Status report after the first six project months

February 2023

At the beginning of February, the first small plants of the sown intercrops are appearing between the olive trees. Furthermore, the first reed plants have also been planted in the constructed wetlands. However, the low temperatures with night frosts in Turkey and Portugal are still challenging for the plants and stronger growth can only be expected in the next few months.

These biomasses of the intercropping and the constructed wetland thus still have to be produced. However, within the other work packages, representative biomass has already been worked with. Different types of biomass were pretreated to separate valuable biobased products and subsequently produce biogas and digestate.

In the following, a literature review is given for the different topics of

Furthermore a short update concerning the current project status with regard to these topics is given in each part.

PRIMA PROJECT INTERIM REPORT (1.-6. MONTH)

A) Lignocellulosic materials and pre-treatment methods

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1. Lignocellulosic Materials

Lignocellulosic materials such as wood, agricultural, or forestry wastes are present in great abundance, which are usually underutilized. However, the lignocellulosic materials offer a high potential as alternative resources for biofuel and chemical production. Lignocellulosic biomass usually composed of three main structural polymers: cellulose, hemicelluloses, and lignin (Figure 1). Depending on the origin of the plant material, these three fractions vary in quantity and quality. In general, a lignocellulosic material contains 40-60 % cellulose, 20-40 % hemicellulose and 10-25 % lignin.
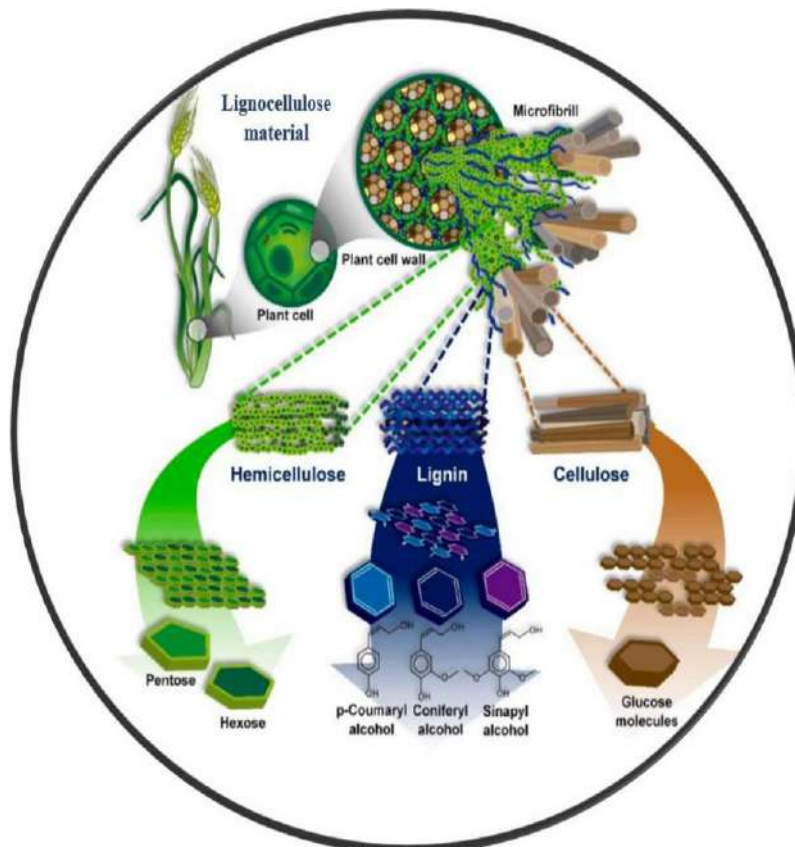

Figure 1. Structure of lignocellulosic materials (Khan et al. 2022)

1.1.   Cellulose

Cellulose is a homo-polysaccharide having a linear chain of repeating β-D-glucopyranose units linked covalently by β-(1, 4) glycosidic bonds which provides stiffness and stability to the cell wall (McDonald and Donaldson 2001). Cellulose chains are connected by hydrogen bonds and van der Waal's forces to form microfibrils which contain both highly ordered (crystalline) and less ordered (amorphous) regions (McDonald and Donaldson 2001). Due to having loosely organized hydrogen bonding, the amorphous structures are 3–30 times easier to lyse compared to the crystalline form (Yang et al. 2019). The measured degree of polymerization of native cellulose is in the order of 10000. The microfibrils are further bonded with hemicelluloses and lignin to form macrofibrils. The high level of bonding results in both high tensile strength and general insolubility in most solvents, which makes it difficult to degrade (McDonald and Donaldson 2001).

1.2.   Hemicellulose

Unlike cellulose, hemicellulose is a branched hetero-ploysaccharide with 300-5000 sugar units. It contains highly branched pentoses (xylose and arabinose), hexoses (mannose, glucose, galactose), low content saccharides (rhamnose and fructose), uronic acids and acetyl groups in varying amounts linked together by hydrogen and covalent bonds (Yousuf et al. 2020). A variety of structural compositions between different types of plants can be observed. For instance, softwood hemicelluloses contain mainly glucomannans, whereas hardwood hemicelluloses contain mainly xylans (Rahmati et al. 2020). It connects microfibrils tightly with non-covalent attractions and forms a complex network of covalent bonds between lignin, enhancing the structural strength (Speight 2020). Hemicellulose has its own acetate groups attached randomly to the hydroxyl groups of sugars with ester linkages and lacks a crystalline structure (Yousuf et al. 2020). Therefore, it can be hydrolyzed easily by enzymes and different types of chemicals.

1.3.   Lignin

Lignin is an aromatic, irregular, non-crystalline, three-dimensional polymer containing various functional groups, such as phenyl-propane, methoxy, and non-carbohydrate polyphenolic substances (Ashokkumar et al. 2022). It is formed by the free-radical polymerization of alcohol precursors namely, p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol, building the three primary units, p-hydrox- yphenyl (H), guaiacyl (G), and syringyl (S) groups, respectively (Del Río et al. 2020). Lignins are linked with hemicellulose via covalent and hydrogen bonds

(Scelsi et al. 2021). Lignin fills the gap between cellulose fibrils and hemicellulose and holds the polymers together. It offers hydrophobic strength and enhance recalcitrance of the lignocellulose. Therefore, lignin has a negative impact on lignocellulosic biomass conversion (Zoghlami and Paës 2019).

2. Pretreatment of Lignocellulosic Materials

An effective fractionation process is vital to convert lignocellulosic biomass effectively and to design an economical, eco-friendly and sustainable biorefinery (Chuetor et al. 2021). Therefore, pretreatment is considered as the most crucial step prior to conversion of lignocellulosic biomass into value added products. Pretreatment alters the structure of biomass and improves the digestibility of cell wall polysaccharides.

Pretreatment affects the biorefinery system in terms of configuration and cost. In addition, the type of the feedstock, environmental concerns and sustainability should also be considered while selecting a pretreatment method. Various pretreatment technologies (physical, chemical, physico-chemical and biological) have been proposed in the last couple of decades (Khan et al. 2022). The different pretreatment techniques have been the subject of many review papers and their principals are summarized in the literature (Ashokkumar et al. 2022; Khan et al. 2022; Cheng and Whang 2022; Saravanan et al. 2022). In this project, four types of pretreatment methods (acid, alkaline, hydrothermal and deep eutectic solvent) will be examined. Therefore, information regarding only the aforementioned techniques are provided in the report.

2.1.1. Acid

Acid treatment is one of the most commonly applied technique to destruct the cellulosic structure. Both inorganic and organic acids (such as sulfuric acid, nitric acid, hydrochloric acid, maleic acid, formic acid and oxalic acid) can be used in this method. Even though both dilute and concentrated acid pretreatment have been practiced, concentrated acid method became less appealing over time owing to its eroding, noxious and expensive nature (Saravanan et al. 2022). Due to its high hemicellulose recovery, reduction in enzyme usage, and being economically friendly, diluted acid pretreatment is considered the most widely employed treatment method in the full-scale plant (López-Linares et al. 2020a).

Hemicellulose is the main point of attack of the acids during pretreatment process (Cai et al. 2021). Dilute acid hydrolysis causes swelling of biomass and hence, loosening the structural linkages between xylan and lignin (Loow et al. 2016). Acid dissociates in water to form H+

protons, which migrates to the reactive sites of lignocellulose and disrupts the structure. It cleaves the glycosidic linkages (defined as the ether bonds) inside hemicellulose polymer by protonating glycosidic oxygen or ring oxygen in formation of carbonium cation (Harmsen 2010). This leads to the conversion of polymers to simple sugars such as glucose, xylose, and arabinose, as well as lignin fragments.

The most important process parameters that affect acid treatment efficiency are solid to liquid ratio, size of the lignocellulose particle, concentration of the acid, pretreatment temperatures and duration (Saravanan et al. 2022).

2.1.2. Alkaline

Alkaline pretreatment has also shown great promise for fractionation of lignocellulosic biomass. Sodium hydroxide (NaOH), potassium hydroxide (KOH), calcium hydroxide, sodium carbonate, aqueous ammonia (e.g., ammonium hydroxide, ammonium carbonate), and hydrogen peroxide are used in alkaline treatment (Rahmati et al. 2020).

Alkali pretreatment breaks down the lignin structure without degrading the polymeric carbohydrates. It promotes structural swelling and causes an increase in the internal surface area and porosity of materials, which also promotes the reactivity of the remaining polysaccharides. This technique demonstrates better efficacy on agriculture non-wood based lignocellulose materials (Reilly et al. 2015).

Similar to acid pretreatment, solid to liquid ratio, size of the lignocellulose particle, concentration of the alkaline, pretreatment temperatures and duration are the most important process parameters for this technique (Saravanan et al. 2022).

2.1.3. Hydrothermal

Hydrothermal pretreatment includes methods that use water as solvent at high temperatures. It has been widely studied due to its simple operation, moderate energy consumption, relatively short treatment time and low cost. It is also considered as one of the best environmentally friendly pretreatment method since, no chemicals are used in this technique (Yang et al. 2018).

Water is polar under normal conditions, and a wide network of hydrogen bonds can be formed between water molecules. The hydrogen bonds slowly weaken as the temperature rises and water molecules dissociates into acidic hydronium ions (H_3O^+) and basic hydroxide ions (OH^-) (Yang et al. 2018). Therefore, water at high temperatures has the function of acid-alkali

autocatalysis (Torres-Mayanga et al. 2019). The hydronium ions depolymerize polysaccharides through selective hydrolysis of heterocyclic ether bonds and cleavage of acetyl groups (Lu et al. 2016). The acetyl groups in hemicelluloses could produce organic acids (formic acid, acetic acid, and levulinic acid) during hydrothermal pretreatment, which further promotes the depolymerization process. This process is more effective for materials with high acetyl content (i.e. hardwoods, agricultural residues), since the hydronium ions generated from acetyl groups play an important role in hydrolysis (Yue et al. 2022).

Solid to liquid ratio, size of the lignocellulose particle, pretreatment temperature and time are the most important process parameters for this technique (Yue et al. 2022).

2.1.4. Deep Eutectic Solvents (DES)

Deep Eutectic Solvents (DES) are a new generation of solvents that have emerged at the beginning of this century. They can be synthesized by simply mixing two compounds (hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA)) together within a certain molar ratio. These two components must be capable of forming a eutectic mixture (Abbott et al. 2003). DES are easy to prepare and biodegradable. They have low toxicity, low melting points, high thermal stability, low volatility, non-flammability, and high air stability (Abbott et al. 2003).

DES are capable of donating and accepting protons, which provide them a high solvation property. This characteristic enables the formation of hydrogen bonds with other compounds (Pandey et al. 2017). The chloride ions in DES disrupt the existing bonding interactions between carbohydrates and lignin and develop new and competing hydrogen bonds with these components (Isci et al. 2021).

