al. (2017) evaluated ethanol production using a sequential enzymatic
39 saccharification and fermentation of ionic liquid and organosolv pretreated AB with cellulolytic enzymes and
40 the ethanologenic *Escherichia coli* strain MS04. This process achieved a conversion of 90% and 84% of
41 glucan and xylan respectively for ionic liquid pretreatment bagasse, and 93% and 90% of glucan and xylan
42 respectively for organosolv pretreatment bagasse. Ethanol production yields were 12.1 and 12.7 kg per 100 kg
43 of untreated AB, with ionic liquid pretreatment and organosolv pretreatment respectively. Another alternative
44 is the genetic modification of microorganisms to give them the capability to process pentoses, for instance, *E.*
45 *coli* has been genetically modified in order to produce ethanol from pentose; nevertheless, implementation of
46 ethanol production using modified microorganism represents high cost attributed to aseptic conditions
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56 (Ingram et al. 1987).
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4 Other microorganism that can use xylose and glucose as carbon source for ethanol production is
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6 *Scheffersomyces stipitis* (formerly known as *Pichia stipites*). Nakasu et al. (2016) used *S. stipitis* to study the
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8 ethanol production from xylose-enriched hemicellulose hydrolysate of sugarcane bagasse. They studied
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10 several pretreatments conditions, and found a maximum xylose conversion of 97.3% when sulfuric acid was
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12 used as pretreatment. In terms of ethanol production, they found a maximum ethanol concentration of 10.6
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14 g/L, with a fermentation yield close to 60% with 33.5 g xylose/L. Interestingly, the authors showed that the
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16 presence of inhibitors (e.g. acetic acid, phenolic compounds, furfural, and others) precluded the ethanol
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18 production. Therefore, low generation of these inhibitory compounds or its previous detoxification is an
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20 important step of the AF process (see section 5.4 Inhibitors). In this regard, ethanol yields are dependent of
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22 pre-treatment and hydrolysate conditions. When xylose is found at high proportion, the ethanol yield is
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24 affected and decreases about 80% of the theoretical value (Olsson and Hahn-Hägerdal 1996; Nigam 2001;
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26 Klinke et al. 2004).

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33 Hydrogen (H₂) production by DF is considered as a promising biotechnology that can be a central keystone
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35 towards the establishment of lignocellulosic biorefineries. The H₂ produced herewith can be highlighted by
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37 two principal reasons: its high energy content (120 kJ/g) and its high-efficient conversion to electricity. DF
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39 has been extensively explored in multiple reactor configurations, different substrates, inocula and operational
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41 conditions (Nissilä et al. 2014; Barca et al. 2015; Ghimire et al. 2015; Ren et al. 2016). In the last decade, DF
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43 started to be considered as part of a revalorization chain of lignocellulosic residues. In this sense, substrates
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45 such as sugarcane bagasse, oat straw, and AB have been investigated (Monlau et al. 2013).
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49 Particularly, the use of AB for hydrogen production has been scarcely reported thus far. In batch experiments,
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51 Arreola-Vargas et al. (2016) used acid and enzymatic hydrolysates of *Agave tequilana* at different
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53 concentrations of 20-100% (v/v). They found that the highest H₂ production rate was obtained with 40% (v/v)
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55 enzymatic hydrolysates and it was equivalent to a volumetric hydrogen production rate (VHPR) of 2400 mL
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57 H₂/L-d. In contrast, when the acid hydrolysates were used, the H₂ production rate was limited by the
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59 increasing concentration of inhibitory compounds (see section 5.4). On the other hand, in continuous mode,
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4 Contreras-Dávila et al. (2017) reported a maximum VHPR of 2530 mL H₂/L-d with a CSTR operated at an
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6 organic loading rate (OLR) of 52.2 g COD/L-d using enzymatic hydrolysates of AB. They also found that a
7
8 notably higher productivity could be achieved with a trickling bed reactor (TBR). Using the TBR
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10 configuration, they found a maximum VHPR of 3450 mL H₂/L-d, at an OLR of 52.9 g COD/L-d.
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12 In comparison with other lignocellulosic materials (Table 3), AB hydrolysates have demonstrated to be a
13
14 feasible feedstock for hydrogen production. Nevertheless, there are important challenges in the DF systems
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16 that must be solved in order to improve the rate and efficiency of the H₂ production. In this regard, three
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18 important constraints are: 1) endogenous H₂ consumption, 2) incomplete substrate utilization and 3) presence
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20 of inhibitory compounds. Endogenous H₂ consumption is considered as any H₂ utilization either direct (i.e.
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22 molecular H₂) or indirect (i.e. NADH, Fd, and others) in the fermentation reactor that leads to the synthesis of
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24 different metabolites (e.g. propionate, ethanol, lactate, etc.) with inherent inefficiency of the process. Hereof,
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26 the metabolic diversification can be minimized with proper control of pH, OLR, temperature, etc. (Ghimire et
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28 al. 2015). The incomplete utilization refers to the fact that DF can only aim to a maximum hydrogen recovery
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30 of 4 molH₂/mol hexose which is only one third of the energy content in hexose-type carbohydrates. Therefore,
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32 it is necessary to use the DF effluents as feedstock for other biotechnologies such as electrochemical systems
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34 (H₂), photofermentation (H₂) and AD (CH₄) to enhance the energy recovery. Other alternatives to revalorize
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36 the DF residues are depicted in the following section. The third potential drawback of DF is its sensitivity to
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38 inhibitory compounds (e.g. Furfural, HMF, phenols, formic acid, etc.) which could result from aggressive
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40 treatment (Monlau et al. 2014). Thus, the implementation of a previous detoxification steps (briefly described
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42 in the next section) is probably required.

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44 Although DF is considered as a promising alternative for the production of hydrogen energy, the current
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46 status of the technology suggests that further evaluation, especially at the large scale, is still necessary. Up to
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48 date, successful operation of pilot-scale reactors have only been carried out for a limited number of waste
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50 materials, i.e. as molasses (Ren et al. 2006) and food wastes (Licata et al. 2011; Sekoai and Gueguim Kana
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52 2014; Elsamadony and Tawfik 2015). To the best of our knowledge, no studies of hydrogen production from
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54 AB or other lignocellulosic wastes in pilot- or full-scale have been conducted so far.

55 56 57 58 5.2.1 Dark fermentation byproducts 59 60 61 62 63 64 65

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4 An important drawback of hydrogen production by DF is that most of the substrate entering the system is
5 transformed to partially oxidized compounds, mainly volatile fatty acids (VFA). Thus, the feasibility of the
6 AB biorefinery is expected to depend on the proper utilization or valorization of such effluents. The
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8 composition of DF effluents will change as function of the operational conditions, type of inoculum, and
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10 intrinsic characteristics of the hydrolysate. From AB enzymatic hydrolysates, literature reports have shown
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12 that acetic and butyric acid account for more than 80% of VFA produced in DF (Arreola-Vargas et al. 2016a;
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14 Contreras-Dávila et al. 2017). Considering such composition, the use of DF effluents as substrate for
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16 polyhydroxyalkanoates (PHA) production can be an attractive alternative that can also be coupled to
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18 additional H₂ production (Venkata Mohan et al. 2010; Venkateswar Reddy et al. 2014; Sarma et al. 2015;
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20 Cardeña et al. 2017). Nevertheless, the PHA production from dark fermentation effluents is still considered as
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22 a young technology that requires important efforts towards its use in the AB biorefinery.
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27 5.3 Biogas 28 29 30