DES type, DES molar ratio, water content in DES, solid to liquid ratio, size of the lignocellulose particle, pretreatment temperature and time are the most important process parameters for this technique.

3. Literature Survey of Feedstock Pretreatment

In this project, the biomass that will be used in conversion processes are oak (*Quercus suber L*) bark waste, cork dust, reed, olive tree waste and olive pomace. The information regarding these feedstock are provided below.

3.1. Pretreatment of oak bark/cork dust

Cork is produced from the outer bark of the cork oak tree (*Quercus suber L.*) (Aroso et al. 2017). Cork consists primarily of lignin and suberin (hydrophobic biopolymers) along with cellulose and hemicellulose (hydrophilic polysaccharides) (Jové et al. 2011; Carriço et al. 2018). It is a natural, renewable, and sustainable raw material that is usually made up of suberin (30-60 wt%), lignin (19-22 wt%), polysaccharides (12-20 wt%), and extractives (9-20 wt%), such as aliphatic, phenolic, and triterpenenic compounds (Soares et al. 2014) (Figure 2). There is growing interest in the extractives found in cork and cork by-products, specifically in bioactive compounds such as polyphenols and triterpenoids (e.g., friedelin), for their anti-inflammatory, anti-cancer, anti-oxidative, antiviral, antifungal, and antibacterial properties (Cunha et al. 2020).
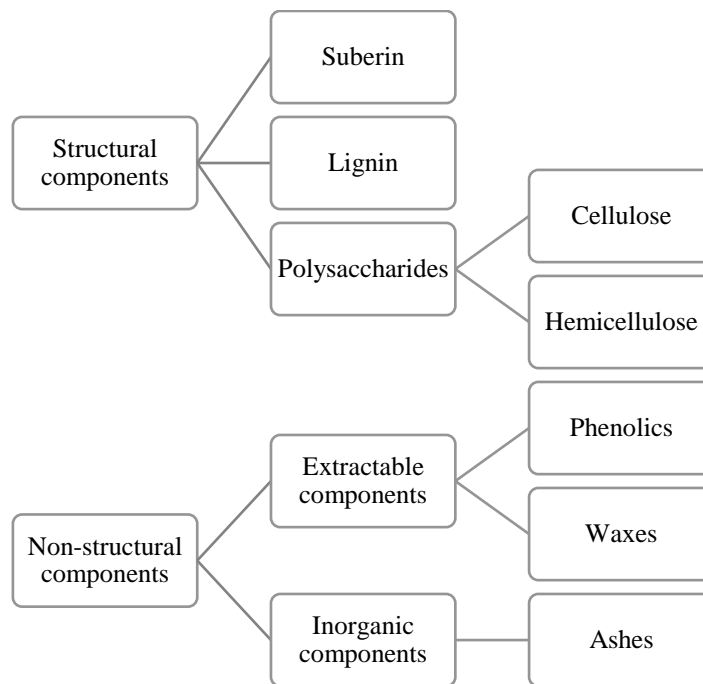
Figure 2. Chemical components of cork (Soares et al. 2014)

In contrast to other woods and barks, where cellulose constitutes approximately 50% of cell wall material, cellulose does not play such an important role in cork (Carriço et al. 2018). Furthermore, Conde et al. (1998) stated in their study that cellulose does not play the same role in cork as it does in other woods and barks, and that hemicellulose, specifically xylans, plays an important role in cork. Polysaccharides are present in non-negligible amounts in cork, and therefore they are one of the least investigated components (Silva et al. 2005). Hence, the

valorization of the polysaccharide fraction derived from cork or cork by-products was not discussed in this section due to a lack of studies.

This renewable material has a wide range of applications, the most important of which is the production of wine stoppers, followed by applications in thermal and/or acoustic insulation materials (Figure 3). The cork industry is an important sector in the Portuguese economy because it is the world's largest cork producer (about 157 000 tons of cork annually, representing 50% of global production) and exporter (representing 60% of global cork exports in 2005) (Santos et al. 2013).

Figure 3. Debarked *Quercus suber* tree, cork products, and alternative ways for the valorization of residues (Aroso et al. 2017).

The cork industry generates a problematic residue known as "cork dust/cork powder," which accounts for a significant portion of the total amount of waste with low commercial value (Mateus et al. 2016). Industrial processing of raw cork yields 20% to 30% residue, primarily in the form of cork powder with small particle size and low valorization. This powder is primarily used to generate heat and energy. As a result, chemical conversion of this waste, cork powder, into a high-value product is an attractive option for increasing the overall value of the cork chain and increasing cork oak sustainability (Esteves et al. 2017). Instead of being burned to produce energy, this by-product could potentially be used more efficiently to produce bio-polymers. Several efforts in this direction have already been made. Suberin, a key component of cork, has been extracted and used in the production of polyurethanes. Cork has also been liquefied using cyclic carbonate methods (oxypropylation), and the resulting polyols have been used to make polyurethanes (Yona et al. 2014).

The molecular structure of cork suberin is complex, and it is intricately linked to other structural components. Because it is insoluble in the majority of common solvents, prior chemical cleavage is required for analysis, extraction, or conversion. As a result, any attempt to valorize suberin will necessitate the development of suitable depolymerization processes to convert it to a usable format. However, little was known about the use of suberin in practical applications until recently, and the depolymerization processes relied heavily on suberin obtained through the same procedures as for characterization studies (Aroso et al. 2017).

Cork liquefaction is an alternative process to traditional chemical depolymerization via methanolysis or hydrolysis that appears to have gained recent interest. Biomass liquefaction, such as the liquefaction of lignocellulosic wastes, is a thermochemical process that transforms lignocellulosic materials into liquid products. Numerous studies on biomass liquefaction and its optimization utilizing various methods may be found in the literature. Thus, liquefaction parameters such as the ratio of raw material to solvent, solvent type, catalyst loading, liquefaction time, and temperature have been extensively studied. In this sense, the possibility of converting cork powder into liquid polyols via liquefaction process was studied in several studies. For example, Soares et al. (2014) investigated the conversion of cork powder into liquid polyols by acid liquefaction using 4% sulfuric acid as a catalyst at 150 °C for 60 min in the presence of a PEG 400-Glycerol solvent mixture. Under these conditions, the liquefaction yield (LY) was 79%, resulting in minimal residue content. They concluded that cork powder can be converted into liquid polyols through acid liquefaction at atmospheric pressure and mild temperatures in the presence of PEG 400. In another study conducted by (Yona et al. 2014), the liquefaction of cork with glycerol was investigated at 150 °C, 180 °C, and 200 °C under acidic (sulphuric acid) or alkaline conditions (sodium hydroxide). It was reported that only the liquefaction of lignocellulosic components (lignin and cellulose fractions) was accomplished in the presence of sulfuric acid, resulting in a liquefaction yield of less than 47%. However, sulfuric acid was ineffective in liquifying suberin. In contrast, sodium hydroxide was very appropriate for the liquefaction of suberin, but less effective for lignocellulosics. Suberin was almost completely liquefied as the reaction temperature was increased from 150 to 200 °C, demonstrating the effectiveness of a basic catalyst at higher temperatures. In contrast, a small amount of PEG added to glycerol caused suberin conversion under acidic conditions, resulting in liquefaction yields of up to 93%, as opposed to 50% when NaOH was used. (Mateus et al. 2015) used ultrasound irradiation to accelerate the liquefaction process. The reaction was carried out on a mixture containing 10% w/w cork powder in a 1:2 (% w/w) mixture of 2-

ethylhexanol and diethylene glycol containing 3% p-toluene sulfonic acid. At the start of the reaction, high frequency sound waves (24 kHz) with amplitudes ranging from 60% to 100% were applied for 3 minutes. The accelerated process achieved 90% conversion in less than 3 minutes, whereas the non-accelerated process was lasted for 60 minutes. In another study, microwave radiation was used as the heating source in cork liquefaction at 160°C in the presence of 2-ethyl hexanol/diethylene glycol and p-toluene sulfuric acid as catalyst (Santos and Mateus, 2015). They also compared the conventional, ultrasound- and microwave- assisted liquefaction methods. It was found that microwave heating showed high product yields and short reaction time even when compared with the ultrasounds-assisted depolymerization. The optimal conditions were set to irradiate the reaction mixture for 5 minutes with a microwave power of 300 W. In another study (Esteves et al. 2017), the optimum conditions to obtain high liquefaction yields from cork powder were determined to be 160 °C for 1 h; glycerol to PEG-400 solvent ratio of 1:9; cork to solvent ratio of 1:3; and 3% H_2SO_4 as catalyst.

3.2.   Pretreatment of olive tree waste (pruning waste)

Olive oil industry is one of the most important industries in the world. Over the past several decades, the healthy properties associated with the consumption of high-quality olive oil have made the cultivation of olive trees to expand all over the world. According to the Food and Agriculture Organization of the United Nations, the land devoted to olive-tree cultivation around the world is 11×10^6 ha, resulting more than 20×10^6 t olives per year. This led to 3.2 million tons of olive oil (García Martín et al. 2020).

Along with olive oil, the main product, several different biomasses are also generated. Olive tree pruning biomass is an agricultural residue generated yearly in olive tree cultivation by the removal of un-productive branches to prepare the tree for next harvest. This operation results in a large amount of biomass that must be removed from the fields. The olive-pruning debris consists of thin branches (usually <5 cm diameter) and leaves. An average of 3 tons of olive-pruning debris is generated from a hectare of olive orchard, leading to producing more than 3.3 10^7 t biomass which have a huge potential as source of energy (García Martín et al. 2020; Martínez-Patiño et al. 2017). Generally, the olive-pruning debris is either ground and ploughed into soil or left on the land to be incinerated. This may not only cause air pollution (CO_2 emissions) but also mineralize the soil and increase the risks of pest propagation and fire accidents.

The olive-derived biomass can be converted to energy, gas, and fuel as a result of several conversion processes. Moreover, production of phenolic compounds, mono- and oligo-saccharides, pectins, proteins, and lignocellulosic fibers from olive by-products have been proposed in the literature.

In the study conducted by Martínez-Patiño et al. (2017), combined two-stage acid/alkali-peroxide pretreatment has applied to olive tree biomass to produce bioethanol. As a first stage olive tree biomass was subjected to different sulfuric acid concentrations (0.59–3.41 w/v) and different reaction time (47.6–132.4 min) at 130°C. In the second stage, the effect of hydrogen peroxide concentration and pretreatment time were investigated at 80°C. 80% delignification and 82 g glucose/100 g yield were reported under the best operating conditions (7% H_2O_2, 90 min). It was stated that 15 g ethanol/100 g OTB was produced using treated olive tree waste (Martínez-Patiño et al. 2017).

Romero et al. (2010), have also studied acid hydrolysis of olive tree biomass. It has aimed to reveal a mathematical model for predicting fiber hydrolysis and sugar production as a function of operational variables such as sulfuric acid concentration (0-32% (w/w)), temperature (60-90°C) and process time (0-240 minutes). Temperature and acid concentration had a significant impact on the hydrolysis yields of olive tree biomass. However, acid concentration above 6% and hydrolysis times over 100 min caused a decrease in the efficiency of the process. The process was modelled by first-order reaction kinetics.

Romero-García et al. (2022), have compared the liquid hot water (LHW) and steam explosion (SE) pretreatment to convert olive tree pruning biomass (OTPB) into edible products. Effect of temperature (180, 200, and 220 °C) has been studied at constant pretreatment time (5 min). An overall sugar recovery of 80% has been reached performing LHW and SE pretreatment at 200 and 220 °C, respectively.

Bioethanol production from olive pruning biomass with the deacetylation step before acid hydrolysis has been studied by Moya et al. (2016). First, the deacetylation process conditions were optimized (60°C, 59 minutes, 0.8% NaOH). Subsequently, oxalic acid was used for acid hydrolysis of deacetylated olive pruning biomass at different process conditions. The highest level of total fermentable sugars (28.1 g/dm^3), were achieved at 150°C pretreatment temperature, 0.075M oxalic acid concentration and 30 min pretreatment time. The liquid fraction was fermented and 22 g/dm^3 bioethanol was produced using *P. Stipitis* (Moya et al. 2016). Similarly, Negro et al. (2014), fermented the steam exploded olive tree pruning biomass.

The highest ethanol concentration (31.9 g/L) which was 80% of the theoretical yield was obtained at 195°C.

López-Linares et al. (2013), have studied the pretreatment of olive tree biomass using $FeCl_3$ prior enzymatic hydrolysis. 0.05–0.275 M $FeCl_3$ was used at 120–180 °C for 0–30 min. Optimum $FeCl_3$ pretreatment conditions were found as 152.6°C, 0.26 M $FeCl_3$ for 30 min. Under such conditions, 100% of hemicellulose fraction was removed and hemicellulosic sugar recovery in the prehydrolysate was 63.2%.

In the study on maximizing the overall glucose yield of olive tree pruning, the effects of steam explosion pretreatment has been evaluated. Experimental conditions such as temperature, pressure and time were chosen between 190-210 °C, 13-23.5 bar and 10-15 min, respectively. The highest theoretical ethanol yield of 14.41 g/100 g dry raw material was achieved at a severity of 4.41 (Barbanera et al. 2015).

Negro et al. (2015), have investigated one-step alkaline–extrusion pretreatment process using olive tree pruning. Pretreatment conditions [temperature (70, 90, 110 °C), screw speed (70 and 140 rpm) and alkaline catalyst to dry matter ratio (5 and 10 g NaOH/100 g DM biomass)] were optimized and it was concluded that enzymatic digestibility was remarkably improved by extrusion. The maximum glucose yield after enzymatic hydrolysis was found as 21 g/100 g raw material.

In the study conducted by Martínez-Patiño et al. (2018), the effect of biological pretreatment with *Irpex lacteus* (BP), dilute acid pretreatment (AP) and combination of these pretreatment methods were evaluated. The biological pretreatment with *I. lacteus* followed by the acid pretreatment (BP + AP) yielded an enriched cellulose solid (35% cellulose content) with an enzymatic hydrolysis yield (EH) 3.5 times higher than that of the un-pretreated olive tree biomass.

3.3. Pretreatment of olive pomace

The industries involved in the production of olive oil generate a large amount of agro-industrial waste every year. This includes olive pomace which is main residue generated in olive oil industry after the extraction of the oil from olive. Although olive pomace is currently used in direct combustion for heating, it has some drawbacks: its low cost provides a limited income, different environmental problems occur during combustion, e.g., particle emission and

hazardous gas generation. Alternatively, olive pomace can be used to obtain bioethanol, xylitol and antioxidants.

Battista et al. (2016), have studied ultrasound assisted and alkaline pretreatment to increase the soluble sugars. NaOH (3 M) pretreatment for 24 h was the best pretreatment method with 9.01 g/L final ethanol concentration. Similarly, Abo Chameh et al. (2016), have investigated the effect of reaction time, temperature, pH and enzyme loading on hydrolysis yield of alkali pretreated (5 % w/v NaOH) of olive pomace. Pretreatment was performed at 50°C for 4 h. It was found that the hydrolysis yield of alkaline pretreated olive pomace was 2.6 times higher than that of untreated olive pomace.

Miranda et al. (2019), focused on hydrothermal treatment (130 °C, 30 min; severity factor log R_0=2.99) of olive pomace and stones. Three different commercial enzymes (Saczyme Yield, Ultimase BWL 40 and Celluclast 1.5 L), were evaluated for saccharification at different biomass loadings (10, 20 and 30%, w/v). Results have shown that Saczyme and Ultimase were active with high solid loadings (30%), achieving 80 and 90% glucan conversion for olive pomace, and 40 and 55% for olive stones, respectively.

In another study López-Linares et al. (2020b), ethanol production from hemicellulosic sugars of olive pomace was investigated. After acid pretreatment (at 170 °C, using 2% H_2SO_4 (w/v)), the fermentability of the liquid fraction was evaluated using *Escherichia coli* SL100. When the hydrolysate was detoxified with activated charcoal, E. coli assimilated 96% of carbohydrates in a fermentation time of 20 h with an ethanol production of 14.5 g/L, corresponding to 0.46 g ethanol/g consumed sugar (89.7% of the theoretical yield) (López-Linares et al. 2020b).

Gómez-Cruz et al. (2021) have studied liquid hot water and organosolv pretreatment with ethanol (catalyzed and un-catalyzed with 1% sulfuric acid). 50 % ethanol with 1% sulfuric acid (at 130 °C for 60 min) was selected as the best pretreatment solvent.

Manzanares et al. (2020), treated hot water extracted olive pomace using Liquid Hot Water (170,190 C and 210 °C) or dilute acid (DA) [1% and 2% (w/v) sulfuric acid]. The water extraction step contribute significantly to EOP valorization by extracting valuable compounds (e.g. mannitol and phenols). Results have revealed that dilute acid (DA) pretreatment (170 °C and 2% acid) provided the highest glucose and xylose yields after enzymatic hydrolysis (85 and 62%, respectively).

3.4. Pretreatment of reed

Reed is a promising biorenewable energy source given its ability to grow rapidly under environmentally challenging conditions. It is an annual plant with large abundance and needs to be harvested every year. Reed has been used in paper industry as a feedstock; however, a large amount of reed wastes is still remained which are emergent to be converted into high-value products. The main components of reed are cellulose, hemicellulose, and lignin, among which cellulose accounts for 35–40 % (Chen et al. 2023). This high cellulose content makes reed a great potential to be manufactured into fermentable sugars. It is well known that enzymatic saccharification is an environmentally friendly process to produce fermentable sugars; nevertheless, enzymatic conversion efficiency is limited by the natural recalcitrance of complex plant structure.

Many pretreatment technologies have been reported for reed in literature. A brief summary of these pretreatments are given below:

Chen et al. (2023) have investigated the combinatorial methods using liquid hot water (LHW) and lactic acid (LA) to treat reed straw for effective lignocellulose digestibility and xylose recovery. The conditions were reported as 180 °C (60 min) for LHW and 170 °C (45 min) for LA. They have demonstrated that LHW-LA pretreatment increased lignocellulose digestibility and glucose yield by 1.95-fold and 1.13-fold, respectively.

In another study, the effect of DES (benzyltriethylammonium chloride/formic acid, BTEAC/FA) pretreatment on glucose yield of reed straw was investigated (Zhang et al. 2022). BTEAC and FA were mixed to synthesize DES in different molar ratios (1:2, 1:3, 1:4, 1:5, 1:6) at ambient temperature. They reported that BTEAC/FA provided significant delignification and xylan removal while preserving most of the cellulose. The glucose yield of cellulose-rich substrates by enzymatic hydrolysis has reached 76.64%, which was about 5.24-fold higher than that of raw reed straw when the optimum molar ratio (1:6) was used for DES preparation. Moreover, it was declared that the treatment showed excellent delignification (69.32%–94.10%) and xylan removal (43.30%–78.34%) with retention of a large amount of cellulose (94.5%–88.58%).

Alkali treatment was also applied on giant reed (Arundo donax L.) (Vasmara et al. 2022). In this study, it was aimed to evaluate the hydrogen production from pretreated giant reed. The alkali treatment was perfomed using NaOH (1.2-10 % w/w) at 121 °C for 20 min. The

pretreated sample was then hydrolyzed with cellulase. The highest hydrogen yield, 2.0 mol/mol of hexoses.

Piccitto et al. (2022) have investigated the effect of fungal pretreatment on giant reeds obtained using two white rot fungi (*Pleurotus ostreatus* and *Irpex lactus*). The samples were incubated at 26 °C for 30 days. The authors reported that pretreatment using *P. ostreatus* showed promising results with the highest biomass lignin degradation after 30 days incubation. In addition, biomethane yields of *P. ostreatus* treated biomass was reported as 130.9 NmL/g VS for the winter harvest.

Ma et al. (2022) investigated the effect acid treatment (p-toluenesulfonic acid) followed by ball milling. The samples were treated at 90 °C for 30 min. The combination pretreatment led to a significant reduction in hemicellulose (78%) and lignin (67%) content. Moreover, enzymatic hydrolysis of the residue led to a glucose content of 12.0 g/L.

Di Fidio et al. (2022) reported a study aiming the optimization of an innovative catalytic high-pressure CO_2 pretreatment of giant reed. They also investigated the effect of synergistic addition of very low amounts of catalyst ($FeCl_3$) and surfactant (polyethylene glycol 400) on the enzymatic hydrolysis yields. The conditions of the experiments were given as temperature of 160 °C, biomass loading ratio of 9 wt%, pressure of 140 bar and reaction time of 6h. Under the optimized pretreatment conditions, the xylan conversion of 82 mol% and xylose and xylo-oligosaccharides yields of 43 and 20 mol% were achieved, respectively. The total glucose yield was 67.8 mol% with respect to the glucan units in the biomass.

The effect of lactic acid pretreatment on enzymatic hydrolysis of reed was investigated by Chen et al. (2022). The lactic acid pretreatment experiments were designed with three factors and three levels. Liquid-solid ratio (1:6, 1:8 and 1:10), the reaction temperature (130, 150 and 170 ∘C), and the holding time (25, 35 and 45 min) were the independent variables. The optimum temperature was reported as 150 ∘C. The lignin removal, glucan recovery and glucan digestibility were presented as 90.46 %, 89.80 % and 66.43 %, respectively. Besides, the final sugar yield and the sugar acids conversion rate were about 60.70 % and 37.89 %.

Scherzinger et al. (2021), have investigated the vapothermal pretreatment and its effect on anaerobic degradability of common reed (*Phragmites australis*). The treatment was applied at different temperatures (122-178 °C) and residence times (18 -102 min). They have declared that vapothermal pretreatment is able to significantly enhance degradation rate during anaerobic digestion. On the other hand, in the study it was concluded that under the conditions applied,

the biogas and methane yields decrease which was related to increase in cellulose crystallinity. The authors (Scherzinger and Kaltschmitt 2021), have also examined whether vapothermal pretreatment is technically feasible and brings economic advantages for a subsequent anaerobic co-fermentation of reeds together with sewage sludge. They have concluded that vapothermal pretreatment prior to anaerobic digestion leads to lower electricity generation costs.

Combined liquid hot water and sodium carbonate-oxygen pretreatment (Lu et al. 2021) was investigated on reed to produce producing high-concentration bioethanol. The pretreatment procedure had two stages: liquid hot water application at 170 C for 60 min, and sodium carbonate with oxygen application. Sodium carbonate with oxygen application had four factors: the concentration of Na_2CO_3 (4-16 wt %), reaction temperature (120-160 °C), residence time (20-80 min) and oxygen pressure (factor D: 0.2 -1 MPa). An oxygen pressure of 0.8 MPa, a Na_2CO_3 concentration of 16 %, a reaction temperature of 160 °C, and a reaction time of 60 min were determined to be optimal, resulting in the highest bioethanol concentration (66.5 g/ L).

Ummalyma et al. (2021) evaluated the potential of the aquatic biomass *Phragmites karka* (tall reed grass) for biofuel production. They applied dilute acid (0.5, 1.0, and 2% (w/v) H_2SO_4) and alkaline pretreatment (1-10% (w/v) NaOH). It was found that the highest educing sugar yield (79%) was obtained using 1% w/v alkali with a sonication frequency of 20 kHz for 25 min. The fermentation efficiency was reported as 78%.