31 The biogas production by AD consists of four steps namely hydrolysis, acidogenesis, acetogenesis and
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33 methanogenesis (Fig. 2). It is an attractive process to integrate into a biorefinery framework that is gaining
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35 attention worldwide. Biogas production from lignocellulosic materials has an important approach due to
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37 carbon neutral concept. Furthermore, it can reduce more than 90% of organic matter of the
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39 bagasse/lignocellulosic waste, therefore it also contributes to reduce the associated pollution which is an
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41 environmental concern. Another important fact is that nowadays the feedstock of AD does not have a
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43 significant value as it is considered a residue from other processes.
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45 Biogas can be produced all around the world, since it does not depends on the geographical position or
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47 weather conditions of each region. Besides, biogas is a stable process that is already working at full scale. For
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49 example, Dussadee et al. (2014) achieved an energy production of 343680 MJ/d. Actually, biogas facilities
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51 have gained attention as integral part of biorefineries, while biomethane/biogas plants have increased
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53 constantly around the world. Until 2015, in Europe existed 459 biogas plants (European Biogas Association)
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55 while in Mexico there were 16 biogas plants (SENER 2016).
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57 In the biorefinery of AB, the pre-treated bagasse could be used for energy recovery in the form of biogas
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59 (Table 4). For example, Arreola-Vargas et al. (2015) achieved a biogas yield of 0.26 L/g COD and a biogas
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4 production rate of 0.3 L/L-d from acid hydrolysates of AB in an ASBR with a OLR of 1.3 g COD/L-d. On the
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6 other hand, Hassan et al. (2016) achieved a biogas yield of 0.32 L/g VS in a digester with 5% TS feed with
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8 corn stover hydrolysates .
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10 One of the most important problems of the AD is the acidification of the medium due to the VFAs
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12 accumulation, which causes the methanogenesis inhibition (Akuzawa et al. 2011). This problem arises for
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14 substrates with high carbohydrate content such as the case of AB hydrolysates. However, increasing the
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16 buffering capacity, adding alkali, regulating the OLR or lowering the substrate concentration can easily solve
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18 the inconvenient. Another potential problem of AD is the lack of important nutrients (e.g. N-compounds) in
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20 the biomass (e.g AB). This is of special relevance since the biogas production is strongly affected by the C/N
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22 ratio (Hassan et al. 2016a). For this problem, Alatrisme-Mondragón et al. (2006) suggested the co-digestion of
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24 substrates (e.g. bagasse hydrolysate or DF effluent) with other high N-content residues that each industry in
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26 particular produce (e.g. sewage sludge or wastewater) allowing the nutrient balancing (e.g. ratio C/N) and
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28 besides, this strategy improve the biogas yield.
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30 5.4 Fermentation inhibitory compounds 31

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33 In general, fermentation inhibitors could be produced during the hydrolysis of AB, especially in the acidic
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35 hydrolysis, as result of the degradation of carbohydrates (Larsson et al. 1999). The presence of these
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37 compounds (e.g. 5-HMF, furfural, vanillin, syringaldehyde, acetic acid, formic acid, etc.) have different grade
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39 of constraints in AF and DF.
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42 In the case of AF, Klinke et al. (2004) reported yields about 40 mg EtOH/g carbohydrate when acetic acid and
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44 Furfural/ 5HMF were in concentrations of 9 g/L and 1 g/L, respectively. They observed that after the
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46 inhibitors removal, the ethanol yields increased 80-90%. Nakasu et al. (2016) studied the ethanol production
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48 under high concentrations of inhibitors (4 g/L furfural). They found that *S. cerevisiae* could metabolize
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50 furfural, but the process does not stimulate ethanol production due to furfuryl-alcohol formation. In fact,
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52 ethanol production decreased to half of the initial value (about 50 %). For the case of DF, Lin et al. (2015)
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54 recently reported that furan derivatives and phenolic compounds at 15 mM decreased the H₂ yield and
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56 production rate in 4-15% and 20-44%, respectively. Quéméneur et al. (2012) performed a series of
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58 experiments to determine the inhibitory effects of furan derivatives, phenolic compounds and lignin
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60 (concentration, 1g/L each) on the hydrogen production performance. They found major impacts of furan
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4 derivatives (69-76% lower hydrogen yield than control) in comparison with phenols (17-23 % lower
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6 hydrogen yield than control). In terms of hydrogen production potential, they reported that HMF, furfural,
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8 vanilline, phenol and syringaldehyde decreased the amount of hydrogen obtained as compared to the xylose
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10 control (1367 mL/L) by 82, 82, 67, 65, and 23%. In another study, Siqueira and Reginatto (2015) studied the
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12 effect of different concentrations of inhibitory compounds (organic acids, furan derivatives, and phenolic
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14 monomers) on the hydrogen production rate. They observed that the concentrations of inhibitors that reduced
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16 by half the maximum hydrogen production rate (IC50) were 0.38, 0.48, 0.62, 0.71, 1.05, and 5.14 g/L for 4-
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18 hydroxybenzoic acid, HMF, furfural, vanillin, syringaldehyde, and acetic acid, respectively.

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21 In the case of AB, Arreola-Vargas et al. (2016) reported appreciable concentrations of total phenols (941.6
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23 mg/L), 5-HMF (95.8 mg/L), and furfural (33.1 mg/L) in acid hydrolysates. These concentrations limited the
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25 utilization of the hydrolysate in the biological production of H₂ in two-stage processes. Specifically, authors
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27 found that H₂ production rate decreased by 85% when the concentration of hydrolysate reached 100% in
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29 comparison with the maximum rate of 35 mL H₂/h found at 20% hydrolysate.

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32 Concerning the effects of these compounds on the anaerobic digestions, Barakat et al. (2012) reported that
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34 they cause no inhibition of the process; rather, the presence of such byproducts can increase the methane
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36 production. Similarly, Ghasimi et al. (2016) reported that concentrations of 0.8 g/L of furfural and HMF
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38 slightly decreased the rate of methane production, but the methane production was similar. At a concentration
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40 of 2 g/L, furfural and HMF strongly inhibited the methanogenesis process. Using AB hydrolysates, Arreola-
41
42 Vargas et al. (2016) reported that the methane production rate decreased from 12.5 mL CH₄/h to 2.5 mL
43
44 CH₄/h when the hydrolysate concentration changed from 20 to 100% i.e. 2200, 7700, 941.6, 95.8, and 33.1
45
46 mg/L for formic acid, acetic acid, total phenol, HMF, and furfural, respectively.

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49 As alternative, inhibitory molecules could be removed through different strategies such as adsorption onto
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51 activated carbon (Chandel et al. 2007; Lee and Park 2016; Saini et al. 2016; Sambusiti et al. 2016), ion
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53 exchange (Gao and Rehmann 2016; Chen et al. 2017), enzymatic treatment (Saravanakumar et al. 2016), and
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55 combined approaches (Vallejos et al. 2016). In an interesting investigation, Gupta et al. (2016) evaluated
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57 different inhibitory abatement methods and found that the use of activated carbon was the most suitable for
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4 the process. In addition, they also showed the feasibility of the strategy at a pilot scale. However, optimization
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6 is required to minimize the carbohydrates adsorption on the activated carbon.
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8 9 6. CONCLUSIONS AND REMARKS

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11 The AB biorefinery is a conceptual approach towards the full revalorization of the residual lignocellulosic
12 biomass produced in one of the most representative industries in Mexico. In general, the route depicted in this
13 review resulted to be the most suitable due to delignification yields (oxidative pretreatment), saccharification
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15 yield (enzymatic hydrolysis) and energy recovery (dark fermentation → anaerobic digestion). Moreover,
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17 along the proposed route, byproducts generated can be used in several applications (cement production,
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19 adsorbent materials, bioplastics, etc.). Overall, the AB biorefinery is an opportunity to revalorize a residue
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21 and obtain energy and valuable products through a sustainable process.
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25 However, there are still important aspects to be considered. Such is the case of separation and purification
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27 technologies as well as storing and transportation issues. Although these topics were not discussed in this
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29 review, they play relevant roles in the overall techno-economical balance of the biorefinery. Special attention
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31 is being gained by the separation of H₂ from CO₂ as well as the dehydration of ethanol.
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44 45 8. CONFLICT OF INTEREST

46
47 The authors declare that they have no conflict of interest.
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50 51 9. REFERENCES

- 52
53 Akuzawa M, Hori T, Haruta S, et al (2011) Distinctive Responses of Metabolically Active Microbiota to
54 Acidification in a Thermophilic Anaerobic Digester. *Microb Ecol* 61:595–605. doi: 10.1007/s00248-
55 010-9788-1
56
57 Alatríste-Mondragón F, Samar P, Cox HHJ, et al (2006) Anaerobic Codigestion of Municipal, Farm, and
58 Industrial Organic Wastes: A Survey of Recent Literature. *Water Environ Res* 78:607–636. doi:
59 10.2175/106143006X111673
60
61 Alvira P, Tomás-Pejó E, Ballesteros M, Negro MJ (2010) Pretreatment technologies for an efficient
62
63
64
65

- 1
2
3
4 bioethanol production process based on enzymatic hydrolysis: A review. *Bioresour Technol* 101:4851–
5 4861. doi: 10.1016/j.biortech.2009.11.093
6
7 Arreola-Vargas J, Alatraste-Mondragón F, Celis LB, et al (2015a) Continuous hydrogen production in a
8 trickling bed reactor by using triticale silage as inoculum: effect of simple and complex substrates. *J*
9 *Chem Technol Biotechnol* 90:1062–1069. doi: 10.1002/jctb.4410
10
11 Arreola-Vargas J, Celis LB, Buitrón G, et al (2013) Hydrogen production from acid and enzymatic oat straw
12 hydrolysates in an anaerobic sequencing batch reactor : Performance and microbial population analysis.
13 *Int J Hydrogen Energy* 38:13884–13894.
14
15 Arreola-Vargas J, Flores-Larios A, González-Álvarez V, et al (2016a) Single and two-stage anaerobic
16 digestion for hydrogen and methane production from acid and enzymatic hydrolysates of Agave
17 tequilana bagasse. *Int J Hydrogen Energy* 41:897–904. doi: 10.1016/j.ijhydene.2015.11.016
18
19 Arreola-Vargas J, Jaramillo-Gante NE, Celis LB, et al (2016b) Biogas production in an anaerobic sequencing
20 batch reactor by using tequila vinasses: Effect of pH and temperature. *Water Sci Technol* 73:550–556.
21 doi: 10.2166/wst.2015.520
22
23 Arreola-Vargas J, Ojeda-Castillo V, Snell-Castro R, et al (2015b) Methane production from acid hydrolysates
24 of Agave tequilana bagasse: Evaluation of hydrolysis conditions and methane yield. *Bioresour Technol*
25 181:191–199. doi: 10.1016/j.biortech.2015.01.036
26
27 Arriaga S, Rosas I, Alatraste-Mondragón F, Razo-Flores E (2011) Continuous production of hydrogen from
28 oat straw hydrolysate in a biotrickling filter. *Int J Hydrogen Energy* 36:3442–3449. doi:
29 10.1016/j.ijhydene.2010.12.019
30
31 Ávila-Lara AI, Camberos-Flores JN, Mendoza-Pérez JA, et al (2015) Optimization of alkaline and dilute acid
32 pretreatment of Agave bagasse by response surface methodology. *Front Bioeng Biotechnol* 3:1–10. doi:
33 10.3389/fbioe.2015.00146
34
35 Badshah M, Lam DM, Liu J, Mattiasson B (2012) Use of an Automatic Methane Potential Test System for
36 evaluating the biomethane potential of sugarcane bagasse after different treatments. *Bioresour Technol*
37 114:262–269. doi: 10.1016/j.biortech.2012.02.022
38
39 Barakat A, Monlau F, Steyer JP, Carrere H (2012) Effect of lignin-derived and furan compounds found in
40 lignocellulosic hydrolysates on biomethane production. *Bioresour Technol* 104:90–99. doi:
41 10.1016/j.biortech.2011.10.060
42
43 Barca C, Soric A, Ranava D, et al (2015) Anaerobic biofilm reactors for dark fermentative hydrogen
44 production from wastewater: A review. *Bioresour Technol* 185:386–398. doi:
45 10.1016/j.biortech.2015.02.063
46
47 Barrera I, Amezcua-Allieri MA, Estupiñan L, et al (2016) Technical and economical evaluation of bioethanol
48 production from lignocellulosic residues in Mexico: Case of sugarcane and blue agave bagasses. *Chem*
49 *Eng Res Des* 107:91–101. doi: 10.1016/j.cherd.2015.10.015
50
51 Cardeña R, Valdez-Vazquez I, Buitrón G (2017) Effect of volatile fatty acids mixtures on the simultaneous
52 photofermentative production of hydrogen and polyhydroxybutyrate. *Bioprocess Biosyst Eng* 40:231–
53 239. doi: 10.1007/s00449-016-1691-9
54
55 Carvajal JC, Gómez Á, Cardona CA (2016) Comparison of lignin extraction processes: Economic and
56 environmental assessment. *Bioresour Technol* 214:468–476. doi: 10.1016/j.biortech.2016.04.103
57
58 Caspeta L, Caro-Bermúdez MA, Ponce-Noyola T, Martínez A (2014) Enzymatic hydrolysis at high-solids
59 loadings for the conversion of agave bagasse to fuel ethanol. *Appl Energy* 113:277–286. doi:
60 10.1016/j.apenergy.2013.07.036
61
62 Cedeño-Cruz M, Alvarez-Jacobs J (1991) Production of tequila from agave: historical influences and
63 contemporary processes. *alcohol Textb a Ref beverage, fuel Ind alcohol Ind* 225–242.
64
65 Chandel AK, Kapoor RK, Singh A, Kuhad RC (2007) Detoxification of sugarcane bagasse hydrolysate
improves ethanol production by *Candida shehatae* NCIM 3501. *Bioresour Technol* 98:1947–1950. doi:
10.1016/j.biortech.2006.07.047
Chen K, Hao S, Lyu H, et al (2017) Ion exchange separation for recovery of monosaccharides, organic acids

- 1
2
3
4 and phenolic compounds from hydrolysates of lignocellulosic biomass. *Sep Purif Technol* 172:100–
5 106. doi: 10.1016/j.seppur.2016.08.004
6
7 Cheng HH, Whang LM, Chung MC, Chan KC (2016) Biological hydrogen and methane production from
8 bagasse bioethanol fermentation residues using a two-stage bioprocess. *Bioresour Technol* 210:49–55.
9 doi: 10.1016/j.biortech.2015.12.084
10
11 Cherubini F (2010) The biorefinery concept: Using biomass instead of oil for producing energy and
12 chemicals. *Energy Convers Manag* 51:1412–1421. doi: 10.1016/j.enconman.2010.01.015
13
14 Contreras-Dávila CA, Méndez-Acosta HO, Arellano-García L, et al (2017) Continuous hydrogen production
15 from enzymatic hydrolysate of Agave tequilana bagasse: Effect of the organic loading rate and reactor
16 configuration. *Chem Eng J* 313:671–679. doi: 10.1016/j.cej.2016.12.084
17
18 Corona-González RI, Varela-Almanza KM, Arriola-Guevara E, et al (2016) Bagasse hydrolyzates from
19 Agave tequilana as substrates for succinic acid production by *Actinobacillus succinogenes* in batch and
20 repeated batch reactor. *Bioresour Technol* 205:15–23. doi: 10.1016/j.biortech.2015.12.081
21
22 Crespo MR, González DR, Rodríguez R, et al (2013) Evaluación de la composta de bagazo de agave como
23 componente de sustratos para producir plántulas de agave azul tequilero. *Rev Mex Ciencias Agrícolas*
24 4:1161–1173.
25
26 CRT (2016) Consumo de Agave para tequila y tequila 100% de Agave. 2016.
27
28 Dan Jiang, Fang Z, Chin S, et al (2016) Biohydrogen Production from Hydrolysates of Selected Tropical
29 Biomass Wastes with *Clostridium Butyricum*. *Sci Rep* 6:27205. doi: 10.1038/srep27205
30
31 Dussadee N, Reansuwan K, Ramaraj R (2014) Potential development of compressed bio-methane gas
32 production from pig farms and elephant grass silage for transportation in Thailand. *Bioresour Technol*
33 155:438–441. doi: 10.1016/j.biortech.2013.12.126
34
35 Elsamadony M, Tawfik A (2015) Potential of biohydrogen production from organic fraction of municipal
36 solid waste (OFMSW) using pilot-scale dry anaerobic reactor. *Bioresour Technol* 196:9–16. doi:
37 10.1016/j.biortech.2015.07.048
38
39 Fache M, Boutevin B, Caillol S (2016) Vanillin Production from Lignin and Its Use as a Renewable
40 Chemical. *ACS Sustain Chem Eng* 4:35–46. doi: 10.1021/acssuschemeng.5b01344
41
42 Gao K, Rehmann L (2016) Combined Detoxification and In-situ Product Removal by a Single Resin During
43 Lignocellulosic Butanol Production. *Sci Rep* 6:1–10. doi: 10.1038/srep30533
44
45 Ghasimi DSM, Aboudi K, de Kreuk M, et al (2016) Impact of lignocellulosic-waste intermediates on
46 hydrolysis and methanogenesis under thermophilic and mesophilic conditions. *Chem Eng J* 295:181–
47 191. doi: 10.1016/j.cej.2016.03.045
48
49 Ghimire A, Frunzo L, Pirozzi F, et al (2015) A review on dark fermentative biohydrogen production from
50 organic biomass: Process parameters and use of by-products. *Appl Energy* 144:73–95. doi:
51 10.1016/j.apenergy.2015.01.045
52
53 Gupta R, Mehta G, Kuhad RC (2016) Scale-up of abatement of fermentation inhibitors from acid hydrolysates
54 for efficient conversion to ethanol as biofuel. *J Chem Technol Biotechnol* 91:1826–1834. doi:
55 10.1002/jctb.4775
56
57 Hassan M, Ding W, Shi Z, Zhao S (2016a) Methane enhancement through co-digestion of chicken manure
58 and thermo-oxidative cleaved wheat straw with waste activated sludge: A C/N optimization case.
59 *Bioresour Technol* 211:534–541. doi: 10.1016/j.biortech.2016.03.148
60
61 Hassan M, Ding W, Umar M, et al (2016b) Methane Enhancement through Liquid Ammonia Fractionation of
62 Corn Stover with Anaerobic Sludge. *Energy and Fuels* 30:9463–9470. doi:
63 10.1021/acs.energyfuels.6b01745
64
65 Hassan M, Ding W, Umar M, et al (2017a) Methane enhancement and asynchronism minimization through
66 co-digestion of goose manure and NaOH solubilized corn stover with waste activated sludge. *Energy*
67 118:1256–1263. doi: 10.1016/j.energy.2016.11.007
68
69 Hassan M, Ding W, Umar M, Rasool G (2017b) Batch and semi-continuous anaerobic co-digestion of goose