Di Fidio et al. (2020) optimized glucose and levulinic acid production from the cellulose fraction of giant reed (*Arundo donax L.*) in the presence of ferric chloride under microwave heating. They have tried two-step exploitation of the giant reed cellulose to glucose and levulinic acid. Microwave-assisted hydrolysis of cellulose to glucose process was optimized by using the effect of temperature, reaction time and catalyst amount as the independent variables. Under the optimized reaction conditions, the glucose yield was 39.9 mol%. Moreover, to obtain levulinic acid the exhausted residue deriving from the chemical hydrolysis was further treated by microwave at harsher reaction conditions and the maximum levulinic acid yield of this process was reported as 64.3 mol%.

Noori and Ismail (2020) have conducted a study which focused on biogas production from giant reed. The effect of particle size, pretreatment method, inoculum type, and thermal conditions were investigated. Two different pretreatment methods were applied namely H_2O_2 and H_2SO_4 pretreatments. They have found that biogas generation from H_2O_2- and H_2SO_4-pretreated biomass has exceeded the untreated reed by 200% and 36%, respectively.

4. Activities performed between 1.-6. months

In the first 6 month of the project, High Performance Anion Exchange Chromatography (HPAEC-PAD) (Thermoscientific ICS-6000) was purchased and commissioned in Ankara University (Figure 4). In addition, all the necessary chemicals and consumables are purchased to perform the experiments and analysis.

4.1. Compositional analysis of biomass

All the feedstock (Figure 5) were collected, dried and milled for the pretreatment experiments. Olive pomace and olive tree pruning waste were supplied by a local olive oil producer in Ayvalık, Turkey. Reed was harvested from a near by lake in Ankara, Turkey during the summer of 2022. Oak tree waste and cork dust was supplied by a company in Portugal in the form of fine powder.

Figure 4. HPAEC-PAD in Ankara University, TURKEY

Figure 5. Feedstock (from left to right: olive pomace, olive tree pruning waste, reed, oak tree waste and cork dust)

The solid agricultural and processing residues from cork and olive oil industry and the reed were first analyzed following established NREL (National Renewable Energy Laboratory, USA) protocols (Sluiter et al., 2008) to determine their chemical composition (cellulose, hemicellulose, lignin, ash). The protocol was modified such that the monosaccharides (glucose, xylose, arabinose, galactose and mannose) were analyzed using a Dionex ICS-6000 system (Thermo Scientific, USA) with pulsed amperometric detection (HPAEC–PAD). The samples were injected into CarboPac PA200 column (Thermo Scientific, USA) with a guard column of the same type. The samples were eluted from the column with an eluent flow rate of 0.35 mL/min at 30 °C. The mono-saccharides were eluted using an isocratic mobile phase of 2 mM sodium hydroxide for 35 min. The amount of detected sugar was determined through peak integration based on a dilution series of sugar standards. The results of compositional analysis are given in Table 1.

Table 1. Compositional analysis results of the feedstock on dry basis (sugars are expressed in terms of monomeric equivalent)

Feedstock	Glucose (%)	Xylose (%)	Galactose (%)	Arabinose (%)	Mannose (%)	Lignin (%)	Ash (%)
Cork dust	8.96%	8.78%	0.83%	1.24%	0.38%	52.0%	2.1%
Reed	27.80%	19.50%	0.05%	2.27%	0.31%	18.4%	5.8%
Olive tree	30.58%	17.73%	1.13%	1.54%	3.61%	24.3%	0.0%
Olive pomace	18.75%	12.79%	0.97%	1.68%	1.04%	30.3%	1.6%
Cork Oak tree	24.62%	14.29%	2.25%	2.16%	1.77%	39.2%	0.0%

The results were compatible with the findings in literature (Cunha et al. 2020; Pereira 2015; Engel et al. 2022; Wang et al. 2022; Requejo et al. 2012; López-Linares et al. 2013; Miranda et al. 2019; Ferreira et al. 2018), except the lignin value of cork dust which was relatively high. This could be due to suberin content of the cork which is insoluble in acidic environment (Yona et al. 2014). The NREL compositional analysis is a two-step sulfuric acid hydrolysis protocol and the solid fraction after the hydrolysis is considered as Klason lignin. Therefore, it could be possible that suberin fraction ended up in the lignin content. Similar to our findings, Engel et al. (2022) have reported 72% lignin content in their cork samples and stated that it was necessary to remove the suberin of the material that will be quantified before determining the lignin content. Based on this, the cork dust samples will be analyzed again on extractive free basis.

4.2. Pretreatment of biomass

In the first 6 month of the project, DES pretreatment of cork dust was performed as described below.

Deep eutectic solvent was synthesized by mixing choline chloride and formic acid (ChCl : FA) at 1 : 2 mole ratio and heated in a shaker incubator at 30 °C and 180 rpm until a homogenous transparent liquid was formed (approximately 2 h) (Abbott et al. 2003). Subsequently, the solvent was heated at 80 °C for overnight to eliminate the unreacted free acids.

10 g of cork dust was mixed thoroughly in a glass bottle with 100 mL of choline chloride and formic acid mixture. The slurry was treated at different temperatures (90, 110, 130 °C) and pretreatment time (20, 40 and 60 s) using an autoclave (NUVE, Turkey). Subsequent to pretreatment, the samples were immediately placed into an ice bath to cool them down to room temperature. The slurry was vacuum filtered using coarse filter papers. The liquid phase was collected for sugar analysis. The solids were washed with 250 mL de-ionized water. The pretreated cork dust solid fractions were dried at 45 °C for at least 2 days and shipped to Germany for biogas experiments.

The solid recovery (SR) was calculated based on the ratio of dry biomass weight after pretreatment to dry biomass weight before pretreatment. The results are depicted in Figure 6. The percent of solids recovered (SR) was decreased as the temperature and pretreatment time was increased. For instance, the highest percentage of solids recovered (99.5%) was observed at 90 °C and 20 min pretreatment time. Recovery was decreased to 75.6 % when the temperature and time was increased to 130 °C and 60 min, respectively. At extreme pretreatment conditions the lignocellulosic structure is destructed harshly and more solids are solubilized into the liquid fraction. Similar results were also reported by (Isci et al. 2020) for DES treatment.
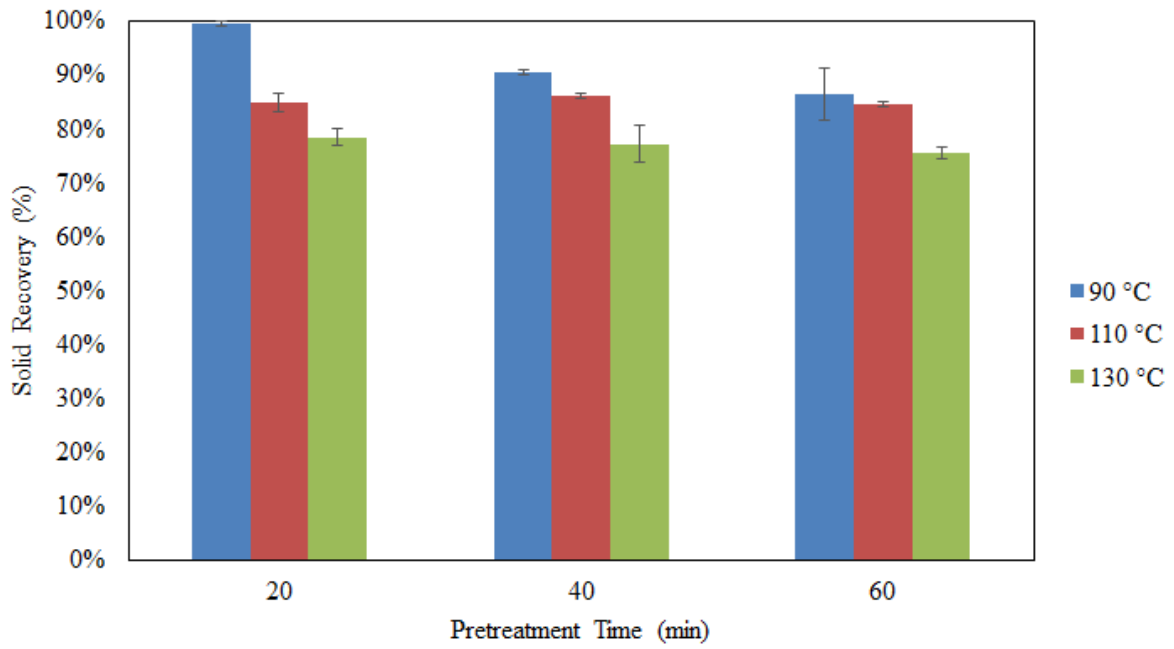
Figure 6. Effect of DES treatment on solid recovery of cork dust samples

5. Future work

The sugar analysis of the liquid fraction of cork dust samples will be performed. In addition, the biomass samples will be subjected to different pretreatment techniques (autohydrolysis, acid, alkaline treatments and deep eutectic solvent pre-treatment) to enhance the biogas production. The solid fractions of the treated biomass will be send to Germany for biogas experiments and liquid fractions will be analyzed at Ankara University.

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B) Downstream-processing: removal of inhibitors

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Lignocellulosic materials (*LCMs*) such as agricultural wastes, forestry residues, agro-industrial wastes, wood wastes, grasses, waste papers, wastes of food industry, municipal wastes, animal manures, and bioenergy crops are the most abundant available natural sources in the world (approximate 10-50 billion ton annually worldwide), which consist of cellulose (40-50%), hemicellulose (25-30%), lignin (15-20%), and the other extractives. Therefore, they are rich in terms of carbohydrates such as pentoses and hexoses and thus they can be converted to value-added products by fermentation. Before fermentation, pretreatment is one of the most significant stages in the production of value-added products from *LCMs*. Many pretreatment methods such as physical, chemical, physicochemical, biological, and thereof combinations are applied for conversion of cellulose and hemicellulose in the *LCMs* to cellobioses and monosaccharides (mainly glucose (*Glc*), xylose (*Xyl*), and arabinose (*Ara*)). Nevertheless, numerous inhibitor compounds such as organic acids (mainly acetic acid (*AA*), formic acid, and levulinic acid), phenolics (*PHs*), and furans (mainly 5-hydroxymethyl furfural (*HMF*) and 2-furaldehyde (*2-F*)) are liberated during pretreatment. Among pretreatment methods, acid pretreatment is one of the chemical pretreatment technologies. Dilute sulfuric acid (*DSA*) pretreatment is also the most commonly used method for pretreatment of *LCMs*. Nonetheless, HCl, H_3PO_4, and HNO_3 have also been studied in the pretreatment of *LCMs*.

Formation of inhibitors

After the physical, chemical and biological treatments, several different inhibitors are formed. The inhibitors type could be varied according to the pretreatment methods or lignocellulosic material sources (Palmqvist and Hahn-Hagerdal, 2000). Inhibitors are generally released degradation of the hemicellulose and lignin structure of lignocelluloses. There are three main groups of inhibitors as furans, phenolics and organic acids.

Furans

The furan compound HMF and furfural are formed by dehydration of monomeric sugars such as hexoses and pentoses. The amount of furans released in the environment varies according to the pretreatment methods and lignocellulosic sources. Furfural is often found at lower levels than HMF, but more effective inhibitor according to the HMF (Almeida et all., 2007). Furans are effected specific growth rate and productivity of cells.

Phenolics

Numerous phenolics compounds are released with lignin degradation. In addition, phenolics are formed by carbohydrate degradation during acid hydrolysis. The amount of lignin in the lignocellulosic raw material varies according to the lignocellulosic source. As a result, the type

and amount of phenolic compounds are generated (Almeida et all., 2007). Phenolic compounds cause loss of integrity by acting on the cell membrane. This adversely affects the ability of the cell membrane to act as selective barriers and enzyme matrices (Klinke et all. 2004).