- 1
2
3
4 manure with alkali solubilized wheat straw: A case of carbon to nitrogen ratio and organic loading rate
5 regression optimization. *Bioresour Technol* 230:24–32. doi: 10.1016/j.biortech.2017.01.025
6
7 He Y, Pang Y, Liu Y, et al (2008) Physicochemical characterization of rice straw pretreated with sodium
8 hydroxide in the solid state for enhancing biogas production. *Energy and Fuels* 22:2775–2781. doi:
9 10.1021/ef8000967
10 Hendriks ATWM, Zeeman G (2009) Pretreatments to enhance the digestibility of lignocellulosic biomass.
11 *Bioresour Technol* 100:10–18. doi: 10.1016/j.biortech.2008.05.027
12
13 Hernández-Salas JM, Villa-Ramírez MS, Veloz-Rendón JS, et al (2009) Comparative hydrolysis and
14 fermentation of sugarcane and agave bagasse. *Bioresour Technol* 100:1238–1245. doi:
15 10.1016/j.biortech.2006.09.062
16
17 Ingram LO, Conway T, Clark DP, et al (1987) Genetic engineering of ethanol production in *Escherichia coli*.
18 *Appl Environ Microbiol* 53:2420–5.
19
20 Iñiguez-Covarrubias G, Lange SE, Rowell RM (2001) Utilization of byproducts from the tequila industry:
21 Part 1: Agave bagasse as a raw material for animal feeding and fiberboard production. *Bioresour*
22 *Technol* 77:25–32. doi: 10.1016/S0960-8524(00)00137-1
23
24 Jiang L, Wu N, Zheng A, et al (2016) The integration of dilute acid hydrolysis of xylan and fast pyrolysis of
25 glucan to obtain fermentable sugars. *Biotechnol Biofuels* 9:1–10. doi: 10.1186/s13068-016-0612-0
26
27 Jong E De, Jungmeier G (2015) Biorefinery Concepts in Comparison to Petrochemical Refineries.
28
29 Kafle GK, Kim SH (2013) Anaerobic treatment of apple waste with swine manure for biogas production:
30 Batch and continuous operation. *Appl Energy* 103:61–72. doi: 10.1016/j.apenergy.2012.10.018
31
32 Kalliola A, Vehmas T, Litiä T, Tamminen T (2015) Alkali-O₂ oxidized lignin - A bio-based concrete
33 plasticizer. *Ind Crops Prod* 74:150–157. doi: 10.1016/j.indcrop.2015.04.056
34
35 Kestur G. S, Flores-Sahagun THS, Dos Santos LP, et al (2013) Characterization of blue agave bagasse fibers
36 of Mexico. *Compos Part A Appl Sci Manuf* 45:153–161. doi: 10.1016/j.compositesa.2012.09.001
37
38 Klinke HB, Thomsen AB, Ahring BK (2004) Inhibition of ethanol-producing yeast and bacteria by
39 degradation products produced during pre-treatment of biomass. *Appl Microbiol Biotechnol* 66:10–26.
40 doi: 10.1007/s00253-004-1642-2
41
42 Larsson S, Palmqvist E, Hahn-Hägerdal B, et al (1999) The generation of fermentation inhibitors during
43 dilute acid hydrolysis of softwood. *Enzyme Microb Technol* 24:151–159. doi: 10.1016/S0141-
44 0229(98)00101-X
45
46 Lee SC, Park S (2016) Removal of furan and phenolic compounds from simulated biomass hydrolysates by
47 batch adsorption and continuous fixed-bed column adsorption methods. *Bioresour Technol* 216:661–
48 668. doi: 10.1016/j.biortech.2016.06.007
49
50 Li H, Ye C, Liu K, et al (2015) Analysis of particle size reduction on overall surface area and enzymatic
51 hydrolysis yield of corn stover. *Bioprocess Biosyst Eng* 38:149–154. doi: 10.1007/s00449-014-1253-y
52
53 Li M, Pu Y, Ragauskas AJ (2016) Current Understanding of the Correlation of Lignin Structure with Biomass
54 Recalcitrance. *Front Chem* 4:1–8. doi: 10.3389/fchem.2016.00045
55
56 Licata B La, Sagnelli F, Boulanger A, et al (2011) Bio-hydrogen production from organic wastes in a pilot
57 plant reactor and its use in a SOFC. *Int J Hydrogen Energy* 36:7861–7865. doi:
58 10.1016/j.ijhydene.2011.01.096
59
60 Lin R, Cheng J, Ding L, et al (2015) Inhibitory effects of furan derivatives and phenolic compounds on dark
61 hydrogen fermentation. *Bioresour Technol* 196:250–255. doi: 10.1016/j.biortech.2015.07.097
62
63 Monlau F, Sambusiti C, Barakat A, et al (2014) Do furanic and phenolic compounds of lignocellulosic and
64 algae biomass hydrolyzate inhibit anaerobic mixed cultures? A comprehensive review. *Biotechnol Adv*
65 32:934–951. doi: 10.1016/j.biotechadv.2014.04.007
66
67 Monlau F, Trably E, Barakat A, et al (2013) Two-stage alkaline-enzymatic pretreatments to enhance
68 biohydrogen production from sunflower stalks. *Environ Sci Technol* 47:12591–12599. doi:
69 10.1021/es402863v