Weak acids

Lignocellulosic hydrolysates were mostly contained acetic acid, formic acid and levulinic acid. Acetic acid is formed by de-acetylation of hemicelluloses, while formic and levulinic acids are products of HMF degradation. Moreover, under the effect of high temperature and acid, furfural breakdown to formic acid. As a result of the decomposition of weak acids, it causes a decrease in pH due to anion accumulation in the fermentation medium. This adversely affects cell growth and product formation (Almeida et aii., 2004).
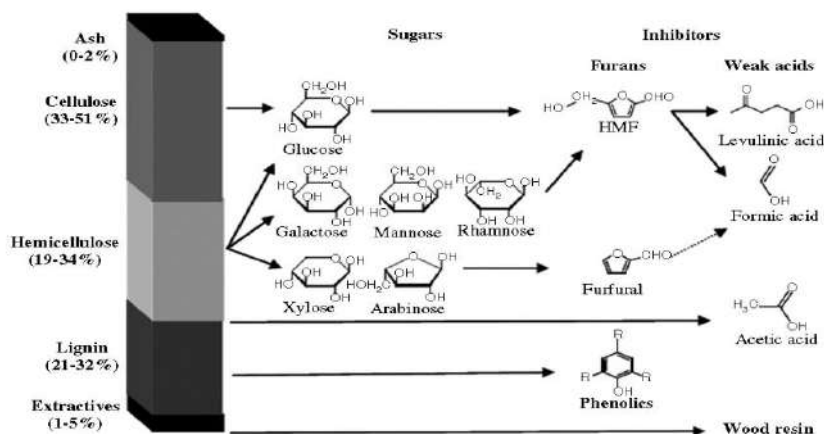

Figure 1. Avarage composition of lignocellulosic biomass and main derived hydrolysis products (Almeida et all., 2004).

Since the high inhibitor concentration in the fermentation medium adversely affects the development, the inhibitors must will be removed. Therefore, inhibitors should be removed from the environment with appropriate detoxification methods. Detoxification treatment can be applied physical, chemical and biological methods. In order to decide the detoxification method and application amount, it is necessary to know the inhibitory tolerance ranges of the microorganism. Because of several researchers focused on inhibitor mechanism in fermentation medium. Erkan et al. 2020 studied to determine the toleration limit of furfural concentration in the ethanol fermentation with immobilized Saccharomyces cerevisiae in bioreactor. It has been observed that *S.cerevisiae* can grow up to 6 g/L furfural concentration. Gürler et al.2020 were investigated the inhibiton effect of phenol concentration on the production of inulinase by *Aspergillus niger* A42. Microorganism growth was limited after 0.5 g/L phenol concentration.

Physical methods

Adsorption, filtration and extraction methods are applied in physical detoxification process. Adsorbents such as bagasse fly ash, activated coal, high-temperature carbon, and polyethyleneimine are used to adsorb inhibitors in the hydrolysate. Generally weak acids could be removed with adsorption methods. On the other hand, the extraction method can be used

with many solvent such as hexane, chloroform and ethyl acetate. Additionally, HMF and furfural can be removed with membrane filtration detoxification method.

Chemical methods

With this method, inhibitors are converted to a less harmful form, eliminating the inhibitory effect. Ammonia and sodium hydroxide can be used for chemical detoxification method.

Biological methods

Another method for the remove inhibitors is the biological method. In biological methods, detoxification is carried out using enzymes. For example, with laccase eliminates phenolic inhibitors. Also, some researchers were showed that its use glucose recovery with this method. On the other hand, microorganisms also used for detoxification in hydrolysate (Kumar, et al. 2020). Singh et al. 2017 were isolated *Bordetella* sp. BTIITR, and it was able to degrade furfural (100%), HMF (94%) and acetic acid (82%) from sugarcane bagasse hydrolysate.

Figure 2. Lab experiment for production of value added product from lignocellulosic materials in Akdeniz University

Nowadays, depending on the increasing world population, since the energy and organic chemical requirements will increase in the future, this has prompted scientists and countries to search for alternative and cheap new resources. Many of research present in literature about value-added product fermentation by using agricultural wastes, which include lignocellulosic structure. Lignocellulosic materials such as agricultural wastes, forestry residues, agro-industrial wastes, wood wastes, grasses, waste papers, wastes of food industry, municipal wastes, animal manures, and bioenergy crops are the most abundant available natural sources in the world. Lignocellulosic materials are rich in terms of carbohydrates such as pentoses and hexoses and thus they can be converted to value-added products by fermentation. Due to those important advantages of lignocellulosic biomass are evaluated as carbon sources to produce enyzmes, bioethanol, oligosaccharides, organic acids, polysaccharides etc.

Some of research about evaluation of lignocellulosic biomass are summarized below.

Different lignocellulosic biomass such as the tea processing waste (Germec et al. 2018a), rice straw (Germec et al. 2018b), rice husk (Germec et al. 2016a, 2016b, Bader et al. 2021a, 2021b) wheat bran (Germec et al. 2019) and carob (Yatmaz et al. 2020) were used for production of bioethanol. In addition to bioethanol fermentation, the other lignocellulosic biomass evaluation research includes inulinase production from sugar beet molasses (Germec 2020, 2021c); mannanase production from barley husk, wheat bran, rye bran, oat husk and coffee grounds (Yilmazer et al. 2021); and lactic acid production from wheat bran and carob extract (Germec et al. 2019).
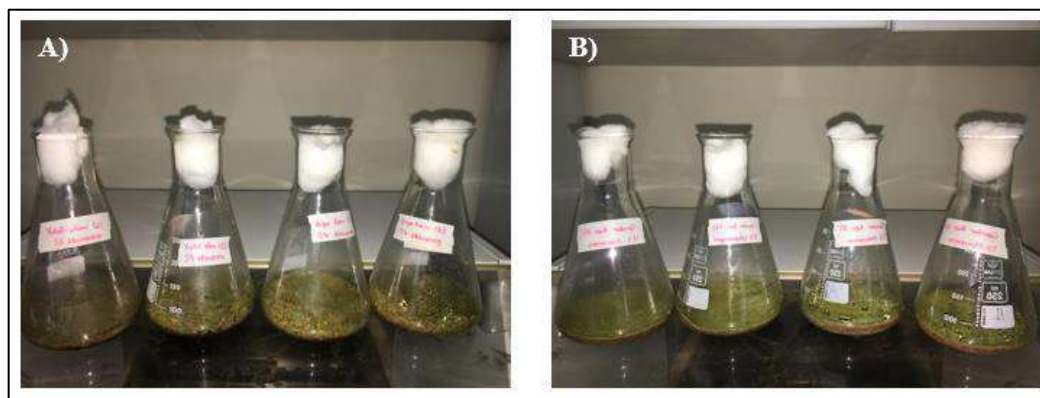

Figure 3. Mannanase and mannooligosaccharide production from a) barley and oat husk b) wheat and rye bran
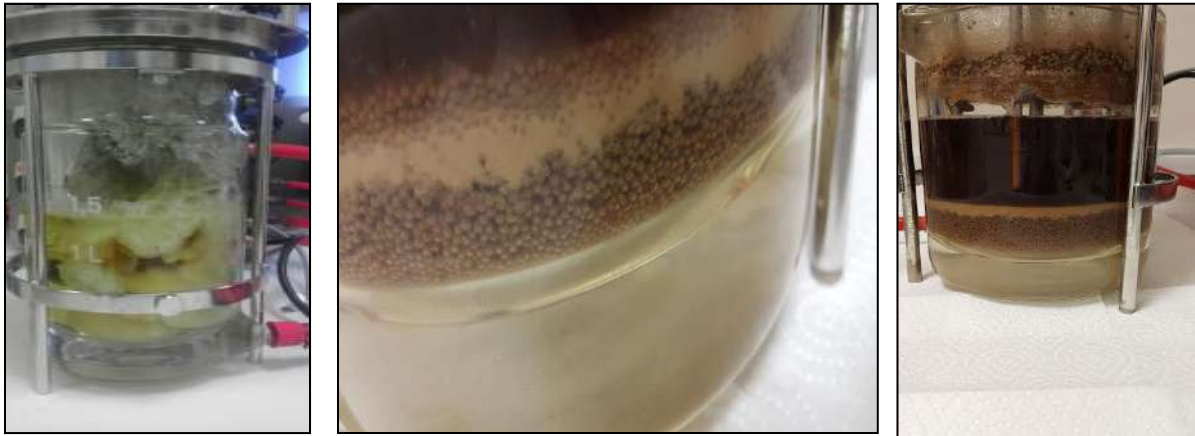
Figure 4. Inulinase and mannanase production from carob extract in bioreactor

Pretreatment is one of the most significant stages in the production of value-added products from by fermentation from lignocellulosic biomass. Many pretreatment methods such as physical, chemical, physicochemical, biological, and thereof combinations are applied for conversion of cellulose and hemicellulose in lignocellulosic biomass to cellobioses and monosaccharides (Germec et al. 2019). Moreover, some oligosaccharides, such as xylooligosaccharides and fructooligosacharides, can be released as a by-product due to pretreatment process of lignocellulosic material and they can be separate before fermentation process of lignocellulosic biomass. For example, fructooligosaccharides can be obtained by ultra-high pressured reactor pretreatment from stevia stem and root, which include high amount of lignin. On the other hand, there are numerous research in the literature about xylooligosaccharides production from lignocellulosic material such as sugarcane bagasse, wheat straw, rice husk, corn straw etc. by using different pretreatment techniques (Poletto et al. 2020). Due to pretreatment techniques, not only various value-added product can be produced by fermentation of lignocellulosic biomass, but also different oligosaccharides released as a by-product.

Figure 5. Stevia leaves, roots and stems are pretreated by ultra-high-pressured reactor to produce fructooligosacharides

At the end of the upstream process as production of value-added product and releasing of oligosaccharides, downstream process, which including purification of these compounds, begin. There are many different purification techniques depending on product are preferred to purify product of fermentation: separation, filtration, or chromatographic purification. For instance, mannanase enzyme, produced from carob extract by fermentation, partially purified with ultrafiltration process. After ultrafiltration, mannanase enzyme was purified with DEAE-cellulose column. (Gürler et al. 2021)

Figure 6. Purification steps of mannanase. Ultrafiltration and chromatographic purification

FUTURE WORKS

In project for lignocellulosic materials, the liquid fraction from the pre-treatment will be analysed in terms of valuable products such as fermentable sugars, phenolics and oligosaccharides. Afterwards, separation and purification of the valuable products from the liquid extract obtained under optimum conditions will be carried out. Possible utilization scenarios of the purified by-products will also be investigated (e.g. antioxidant potential of purified phenolics, the effect of the purified oligosaccharides on the growth of different probiotic bacteria, production of ethanol and enzymes (e.g. inulinase) from the fermentable sugars in the extract).

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Interim report month 1 to 6

C) Anaerobic digestion of biomass in lab and pilot-scale

January 2023

TUHH – Hamburg University of Technology

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1. Summary of the objectives of work package 6

Within the scope of work package 6 it is to be demonstrated that the residual materials produced in the considered systems (i. e. constructed wetlands, olive and cork farms) can be used energetically by means of anaerobic digestion. The respective biomass feedstocks are tested with regard to their biogas potential in anaerobic digestion batch tests. In accordance with work package 5, the proposed pre-treatment methods (i.e. acid pre-treatment, alkaline pre-treatment, hydrothermal pre-treatment and deep eutectic solvent pre-treatment) are investigated for their suitability and efficiency to improve the biogas yield from the different biomass feedstocks. In addition to the determination of the biogas potential, the anaerobic digestion in a continuous anaerobic digestion process is to be demonstrated. Thereby, various operation parameters are to be tested to optimize the biogas production from the pre-treated and non-pre-treated residual materials. Resulting from this, an appropriate process management and control are to be developed.

Table 1 and Table 2 present an overview and the status of all tasks (Table 1) and deliverables (Table 2). Task T6-1 was successfully processed, the respective deliverable D6.1 is to be uploaded. T6-2 is in progress.

Table 1: Overview and status of tasks within WP 6.

#	Task	Start Date	End Date	Status
T6-1	Determination of the specific biogas formation potential of the arising residual materials	01.09.2022	01.12.2022	processed, upload pending
T6-2	Evaluation of the influence of pre-treatments on anaerobic digestibility	01.12.2022	01.09.2023	in progress
T6-3	Investigation of the continuous anaerobic degradation of (pre-treated) residual materials	01.05.2023	01.05.2024	pending
T6-4	Biogas plant optimization	01.07.2024	01.04.2025	pending

Table 2: Overview and status of deliverables within WP6.

#	Delivery	Type	Description	Status
D6.1	01.12.2022	Presentation	Proof of anaerobic degradability	processed, upload pending
D6.2	01.11.2023	Presentation	Improved degradability by pre-treatment	in progress
D6.3	01.06.2024	Presentation	Prove of stable continuous degradation process	pending
D6.4	01.05.2025	Working Paper	Measurement and control strategy for anaerobic digestion	pending

2. Review: Anaerobic digestion of agricultural residue biomass

Anaerobic digestion of residual biomass is a strategy of waste treatment and, at the same time, can be a way of valorisation of the material. It can provide affordable and environmental friendly energy and nutrient-rich digestate for fertilisation or further valorisation. Hence, anaerobic digestion can contribute to the overall sustainability of the agricultural systems investigated within the scope of the ReMe-diation project.

2.1. Anaerobic digestion of olive residues, cork residues and common reed

In the following, the residual biomasses of olive production, cork production and constructed wetlands are discussed with regard to their usability as substrates for biogas production via anaerobic digestion.

Olive production. The main by-products of the olive producing industry are olive-tree pruning debris (olive pruning, 3 t ha^{-1}), olive stones (0.5 t a^{-1}) and olive pomace (1.73 t ha^{-1}), which is a residue remaining after the olive oil has been pressed (S. Sánchez et al. 2002; García Martín et al. 2020). Bigger branches and trunks are usually used in home boilers and incinerated. Further olive tree pruning debris are ground and ploughed into the soil. Similarly, olive stones are usually incinerated. Olive pomace can undergo another oil extraction from which crude pomace oil is obtained. Most commonly, olive pomace is then used as solid biofuel (García Martín et al. 2020). Research has been conducted on its use for pellet production, biodiesel and bioethanol production (García Martín et al. 2020). All substrates mentioned are principally suitable for anaerobic digestion and biogas production, but the process is not regularly applied in industrial scale. Biogas production from e.g. olive pruning is not well studied, even though its relatively low C/N ratio represents a favourable substrate characteristic (García Martín et al. 2020; COSTA et al. 2018). Olive pomace shows sufficient biodegradability and humidity, but due to its higher lignin content it is proposed to be only used as a co-substrate in anaerobic digestion plants to make use of it (García Martín et al. 2020). Anaerobic co-digestion of olive pomace and olive mill waste water with multiple co-substrates like animal manure, food waste, municipal solid waste and agricultural wastes (Orive et al. 2016; Riggio et al. 2015; Milanese et al. 2014; El Gnaoui et al. 2020; Azaizeh und Jadoun 2010; Fountoulakis et al. 2008) are well studied. Olive stones contain a high lignin content and are not regarded as promising substrate for biogas production.

Cork production. A waste product of the cork industry, which has been proposed and studied for the use as biogas substrate, is cork boiling wastewater (Marques und Gil 2013; Gil et al. 2013; Gonçalves et al. 2012). Anaerobic digestion would be functioning as a waste treatment technology of the cork boiling waste water, which contains a high organic load that could be reduced in this process (Marques und Gil 2013). Cork tree residues and cork saw dust were not yet considered for biogas production.

Constructed wetlands. Constructed wetlands use reed beds to remove contaminations from waste water. The reeds grown in the process must be harvested after a certain period of time; at present, the potential uses for the biomass produced are often not yet fully exploited. Common reed, which is very often used in such plants, has a lignocellulosic structure andshows a certain potential for anaerobic digestion. However, lignocellulosic wet plants like reed have a lower biogas potential compared to conventional biogas substrates (i. e. maize, sugar beet); also, the C/N ratio often lies in an unfavourable range. Nevertheless, biogas production by anaerobic digestion is feasible and these materials may still provide a commonly available

feedstock for biogas plants (Roj-Rojewski et al. 2019). Usually, a co-digestion e.g. with animal manure is proposed to make use of such materials in a biogas plant (van Tran et al. 2022; van Tran et al. 2019).

Exemplarily, the methane or biogas potential of some substrates of interest in this study are presented in Table 3.

Table 3: methane and biogas potential of studied substrates or related substrates.

Substrate	Methane or Biogas Potential	Unit	Source
Olive pruning	91.2 ($\pm$ 5.7)	$m_N^3\ CH_4\ t_{VS}^{-1}$	(Bianchini et al. 2021)
Olive pruning	176.5 ($\pm$ 4.2)	$m_N^3\ CH_4\ t_{VS}^{-1}$	(Bianchini et al. 2021)
Olive pomace	803	$m_N^3\ t_{VS}^{-1}$	(Ulusoy und Ulukardesler 2017)
Olive pomace	77	$m^3\ CH_4\ t_{VS}^{-1}\ d^{-1}$	(Orive et al. 2016)
Olive pomace	180	$m^3\ t_{VS}^{-1}$	(Alagöz et al. 2015)
Cork boiling water	126 – 142	$m^3\ CH_4\ t_{COD}^{-1}$	(Marques et al. 2014)
Common reed	77	$m_N^3\ CH_4\ t_{VS}^{-1}$	(Scherzinger et al. 2021)
Common reed	107.6 ($\pm$ 3.9) – 172.4 ($\pm$ 15.3)	$m^3\ CH_4\ t_{VS}^{-1}$	(Baute et al. 2018)

2.2. Pre-treatment of residue biomass to improve anaerobic digestion

Various methods of pre-treatment can improve the biogas yield obtained from the anaerobic digestion of lignocellulosic materials. The biomass consists of structures which are hard to degrade and have a natural resistance towards degradation. Certain pre-treatments can break these structures and improve availability of biopolymers for biogas formation. Pre-treatment may often even be unavoidable to produce biogas, bio-methane or liquid bio-fuels from lignocellolosic biomass. Different methods affect the chemical and physical properties of substrates in different ways and they generally have varying impact in terms of intensity and effectivity. The basic idea of pre-treatment is to improve the accessibility of degradable structures through providing a higher surface area (Zheng et al. 2014; Arranz et al. 2015; Akhand und Méndez Blancas 2019), decrystallization of cellulose (Salehian und Karimi 2013; Mirahmadi et al. 2010; Zheng et al. 2014), solubilisation of biopolymers (Zheng et al. 2014) as well as degradation of hemicellulose and cellulose macromolecules and delignification or alteration of lignin structures (Michalska et al. 2012; Zheng et al. 2014).

Pre-treatment methods proposed for the processing of the biomass residue streams have been studied and applied for multiple lignocellulosic materials. The general suitability of autohydrolysis, alkaline and acid pre-treatment was demonstrated and reported in literature. For instance, thermal pre-treatment and subsequent autohydrolysis of grass increased the resulting methane yield by more than 4.5 % compared to the untreated substrate (Li et al. 2012). Similarly, alkaline pre-treatment may improve biogas yield because it reduces the crystallinity of the structures which is in turn associated with improved substrate conversion to biogas (Salehian und Karimi 2013; Mirahmadi et al. 2010). The recovery of fermentable sugars and thus increased CH_4 yield is impacted by time, temperature and alkali strength during the pre-treatment (McIntosh und Vancov 2010). (Khor et al. 2015) found an increase in CH_4 yield of 15.2 % from alkaline ($Ca(OH)_2$) pre-treated grass and an increase of 11.2 % for pre-treated maize straw compared to the respective untreated material. Alkali pre-treatment with NaOH was shown to improve fermentable sugar recovery from Bermuda grass (Wang et al. 2010) and

(Elalami et al. 2020) found a positive effect of alkali pre-treatment on fibre degradation and lipid solubilisation and subsequent methane production. Dilute acid pre-treatment by e.g. sulphuric or phosphoric acid may also improve the digestibility. Among others, this was shown for olive tree lignocellulosic biomass and potato peels (Lenihan et al. 2010; Cara et al. 2008).

Less research was performed on the effect of deep eutectic solvent pre-treatment on biogas yield from lignocellulosic materials, yet. Still, recent studies showed that DES pre-treatment can lead to delignification (Lima et al. 2021) and DES pre-treatment enhanced lignin extraction and biomass saccharification (Wang und Lee 2021). However, it was observed that DES pre-treatment may facilitate but can also inhibit biogas production from lignocellulosic material (Lima et al. 2021; Olugbemide et al. 2021).

3. Report of the activities in work package 6 until month 6

3.1. Anaerobic batch tests of substrates and DES-pre-treated cork dust

During the first six project months, five major residual biomasses were tested for their biogas potential. The tested biomasses were reed stalks, olive tree residues, olive pomace, cork tree residues and cork dust. The biomass potential was determined in batch tests according to the standard method for "Fermentation of organic materials" (VDI 4630). The reed stalks were comminuted to a particle size < 1 mm, the remaining four materials were powder-like with the cork dust and olive tree residues consisting of the finest powder. Additionally, DES pre-treated cork dust samples were investigated for their biogas potential. The sample pre-treatment was carried out as described in the interim report for work package 5. The samples investigated underwent the following pre-treatment conditions: The deep eutectic solvent used consisted of choline chloride and formic acid at a molar ratio of 1:2. Cork dust was treated at a temperature of 90, 110 and 130 °C, respectively. The applied pre-treatment durations were 10, 40 and 60 minutes. Prior to the investigation of the biogas potential, the water content (W) and volatile solids content (VS) of the samples were determined in triplicates according to (DIN EN ISO 18134) and (DIN EN 15935), respectively. For the water content analysis, 0.7 g of sample were dried at 105 °C in a standard drying oven (U80, Memmert, Germany). The subsequent analysis of the volatile solids content was performed in a muffle furnace (M104, Thermo Scientific Heraeus, USA) at 550 °C.

The biogas potential of the samples was determined according to the guideline VDI 4630. The analysis was run in triplicates at mesophilic conditions (i. e. 37 ± 1 °C) in 500 mL glass bottles. The bottles were filled with 400 ± 0.5 g sewage sludge, which was obtained from the wastewater treatment plant Seevetal located South of Hamburg. The sludge was out gasing for 10 days. Based on the determined water and volatile solids content, 2 g_{vs} of each sample were added to the reactors after this time period. This accounts for a ratio of 0.4 of the organic component of the sample substrate to the organic components of the inoculum (the VDI guideline 4630 requires a ratio < 0.5). The reactors were purged with N_2 at the beginning of the experiment. Over the course of the experiment, the content of the bottles was stirred daily. The produced biogas was collected in eudiometer tubes, which were connected to the reactor bottles and filled with a sealing liquid consisting of sodium chloride solution acidified with citric acid. The amount of biogas was measured via reading the filling level of the eudiometer tubes every day. The composition of the biogas was measured with a portable gas analyser (Biogas5000, Geotech, United Kingdom) equipped with a dual-wavelength IR sensor whenever the headspace above the reactors was filled to the top or at the end of the experiment.