- 1
2
3
4 Montiel C, Hernández-Meléndez O, Vivaldo-Lima E, et al (2016) Enhanced Bioethanol Production from Blue
5 Agave Bagasse in a Combined Extrusion–Saccharification Process. *Bioenergy Res* 1–10. doi:
6 10.1007/s12155-016-9747-x
7
8 Murillo-Alvarado PE, Ponce-Ortega JM, Castro-Montoya AJ, et al (2014) Biofuels from residues of the
9 tequila industry of Mexico. Elsevier
10
11 Mussatto SI, Roberto IC (2004) Alternatives for detoxification of diluted-acid lignocellulosic hydrolyzates for
12 use in fermentative processes: A review. *Bioresour Technol* 93:1–10. doi:
13 10.1016/j.biortech.2003.10.005
14
15 Nakasu PYS, Ienczak LJ, Costa AC, Rabelo SC (2016) Acid post-hydrolysis of xylooligosaccharides from
16 hydrothermal pretreatment for pentose ethanol production. *Fuel* 185:73–84. doi:
17 10.1016/j.fuel.2016.07.069
18
19 Nigam JN (2001) Ethanol production from wheat straw hemicellulose hydrolysate by *Pichia stipitis*. *J*
20 *Biotechnol* 87:17–27. doi: 10.1016/S0168-1656(00)00385-0
21
22 Nissilä ME, Lay CH, Puhakka JA (2014) Dark fermentative hydrogen production from lignocellulosic
23 hydrolyzates - A review. *Biomass and Bioenergy* 67:145–159. doi: 10.1016/j.biombioe.2014.04.035
24
25 Nualsri C, Kongjan P, Reungsang A (2016a) Direct integration of CSTR-UASB reactors for two-stage
26 hydrogen and methane production from sugarcane syrup. *Int J Hydrogen Energy* 41:17884–17895. doi:
27 10.1016/j.ijhydene.2016.07.135
28
29 Nualsri C, Reungsang A, Plangklang P (2016b) Biochemical hydrogen and methane potential of sugarcane
30 syrup using a two-stage anaerobic fermentation process. *Ind Crops Prod* 82:88–99. doi:
31 10.1016/j.indcrop.2015.12.002
32
33 Olsson L, Hahn-Hägerdal B (1996) Fermentation of lignocellulosic hydrolysates for ethanol production.
34 *Enzyme Microb Technol* 18:312–331. doi: 10.1016/0141-0229(95)00157-3
35
36 Ouyang X, Tan Y, Qiu X (2014) Oxidative degradation of lignin for producing monophenolic compounds. *J*
37 *Fuel Chem Technol* 42:677–682. doi: 10.1016/S1872-5813(14)60030-X
38
39 Perez-Pimienta JA, Flores-Gómez CA, Ruiz HA, et al (2016) Evaluation of agave bagasse recalcitrance using
40 AFEX, autohydrolysis, and ionic liquid pretreatments. *Bioresour Technol* 211:216–223. doi:
41 10.1016/j.biortech.2016.03.103
42
43 Perez-Pimienta JA, Lopez-Ortega MG, Chavez-Carvayar JA, et al (2015) Characterization of agave bagasse
44 as a function of ionic liquid pretreatment. *Biomass and Bioenergy* 75:180–188. doi:
45 10.1016/j.biombioe.2015.02.026
46
47 Perez-Pimienta JA, Lopez-Ortega MG, Varanasi P, et al (2013) Comparison of the impact of ionic liquid
48 pretreatment on recalcitrance of agave bagasse and switchgrass. *Bioresour Technol* 127:18–24. doi:
49 10.1016/j.biortech.2012.09.124
50
51 Pérez-Pimienta JA, Vargas-Tah A, López-Ortega KM, et al (2017) Sequential enzymatic saccharification and
52 fermentation of ionic liquid and organosolv pretreated agave bagasse for ethanol production. *Bioresour*
53 *Technol* 225:191–198. doi: 10.1016/j.biortech.2016.11.064
54
55 Quéméneur M, Hamelin J, Barakat A, et al (2012) Inhibition of fermentative hydrogen production by
56 lignocellulose-derived compounds in mixed cultures. *Int J Hydrogen Energy* 37:3150–3159. doi:
57 10.1016/j.ijhydene.2011.11.033
58
59 Quintero M, Castro L, Ortiz C, et al (2012) Enhancement of starting up anaerobic digestion of lignocellulosic
60 substrate: Figue's bagasse as an example. *Bioresour Technol* 108:8–13. doi:
61 10.1016/j.biortech.2011.12.052
62
63 Ren N-Q, Zhao L, Chen C, et al (2016) A review on bioconversion of lignocellulosic biomass to H₂: Key
64 challenges and new insights. *Bioresour Technol* 215:92–99. doi: 10.1016/j.biortech.2016.03.124
65
66 Ren N, Li J, Li B, et al (2006) Biohydrogen production from molasses by anaerobic fermentation with a pilot-
67 scale bioreactor system. *Int J Hydrogen Energy* 31:2147–2157. doi: 10.1016/j.ijhydene.2006.02.011
68
69 Rencoret J, Pereira A, del Río JC, et al (2016) Laccase-Mediator Pretreatment of Wheat Straw Degrades

- 1
2
3
4 Lignin and Improves Saccharification. *Bioenergy Res* 9:917–930. doi: 10.1007/s12155-016-9745-z
- 5 Reungsang A, Sittijunda S, Sreela-or C (2016) Methane production from acidic effluent discharged after the
6 hydrogen fermentation of sugarcane juice using batch fermentation and UASB reactor. *Renew Energy*
7 86:1224–1231. doi: 10.1016/j.renene.2015.09.051
- 8
9 RFA (2017) BUILDING PARTNERSHIPS|GROWING MARKETS 2016 ETHANOL INDUSTRY
10 OUTLOOK.
- 11 Rios-González LJ, Morales-Martínez TK, Rodríguez-Flores MF, et al (2017) Autohydrolysis pretreatment
12 assessment in ethanol production from agave bagasse. *Bioresour Technol*. doi:
13 10.1016/j.biortech.2017.03.039
- 14
15 Saha BC (2004) Lignocellulose biodegradation and applications in biotechnology. In: ACS symposium series.
16 pp 2–34
- 17 Saini JK, Patel AK, Adsul M, Singhania RR (2016) Cellulase adsorption on lignin: A roadblock for economic
18 hydrolysis of biomass. *Renew Energy* 98:29–42. doi: 10.1016/j.renene.2016.03.089
- 19
20 Sambusiti C, Monlau F, Antoniou N, et al (2016) Simultaneous detoxification and bioethanol fermentation of
21 furans-rich synthetic hydrolysate by digestate-based pyrochar. *J Environ Manage* 183:1026–1031. doi:
22 10.1016/j.jenvman.2016.09.062
- 23
24 Sannigrahi P, Pu Y, Ragauskas A (2010) Cellulosic biorefineries-unleashing lignin opportunities. *Curr Opin*
25 *Environ Sustain* 2:383–393. doi: 10.1016/j.cosust.2010.09.004
- 26
27 Santos JRA, Lucena MS, Gusmão NB, Gouveia ER (2012) Optimization of ethanol production by
28 *Saccharomyces cerevisiae* UFPEDA 1238 in simultaneous saccharification and fermentation of
29 delignified sugarcane bagasse. *Ind Crops Prod* 36:584–588. doi: 10.1016/j.indcrop.2011.10.002
- 30
31 Saravanakumar T, Park HS, Mo AY, et al (2016) Detoxification of furanic and phenolic lignocellulose
32 derived inhibitors of yeast using laccase immobilized on bacterial cellulosic nanofibers. *J Mol Catal B*
33 *Enzym* 134:196–205. doi: 10.1016/j.molcatb.2016.11.006
- 34
35 Sarma SJ, Pachapur V, Brar SK, et al (2015) Hydrogen biorefinery: Potential utilization of the liquid waste
36 from fermentative hydrogen production. *Renew Sustain Energy Rev* 50:942–951. doi:
37 10.1016/j.rser.2015.04.191
- 38
39 Saucedo-Luna J, Castro-Montoya AJ, Martinez-Pacheco MM, et al (2011) Efficient chemical and enzymatic
40 saccharification of the lignocellulosic residue from Agave tequilana bagasse to produce ethanol by
41 *Pichia caribbica*. *J Ind Microbiol Biotechnol* 38:725–732. doi: 10.1007/s10295-010-0853-z
- 42
43 Saucedo-Luna J, Castro-Montoya AJ, Rico JL, Campos-Garcia J (2010) Optimization of Acid Hydrolysis of
44 Bagasse from Agave tequilana Weber. *Rev Mex Ing Química* 9:91–97.
- 45
46 Sekoai PT, Gueguim Kana EB (2014) Semi-pilot scale production of hydrogen from Organic Fraction of
47 Solid Municipal Waste and electricity generation from process effluents. *Biomass and Bioenergy*
48 60:156–163. doi: 10.1016/j.biombioe.2013.11.008
- 49
50 Silva EAB da, Zabkova M, Araújo JD, et al (2009) An integrated process to produce vanillin and lignin-based
51 polyurethanes from Kraft lignin. *Chem Eng Res Des* 87:1276–1292. doi: 10.1016/j.cherd.2009.05.008
- 52
53 Siqueira MR, Reginatto V (2015) Inhibition of fermentative H₂ production by hydrolysis byproducts
54 of lignocellulosic substrates. *Renew Energy* 80:109–116. doi: 10.1016/j.renene.2015.01.070
- 55
56 Stewart D (2008) Lignin as a base material for materials applications: Chemistry, application and economics.
57 *Ind Crops Prod* 27:202–207. doi: 10.1016/j.indcrop.2007.07.008
- 58
59 Su Y, Du R, Guo H, et al (2015) Fractional pretreatment of lignocellulose by alkaline hydrogen peroxide:
60 Characterization of its major components. *Food Bioprod Process* 94:322–330. doi:
61 10.1016/j.fbp.2014.04.001
- 62
63 Sun RC, Sun XF, Fowler P, Tomkinson J (2002) Structural and physico-chemical characterization of lignins
64 solubilized during alkaline peroxide treatment of barley straw. *Eur Polym J* 38:1399–1407. doi:
65 10.1016/S0014-3057(01)00303-2
- Taherzadeh MJ, Karimi K (2008) Pretreatment of Lignocellulosic Wastes to Improve Ethanol and Biogas