Microcrystalline cellulose (MC) was used as a reference material; it was analysed for its biogas production in parallel to the pre-treated samples. To confirm the suitability of the inoculum the guideline requires that 90 % of the biogas to be theoretically formed from the reference material (745 mL_N g_{VS}^{-1} for MC) is produced over the course of the experiment. Even though this requirement was not met with a formation of 88 % of the theoretically formable biogas, the inoculum proved to be suitable for the batch fermentation experiment.

3.2. Results: Biogas potential of substrates and DES-pre-treated cork dust

Among the five untreated substrates, reed had the highest biogas potential of 482.8 ± 6.4 mL$_N$ g$_{VS}^{-1}$, followed by olive tree pruning (435.6 ± 13.4 mL$_N$ g$_{VS}^{-1}$). Cork tree and olive pomace had a clearly lower biogas potential of 179.5 ± 15.9 mL$_N$ g$_{VS}^{-1}$ and 144.0 ± 13.4 mL$_N$ g$_{VS}^{-1}$ and the lowest biogas potential was determined for cork dust (51.6 ± 15.9 mL$_N$ g$_{VS}^{-1}$) (see Table 4). The cumulative biogas formation is shown in Figure 1. A lag phase in the biogas production was observed for olive tree pruning only. The remaining substrates showed no delay in biogas production. **Fehler! Verweisquelle konnte nicht gefunden werden.** displays the biogas potential of all five untreated substrates.
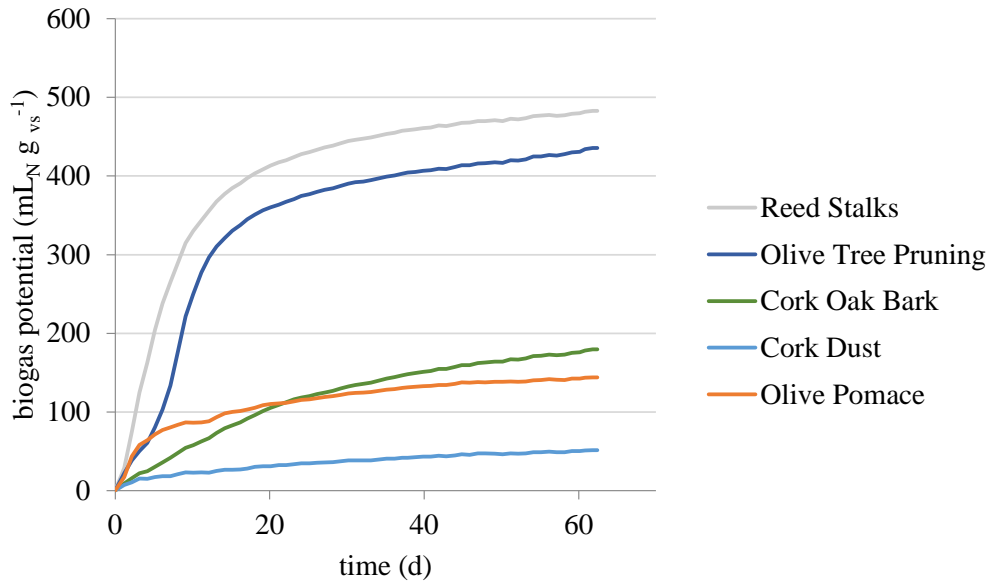

Figure 1: Cumulative biogas formation (mL$_N$ g$_{VS}^{-1}$) of agricultural residues reed, olive tree, cork tree, cork dust and olive pomace in anaerobic batch tests.
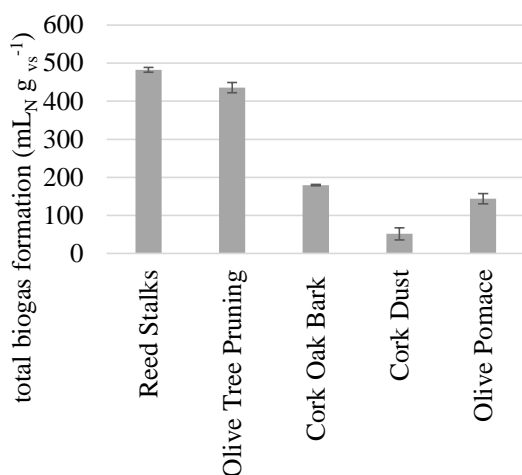

Figure 2: Biogas potential (mL$_N$ g$_{VS}^{-1}$) of substrates reed, olive tree, cork tree, cork dust and olive pomace after 62 days of anaerobic digestion in batch tests.

The first series of DES pre-treatment of cork dust revealed a substantial influence on the biogas potential of the respective substrate. As shown in Figure 3 and Figure 4 the biogas yield of the

substrate could be improved through the pre-treatment with deep eutectic solvents. As the Figures both show, the pre-treatment duration and the pre-treatment temperature had an influence on the biogas yield. While the pre-treatment at 90 and 110 °C increased the biogas yield at max by 22 %, the pre-treatment at 130 °C led to far more substantial improvement of the biogas yield. A DES pre-treatment at 130 °C for 20 min increased the biogas yield by 42 % to 73.1 ± 7.6 mL$_N$ g$_{VS}^{-1}$, A duration of 40 minutes at 130 °C further increased the biogas potential by 61 % to 82.8 ± 9.3 mL$_N$ g$_{VS}^{-1}$ and a treatment for 60 minutes at this temperature led to a biogas yield more then twice as high compared to the biogas potential of the untreated material (increase of 123 % to 114.9 ± 6.2 mL$_N$ g$_{VS}^{-1}$).
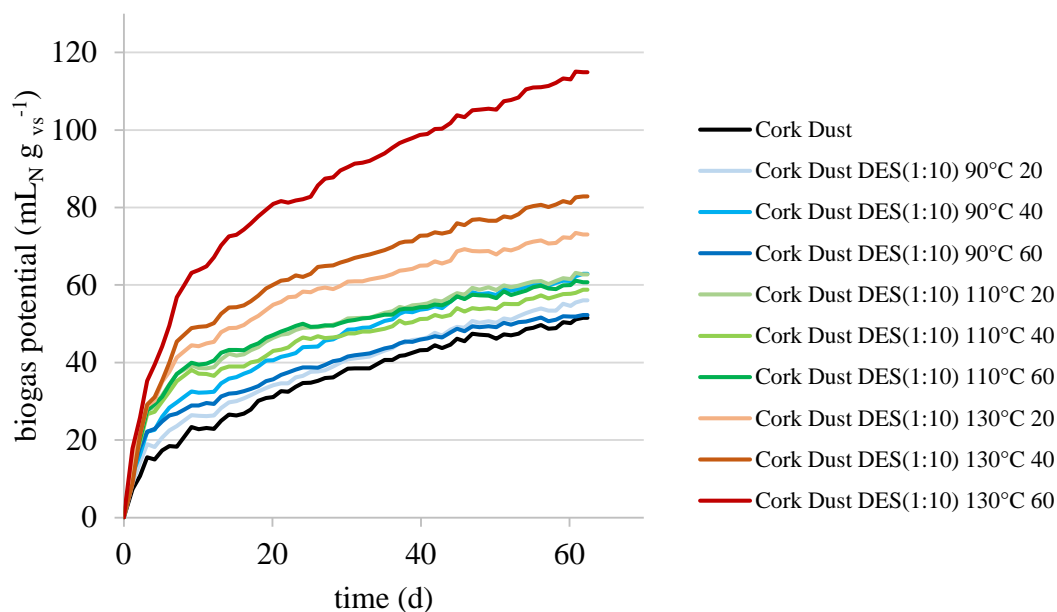

Figure 3: Cumulative biogas formation (mL$_N$ g$_{VS}^{-1}$) of untreated cork dust and pre-treated cork dust at 90, 110 and 130 °C for 20, 40 and 60 min in anaerobic digestion batch tests.

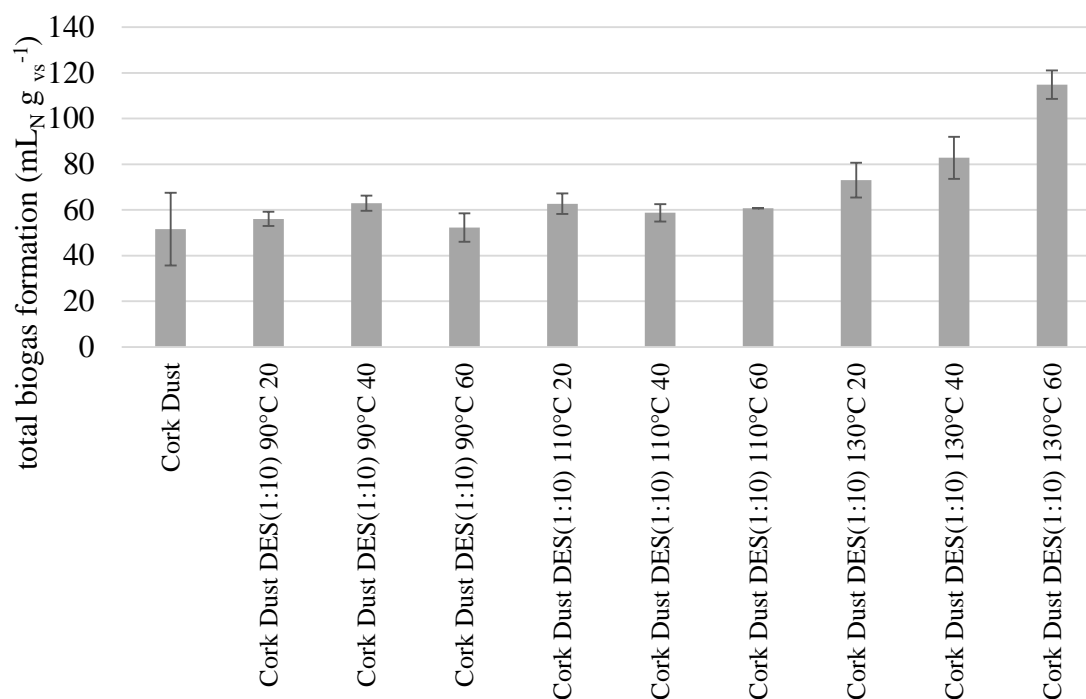Figure 4: biogas potential (mL$_N$ g$_{VS}^{-1}$) of untreated cork dust and pre-treated cork dust at 90, 110 and 130 °C for 20, 40 and 60 min after 62 days of anaerobic digestion in batch tests.

Table 4: dry mass content (% FM), volatile solids content (% DM) and biogas potential (mL$_N$ g$_{VS}$$^{-1}$) of the investigated substrates.

Substrate	Pre-treatment	Dry mass (% FM)	Volatile solid (% DM)	Biogas potential (mL$_N$ g$_{VS}$$^{-1}$)
Reed	None	97.4	91.1	482.8 ± 6.4
Olive Tree Pruning	None	98.9	96.8	435.6 ± 13.4
Cork Tree Pruning	None	97.9	96.9	179.5 ± 15.9
Olive Pomace	None	95.9	93.1	144.0 ± 13.4
Cork Dust	None	94.7	88.9	51.6 ± 15.9
Cork Dust	DES(1:10) 90°C 20	95.2	79.9	56.1 ± 3.3
Cork Dust	DES(1:10) 90°C 40	95.9	89.1	62.9 ± 3.3
Cork Dust	DES(1:10) 90°C 60	96.6	93.5	52.3 ± 6.2
Cork Dust	DES(1:10) 110°C 20	97.6	91.0	62.7 ± 4.5
Cork Dust	DES(1:10) 110°C 40	98.8	86.6	58.8 ± 3.8
Cork Dust	DES(1:10) 110°C 60	95.7	90.5	60.8 ± 0.0
Cork Dust	DES(1:10) 130°C 20	96.4	83.9	73.1 ± 7.6
Cork Dust	DES(1:10) 130°C 40	94.7	88.2	82.8 ± 9.3
Cork Dust	DES(1:10) 130°C 60	97.1	78.2	114.9 ± 6.2

3.3. Other activities

X-ray diffraction spectra of the cork dust and pre-treated cork dust samples were recorded. However, the spectra did not allow a clear statement to be made about the difference between the samples in terms of their crystallinity and the possible improvement or reduction in biogas production as a result.