- 1
2
3
4 Production: A Review. *Int J Mol Sci* 9:1621–1651. doi: 10.3390/ijms9091621
- 5
6 Torres-Tello E V., Robledo-Ortíz JR, González-García Y, et al (2017) Effect of agave fiber content in the
7 thermal and mechanical properties of green composites based on polyhydroxybutyrate or
8 poly(hydroxybutyrate-co-hydroxyvalerate). *Ind Crops Prod* 99:117–125. doi:
9 10.1016/j.indcrop.2017.01.035
- 10 Tovar-Carrillo KL, Nakasone K, Sugita S, et al (2014) Effects of sodium hypochlorite on Agave tequilana
11 Weber bagasse fibers used to elaborate cyto and biocompatible hydrogel films. *Mater Sci Eng C*
12 42:808–815. doi: 10.1016/j.msec.2014.06.023
- 13 Tovar-Carrillo KL, Sueyoshi SS, Tagaya M, Kobayashi T (2013) Fibroblast compatibility on scaffold
14 hydrogels prepared from agave tequilana weber bagasse for tissue regeneration. *Ind Eng Chem Res*
15 52:11607–11613. doi: 10.1021/ie401793w
- 16
17 Valenzuela A (2011) A new agenda for blue agave landraces: food, energy and tequila. *GCB Bioenergy* 3:15–
18 24. doi: 10.1111/j.1757-1707.2010.01082.x
- 19 Vallejos ME, Chade M, Mereles EB, et al (2016) Strategies of detoxification and fermentation for
20 biotechnological production of xylitol from sugarcane bagasse. *Ind Crops Prod* 91:161–169. doi:
21 10.1016/j.indcrop.2016.07.007
- 22
23 Velazquez-Jimenez LH, Pavlick A, Rangel-Mendez JR (2013) Chemical characterization of raw and treated
24 agave bagasse and its potential as adsorbent of metal cations from water. *Ind Crops Prod* 43:200–206.
25 doi: 10.1016/j.indcrop.2012.06.049
- 26 Velázquez-Valadez U, Farías-Sánchez JC, Vargas-Santillán A, Castro-Montoya AJ (2016) Tequilana weber
27 Agave Bagasse Enzymatic Hydrolysis for the Production of Fermentable Sugars: Oxidative-Alkaline
28 Pretreatment and Kinetic Modeling. *Bioenergy Res* 9:998–1004. doi: 10.1007/s12155-016-9757-8
- 29 Venkata Mohan S, Venkateswar Reddy M, Venkata Subhash G, Sarma PN (2010) Fermentative effluents
30 from hydrogen producing bioreactor as substrate for poly(??-OH) butyrate production with
31 simultaneous treatment: An integrated approach. *Bioresour Technol* 101:9382–9386. doi:
32 10.1016/j.biortech.2010.06.109
- 33
34 Venkateswar Reddy M, Amulya K, Rohit MV, et al (2014) Valorization of fatty acid waste for bioplastics
35 production using *Bacillus tequilensis*: Integration with dark-fermentative hydrogen production process.
36 *Int J Hydrogen Energy* 39:7616–7626. doi: 10.1016/j.ijhydene.2013.09.157
- 37
38 Wilkinson S, Smart KA, Cook DJ (2014) Optimisation of alkaline reagent based chemical pre-treatment of
39 Brewers spent grains for bioethanol production. *Ind Crops Prod* 62:219–227. doi:
40 10.1016/j.indcrop.2014.08.036
- 41 Xuebing Z, Zhang L, Liu D (2012) Biomass recalcitrance. Part I: the chemical compositions and physical
42 structures affecting the enzymatic hydrolysis of lignocellulose. *Biofuels, Bioprod Biorefining* 6:465–
43 482. doi: 10.1002/bbb
- 44
45 Zhao X, Cheng K, Liu D (2009) Organosolv pretreatment of lignocellulosic biomass for enzymatic
46 hydrolysis. *Appl Microbiol Biotechnol* 82:815–827. doi: 10.1007/s00253-009-1883-1
- 47
48 Zhou S, Xue Y, Sharma A, Bai X (2016) Lignin Valorization through Thermochemical Conversion:
49 Comparison of Hardwood, Softwood and Herbaceous Lignin. *ACS Sustain Chem Eng* 4:6608–6617.
50 doi: 10.1021/acssuschemeng.6b01488
- 51
52 Zhu L, O'Dwyer JP, Chang VS, et al (2008) Structural features affecting biomass enzymatic digestibility.
53 *Bioresour Technol* 99:3817–3828. doi: 10.1016/j.biortech.2007.07.033
- 54
55
56
57
58
59
60
61
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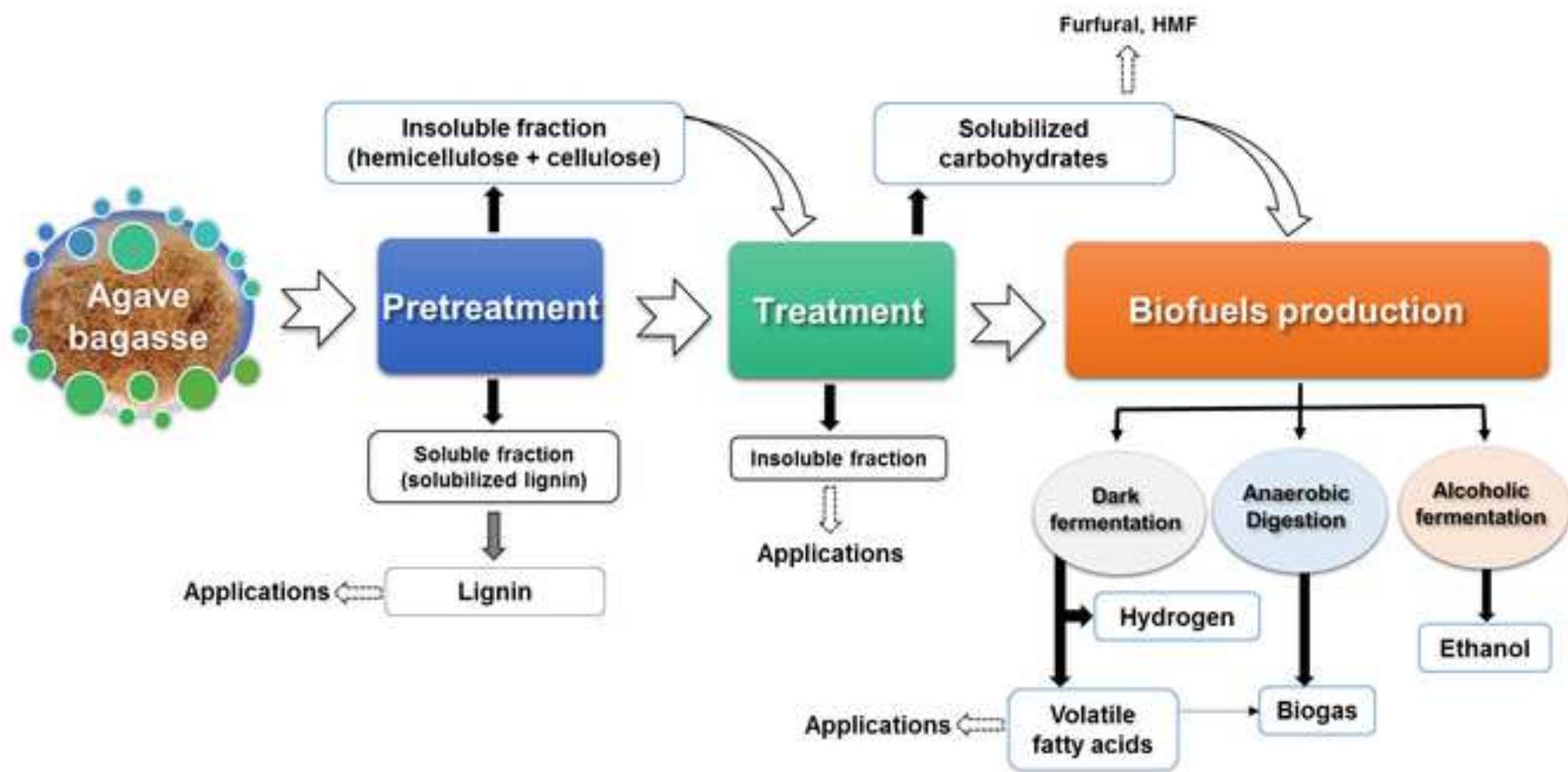
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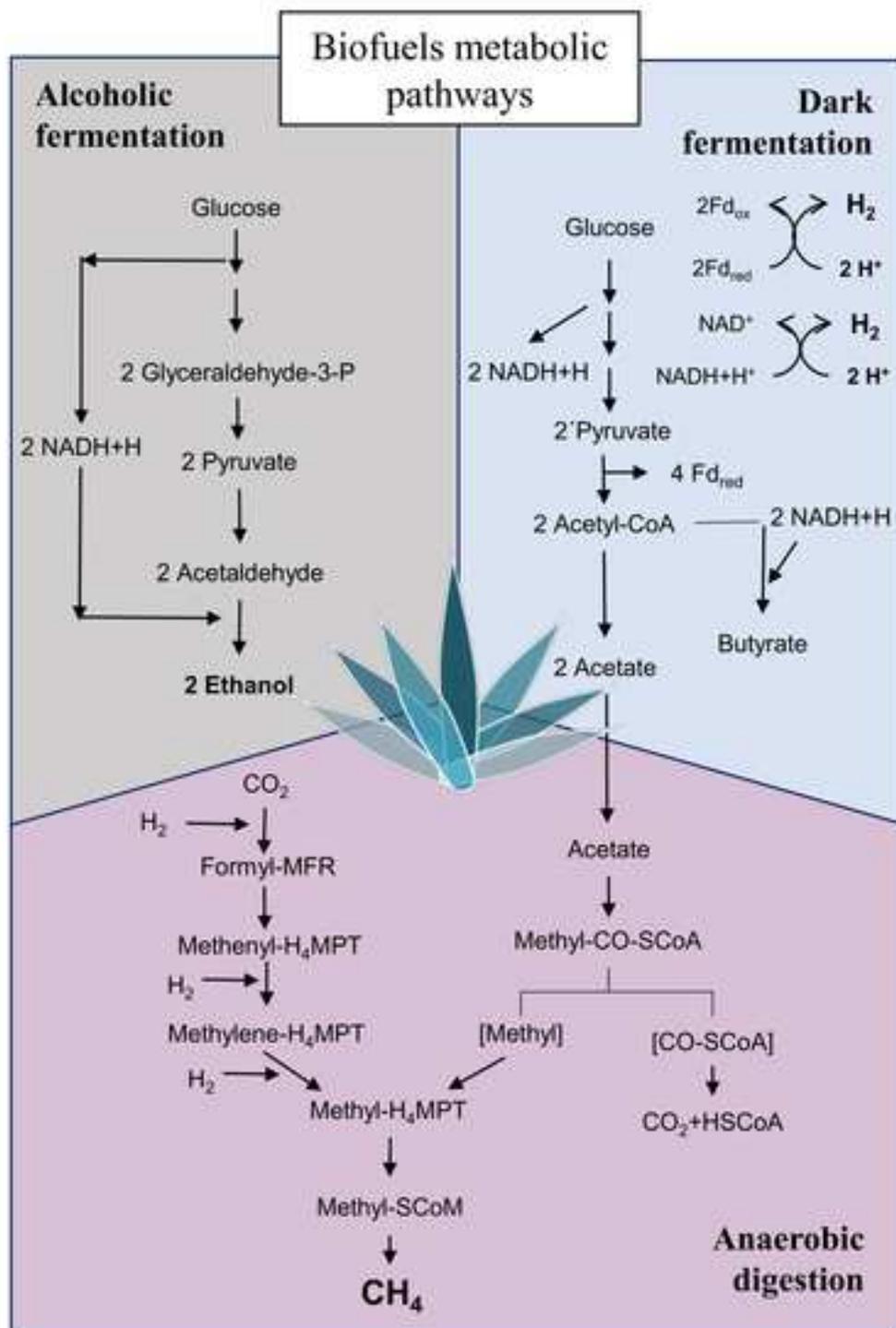
FIGURE CAPTIONS

Fig. 1 Agave bagasse biorefinery scheme

Fig. 2 Metabolic pathways for the production of ethanol, hydrogen and methane by anaerobic processes

Figure 1





LIST OF TABLES

Table 1. Delignification pretreatments for lignocellulosic biomass.

Lignocellulosic biomass	Delignification pretreatment	Delignification yield (%)	Disadvantages	Reference
Wheat straw	Enzymatic (laccases)	48	Their use are restricted by lignin content in lignocellulosic biomass	Rencoret et al. 2016
Agave bagasse ^a	Alkaline	N.R	High temperature and pressure	Hernández-Salas et al. 2009
Agave bagasse	Alkaline	N.R	High temperature and pressure	Ávila-Lara et al. 2015
Rice straw	Alkaline	28.4	Low delignification yields even with high NaOH concentrations (6%) Removal of hemicellulose (36.8%)	He et al. 2008
Agave bagasse	Ethanosolv (similar to organosolv)	69	Removal of hemicellulose by use of sulfuric acid High temperature and pressure	Caspeta et al. 2014
Agave bagasse	Organosolv	45	Removal of hemicellulose (86%) by use of sulfuric acid High temperature and pressure	Pérez-Pimienta et al. 2017
Agave bagasse	Alkaline-oxidative	82.62	High amounts of reagents and temperature Long reaction time	Velázquez-Valadez et al. 2016
Agave bagasse	Oxidative	19.6	Long reaction time (48 h)	Perez-Pimienta et al. 2016
Corncob	Oxidative	74	Removal of hemicellulose (39%)	Su et al. 2015

N. R: Not reported

^a*Agave atrovirens*

Table 2. Enzymatic hydrolysis of agave bagasse after pre-treatment

Pretreatment	Enzymatic hydrolysis conditions	Enzyme	Sugars concentration	Saccharification yield	References
Acid hydrolysis (25.8 g/L of sugars)	pH 5, 40 °C, 72 h	Celluclast 1.5L ¹ (80 U/g of TS*) + Novozyme 188 ² (100 U/g of TS)	41 g/L	73.6 %	Saucedo-Luna et al. 2011
Ionic liquids	pH 5.5, 55 °C, 72 h, solid loadings 15%	40 mg of Cellic® CTec2 ³ /g glucan + 4mg of Cellic® HTec2 ⁴ /g xylan	7.6 g/L		Perez-Pimienta et al. 2013
Ethanosolov	pH 4.8, 50 °C, 72 h, solid loadings of 3 % (w/w)	NS50013 ⁵ 10 PFU/g TS + NS50010 ⁶ 20 CBU/g TS	225 g/L	91 % 0.51 g/g bagasse	Caspeta et al. 2014
Alkaline Acid hydrolysis	pH 4.8, 55 °C, 72 h, solid loadings of 3 % (w/w)	Cellic® CTec2 ³ 35 FPU/g + Cellic® HTec2 ⁴ 60CBU/g biomass		460 mg/g dry matter 457 mg/g dry matter	Ávila-Lara et al. 2015
Alkali extrusion	pH 5.5, 50 °C, 24 h, solid loadings of 2.5 % (w/w)	80% Cellic CTec2 ³ + 20% Viscozyme ⁷ 10 mg/g DM	69.5 g/L	73 %	Montiel et al. 2016
Alkali-Oxidative	pH 5.0, 50 °C, 72 h	6% Cellic® CTec3 ³ + 6% Cellic® HTec3 ⁴	165.7 g/L 136.4 g/L of glucose and 29.2 g/L of xylose	82.2 %, 352.2 g/kg	Velázquez-Valadez et al. 2016
Ionic liquids	pH 4.8, 55 °C, 48 h, solid loadings 5 g glucan/L	Cellic® CTec2 + Cellic® HTec2: 20 g protein per kg glucan and 2 g protein per kg xylan, respectively	6.7 g/L		Perez-Pimienta et al. 2015
Ionic liquids			25.5 g/L	397 g/kg	
Ammonia fiber expansion	pH 4.8, 50 °C, 24 h, solid loadings 20 g/L	Cellic® CTec2 + Cellic® HTec2 40 mg protein per g glucan and 4 mg protein per g xylan, respectively	21.4 g/L	425 g/kg	Perez-Pimienta et al. 2016
Autohydrolysis			14.4 g/L	269 g/kg	
Ionic Liquids	pH 4.8, 50 °C, 18 h, solid loadings 10% (w/w)	Cellic® CTec2 8 FPU/g + Cellic® HTec2 15 CBU/g	36.3 g glucose/L and 14.4 g xylose/L	71.2 %	Pérez-Pimienta et al. 2017
Organosolov			67.7 g glucose/L and 5.6 g xylose/L	53.8 %	

¹Celluclast 1.5L, ³Cellic® CTec, ⁵NS50013 (Cellulases); ²Novozyme 188, ⁶NS50010 (Cellobiase); ⁴Cellic® HTec2, ⁷Viscozyme (Hemicellulase) *TS – Total Solids

Table 3. Selection of AF, DF, and AD of lignocellulosic materials.