All untreated biomasses and the DES pre-treated cork dust samples were handed over to the central laboratory of the Hamburg University of Technology for a NCHS-analysis.

The design and equipment of a pilot biogas plant (btp basic, UIT GmbH) for the continuous anaerobic digestion experiments was set and a respective plant was purchased. The plant is expected to be delivered in March 2023.

4. Upcoming activities work package 6

The equipment training for the scanning electron microscope (SEM) at TUHH took place already. This will be used to take SEM images of the DES pre-treated samples.

As the cork dust samples generally had a comparably low biogas potential, but the results of the biogas batch tests suggested that DES pre-treatment was effective in improving the biogas yield of the samples, another series of DES pre-treated samples will be analysed in batch tests. This series will comprise of DES pre-treated olive pruning samples. The pre-treatment conditions will be as reported for the cork dust samples. Moreover, further pre-treatment methods will be investigated accordingly.

From spring/summer 2023, two biogas pilot plants will be available for continuous fermentation experiments. The co-fermentation of the different residue substrates will be of particular interest. The goal is to proof the feasibility of the various residues for co-fermentation and thus provide an effective and environmentally friendly strategy of waste treatment, which provides energy and a digestate as fertilizer.

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Interim report month 1 to 6

D) Valorisation of the digestate and lignin-enriched residue streams

January 2023

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1. Summary of the objectives of work package 7

The digestate leaving the continuous biogas plant after anaerobic fermentation in work package 6 contains a liquid and a solid phase, which will be separated from each other. The liquid phase is predominantly recirculated to the biogas plant in order to save water and recycle nutrients; in part, it can also be applied as liquid fertilizer. This work package 7 is focusing on the remaining solid, fibrous digestate which contains mainly accumulated nutrients, lignin and non-degraded carbohydrates (their respective content depends on the type of treatment) and thus seems to be promising for different applications. Two potential applications, the use for **humus fertilization** and the further valorisation by upgrading **lignin to bio-based aromatic chemicals** will be investigated within this work package. The potential application for biochar production will be investigated within work package 3.

For the determination of the **fertiliser and humus potential**, the composition of the digestates produced by continuous biogas production in WP 6 will be analysed in detail, by means of elemental composition, lignin- /cellulose- /hemicellulose- /protein- /ash- /extractives- content and the content of specific metals. This allows a theoretical estimation of the fertilizer and humus effect and also provides insights into the performance of anaerobic digestion.

In order to determine the potential to produce **bio-based aromatic chemicals**, digestates and other lignin-rich biomasses arising within the ReMe-diation project will be analysed for their lignin content and lignin structure (i.e. content of ether bonds). Based on the obtained results, possible concepts for lignin valorisation will be developed and evaluated.

Table 1: Overview and status of tasks within WP 7.

#	Tasks	Start Date	End Date	Status
T7-1	Determination of the fertiliser and humus potential	01.05.2023	31.07.2025	pending
T7-2	Characterisation of lignin structure in untreated, pre-treated and digested biomasses	01.03.2024	30.04.2025	pending
T7-3	Selective lignin depolymerisation into monomers and oligomers in lab-scale	01.04.2024	30.04.2025	pending

Table 2: Overview and status of deliverables within WP7.

#	Delivery	Type	Description	Status
D7.1	01.11.2023	Report	(Continuous) Characterisation of digestates	pending
D7.2	01.02.2024	Report	Identification of lignin-rich streams suitable for the production of aromatic chemicals	pending
D7.3	01.10.2024	Report	Production of aromatic monomers and oligomers from suitable lignin-rich streams	pending

2. Review: Valorization of digestates and lignin

2.1 Fertilizer and humus potential of digestates

A huge variety of different biomasses can be used for biogas production. Moreover, there are different ways to produce biogas from biomass (e.g. dry fermentation vs. wet fermentation). Consequently, there is a huge variety of digestates with very different compositions. In the following, the focus is set on the solid digestate obtained after the anaerobic digestion of lignocellulosic biomass and the subsequent separation of the liquid phase. In a possible process design for the fermentation of lignocellulosic biomasses, the liquid phase might be predominantly recirculated after the anaerobic digestion, in order to achieve sufficient nitrogen supply (Andersen et al. 2020; Dieckmann et al. 2016).

It is common practice to use the solid digestates as fertilizer, either with or without previous composting and/or drying (Drosg et al. 2015). Minerals and anaerobically hardly degradable organics accumulate in these digestates, thus making them an interesting source for nutrients and stabilized organic matter. A ten-year study by the TFZ (Technology and Funding Center in the Competence Center for Renewable Resources, Bavaria) showed that fertilization with digestate could result in positive effects on all soil properties studied (for example, aggregate stability and infiltration performance of the soil, soil fauna, humus effect, ...), but is not sufficient to achieve a balanced humus balance in intensive crop rotations (Burmeister et al. 2020). However, digestates showed significantly higher humus efficiencies than typical other organic substrates like untreated animal feces and plant materials like coarse roots, crop residues and litter (Gasser et al. 2022). Some important parameters to assess the fertilizer potential and applicability of a digestate are the content in nutrients (mainly total N, P, K, S, Na, Ca, Mg, Fe, Cu, Mn, and Zn), heavy metals (e.g. Pb, Cd, Cr and Ni) and organic carbon (Alburquerque et al. 2012; Nielsen et al. 2017; Roß et al. 2020). The lignin content of digestates can be used as parameter to roughly estimate their humus reproduction capacity, however, this single factor is not sufficient for a precise analysis (Nielsen et al. 2020; Nielsen et al. 2017). Besides the lignin content, the C/N-ratio and especially incubation experiments analyzed by the ensemble of different models allow a more precise statement about the humus reproduction capacity (Gasser et al. 2022).

2.2 Valorization of lignin

Lignin is a heterogeneous aromatic polymer, which typically constitutes 15 to 30 wt% of lignocellulosic plants like wood and herbaceous plants. It forms by the radical polymerization of mainly three phenylpropanoids: p-coumaryl alcohol (H-constituent), coniferyl alcohol (G-constituent) and sinapyl alcohol (S-constituent) (Boerjan et al. 2003). The corresponding lignin structure varies depending on the biomass type: the proportions of the individual building blocks are different, moreover, they may have different side groups and other building blocks

may be added to a lesser extent. In particular, the lignin in stalk-type plants often shows strong acylation with acetic, p-coumaric, and ferulic acids. And in wheat straw, for example, the incorporation of the flavone tricin as a further building block was found (del Río et al. 2008; del Río et al. 2015; del Río et al. 2012). Further heterogeneity is provided by the different types of bonds between the monomers. Depending on the biomass, ether bonds (C-O bonds) predominate in native lignin with a share of between about 50 and 80 %; however, different types of C-C bonds are also formed (Rinaldi et al. 2016).

The potential of lignin is particularly seen in its use as a feedstock for the production of aromatic chemicals due to its aromaticity. Aromatic chemicals have so far been produced almost exclusively from fossil petroleum, and the synthesis route usually involves substances (such as benzene) that pose a severe hazard to humans and the environment. At the same time, numerous attractive properties of aromatic building blocks are leading to increased interest in these molecules in various industries (Rahim et al. 2019). Therefore, using lignin as a renewable source for bio-based chemicals appears attractive, but has not yet become established on a large scale. One major hurdle in lignin utilization is its strong, already described heterogeneity, Another major hurdle is its reactivity: the comparatively labile ether bonds are cleaved under harsh conditions that are necessary for lignocellulose fractionation (Rinaldi et al. 2016). This produces reactive intermediates that continue to react rapidly under the harsh conditions and form various new, much more stable bonds (Lan and Luterbacher 2019). These newly formed bonds are typically C-C bonds, which are novel, thus not found in native lignin, and have not yet been fully characterized (Shuai and Saha 2017; Lancefield et al. 2018). This process of structural degradation is referred to as condensation - and lignins condensed by harsh processes are referred to as technical lignins. Thus, this condensation results in a decreased content of C-O bonds and an increased content of various C-C bonds, negatively affecting the depolymerizability of the lignin. The extent of condensation depends on the severity of the treatment. There are many different biorefinery setups providing very different kinds of such condensed technical lignins and these processes and possible depolymerization strategies are described in detail in many different reviews, e.g. by Schutyser et al. (2018) or by Rinaldi et al. (2016). The most prominent processes yielding technical lignins are pulping processes as the Kraft and the sulfite process.

With regard to the valorization of lignin from still untreated biomass, so-called lignin-first processes are currently being researched. The idea behind these processes is to focus on lignin valorization first (i.e. prior to carbohydrate valorization) and to avoid the lignin condensation described above. This kind of processes is reviewed for example by Abu-Omar et al. (2021) and by Schutyser et al. (2018). One example for such a lignin-first process is the reductive catalytic fractionation (RCF), which will also be used within the ReMe-diation project. During RCF an organic solvent (e.g. methanol or ethanol) at high temperatures (typically 180-250 °C) is used to extract and fragment lignin. Additionally, a hydrogenation catalyst and often hydrogen gas are added in order to reductively stabilize lignin fragments and avoid lignin condensation (Renders et al. 2018). Most used substrates for RCF are hardwoods as birch and poplar (Schutyser et al. 2018).

Within the ReMe-diation project, however, different agricultural residual biomasses are arising, partly having a high lignin content. Besides cork tree waste, cork dust, reed, olive tree waste and olive pomace, the digestates after anaerobic digestion are especially interesting in the context of lignin valorization, as lignin is not degraded during anaerobic digestion and thus accumulating in its native structure (Steinbrecher et al. 2022). Among these biomasses, the RCF

of cork bark (which is comparable in its composition to cork dust) has already been investigated in some studies (Cao et al. 2023; Garrett et al. 2013). These studies show that suberin-derived lipids make up a huge part of bio-oil derived by RCF from cork. The yield of aromatics is rather low, with a value of around 2 wt-% based on the whole biomass weight (Garrett et al. 2013). One study was found dealing with the RCF of olive pomace, using a water/ethanol 1:1 (v/v) mixture at 200 °C (Cequier et al. 2020). A maximum yield of 15 wt-% mono-aromatics based on lignin content (corresponds to around 5.6 wt-% of whole biomass as the lignin content was around 37 wt-%) was achieved within the investigated process parameters and mainly 4-propylguaiacol, 4-propylsyringol, (Z)-4-propenylsyringol, (Z)-isoeugenol, dihydrosinapyl and dihydroconiferyl alcohol were produced. Also the RCF of reed has recently been investigated but not further optimized: Using a Ru/C catalyst in methanol at 250 °C, a monomer yield around 36 % based on lignin content (corresponds to around 7.6 wt-% of whole biomass as the lignin content was around 21 wt-%) (Wang and Deuss 2021). The main lignin monomers obtained were dihydrosinapyl and dihydroconiferyl alcohol, methyl 3-(4-hydroxyphenyl)-propanoate and methyl 3-(4-Hydroxy-3-methoxyphenyl)-propanoate. To the best of our knowledge, the RCF of cork and olive tree waste has not been investigated yet. Furthermore, there are no published results dealing with the RCF of any kind of anaerobically digested material. Recently, our research group at TUHH investigated the RCF of solid straw digestate and it was found, that slightly higher yields of mono-aromatics based on the biomass weight could be obtained from the straw digestates compared to the unfermented straw; however, these results are not published yet.

3. Report of the activities in work package 7 until month 6

As originally planned, the processing of work package 7 has not yet started. It will start as soon as the first fermentation residues are produced by continuous fermentation.

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