Biofuel process	Type of system	Inoculum	Substrate	Operational conditions	Fuel production rate	Energy production rate kJ/gCOD-d	Fuel production yield	Energy yield kJ/gCOD	Reference
DF	Batch	Anaerobic sludge	Agave bagasse (EH)	40 % (v/v) hydrolysate, S ⁰ : 16 gCOD/L, 37 °C, pH: 7	2.4 L/L-d	1.6	3.4 molH ₂ /molHex	4.25 ^a	Arreola-Vargas et al. 2016a
DF	CSTR	Anaerobic sludge	Agave bagasse (EH)	HRT: 6 h, S ⁰ : 13 gCOD/L, OLR: 52.5 gCOD/L-d, 37 °C, pH: 5.5	2.53 L/L-d	2.1	0.79 molH ₂ /molHex	0.99 ^a	Contreras-Dávila et al. 2017
DF	TBR	Anaerobic sludge	Agave bagasse (EH)	HRT: 4 h, S ⁰ : 8.8 g COD/L, OLR: 52.9 gCOD/L-d, 37 °C, pH: 5.5	3.45 L/L-d	4.2	1.53 molH ₂ /mol Hex	1.91 ^a	Contreras-Dávila et al. 2017
DF	BTF	Anaerobic sludge	Oat straw (AH)	HRT: 12 h, S ⁰ : 35 gCOD/L, OLR: 70 gCOD/L-d, 28 °C, pH: 5.5	1.95 L/L-d	0.60	0.4 molH ₂ /molHex	0.5 ^a	Arriaga et al. 2011
DF	ASBR	Anaerobic sludge	Oat straw (EH)	HRT: 8 h, S ⁰ : 5 gCOD/L, OLR: 15 gCOD/L-d, 35°C, pH: 4.5	0.71 L/L-d	1.52	0.81 molH ₂ /molHex	1.01 ^a	Arreola-Vargas et al. 2013
DF	TBR	Triticale silage	Oat straw (EH)	HRT: 12 h, S ⁰ : 5 gCOD/L, OLR: 10 gCOD/L-d, 35°C, pH: 5,	0.624 L/L-d	1.33	2.3 molH ₂ /molHex	2.87 ^a	Arreola-Vargas et al. 2015a
DF	Batch	<i>C. butyricum</i>	Jatropha hulls (AH)	S ⁰ : 15.64 gRS/L, 35°C, pH: 6.5	4.29 L/L-d	2.9	1.95 molH ₂ /molHex	2.44 ^a	Dan Jiang et al. 2016
DF	Batch	<i>C. butyricum</i>	Sugarcane bagasse (AH)	S ⁰ : 15.64 gRS/L, 35°C, pH: 6.5	4.52 L/L-d	3.1	2.06 molH ₂ /molHex	2.58 ^a	Dan Jiang et al. 2016
AD	Batch	Anaerobic sludge	Goose manure + corn stover (Alk)	37 °C, pH: 7.0-7.8	0.01 L/g VS-d ^b	-	0.39 L/gVS	13.96	Hassan et al. 2017a
AD	Semi-continuous STR	Anaerobic sludge	Goose manure + wheat straw (Alk)	S ⁰ : 30 gVS/L, OLR: 3 gVS/L-d, HRT: 10 d	~ 8 L/L-d	-	0.26 L/gVS	9.31	Hassan et al. 2017b
AD	Batch	Anaerobic sludge	Agave bagasse → DF Effluent	S ⁰ : 20 % (v/v), pH:8, 37°C	0.96 L/L-d	34.4	0.24 L/gCOD	8.60	Arreola-Vargas et al. 2016a

AD	Continuos	Anaerobic sludge	Agave bagasse → DF Effluent	HRT: 8.7 h, S ⁰ : 0.9 gCOD/L, OLR: 2.5 g COD/L-d, 37°C, pH: 7	0.9 L/L-d	35.8	0.35 L/gCOD	12.53	Cheng et al. 2016
AD	ASBR	Anaerobic sludge	Tequila vinasses	S ⁰ : 8 gCOD/L, X ⁰ : 16.5g VSS/L, 32°C, pH:7	2.25 L/L-d	15.8	0.29 L/gCOD	10.38	Arreola-Vargas et al. 2016b
AD	Batch	Anaerobic sludge	Sugarcane syrup → DF effluents	S ⁰ : 25 gCOD/L, 30 °C, pH: 7	0.022 L/gCOD-d	0.78	0.31 L/gCOD	11.10	Nualsri et al. 2016b
AD	UASB	Anaerobic sludge	Sugarcane syrup → DF effluents	HRT: 3 d, S ⁰ : 25 gCOD/L	2.25 L/L-d	3.24	0.27 L/gCOD	9.66	Nualsri et al. 2016a
AD	UASB	Anaerobic sludge	Sugarcane syrup → DF effluents	HRT: 4 d, OLR: 5.25 g COD/L-d, S ⁰ : 20 g COD/L	1.27 L/L-d	2.27	0.35 L/gCOD	12.53	Reungsang et al. 2016
AD	ASBR	Anaerobic sludge	A. tequilana bagasse (AH)	S ⁰ : 5 g COD/L, VSS/L, pH: 7.5, 32 °C	0.3 L/L-d	2.14	0.26 L/gCOD	9.31	Arreola-Vargas et al. 2015b
AD	Batch	Anaerobic sludge	Sugar cane bagasse → AH + EH	S ⁰ : 5.5 g VS/L, pH: not controlled (7-8.1), 37°C	-	-	0.2 L/gVS	7.16	Badshah et al. 2012
AD	Batch	Ruminal liquid + pig waste sludge	Fique`s bagasse (<i>Furcraea</i> sp.)	39°C	0.14 g COD-CH ₄ /g VSS	-	0.3 L/gVS _{added}	10.74	Quintero et al. 2012
AD	CSTR	Anaerobic sludge	Apple waste (25% VS) + swine manure (75% VS)	HRT:30 d, OLR: 1 g VS/L-d, 36-38 °C, pH:7.8	-	-	0.24 L/gCOD _{added}	8.59	Kafle and Kim 2013
AF	Batch	<i>S. cerevisiae</i>	Agave (AH)	S ⁰ : 35.4 g RS/L, 30°C, pH 5	3.7 g/L-d ^b	0.35	0.14 g/gCH	0.6	Hernández-Salas et al. 2009
AF	Batch	<i>P. caribbica</i>	Agave bagasse (EH)	S ⁰ : 25.8 gCH ₅ s, 30 °C, pH 5	3.2 g/L-d ^b	0.5	0.56 g/gCH	0.91	Saucedo-Luna et al. 2011
AF	Batch	<i>S. cerevisiae</i>	Agave bagasse (EH)	S ⁰ : 93 g _{glucose} /L, 30 °C, pH 5	38.4 g/L-d ^b	0.61	0.96 g/gGlucose	0.58	Caspeta et al. 2014
AF	SSF	<i>E. coli</i>	Agave bagasse (Organosolv)	37 °C, pH 7	28.8 g /L-d	1.12	0.85 g/gCH	1.52	Pérez-Pimienta et al. 2017
AF	SSF	<i>E. coli</i>	Agave bagasse (IL)	37 °C, pH 7	16.3 g/L-d	1.02	0.82 g/gCH	2.52	Pérez-Pimienta et al. 2017
AF	Batch	<i>S. cerevisiae</i>	Agave bagasse (Autohydrolysis)	S ⁰ : 130 g _{glucose} /L, 32 °C, pH 5.5	156 g/L-d ^b	2.64	0.95 g/gGlucose	1.0	Rios-González et al. 2017
AF	Batch	<i>S. cerevisiae</i>	Sugarcane bagasse (EH)	S ⁰ : 88.8 g delignificated bagasse/L, 37 °C, pH 4.8	13.8 g/L-d ^b	2.45	0.12 g/gBagasse	0.93	Santos et al. 2012

DF: Dark fermentation; AD: Anaerobic digestion; AF: Alcoholic fermentation.

BTF: Biotrickling filter; ASBR: Anaerobic sequencing batch reactor; CSTR: Continuous stirred tank reactor; TBR: Trickling bed reactor; UASB: Up-flow anaerobic sludge blanket; STR: Stirred tank reactor; SSF: Simultaneous saccharification and fermentation.

EH: Enzymatic hydrolysate; AH: Acid hydrolysate; Alk: Alkaline hydrolysate; CH: Carbohydrates; RS: Reducing sugars; S^0 : initial substrate concentration;

VFA: Volatile fatty acids; VS: Volatile solids; VSS: Volatile suspended solids; X^0 : Initial microorganism's concentration.

^a Calculated from COD of hexoses; ^b computed using the total fermentation/digestion time