OPTIMIZATION OF PRETREATMENT/HYDROLYSIS PROCESSES OF AGRO-FOOD WASTES TO SUPPORT BIOREFINERY DEVELOPMENTS

Saverio Niglio

Dottorato in Biotecnologie – XXXI ciclo

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Dottorando:	Saverio Niglio
Relatore:	Prof. Antonio Marzocchella Prof. Giovanni Sannia
Coordinatore:	Prof. Giovanni Sannia

Se pensi in termini di anni, pianta il riso.
Se pensi in termini di decenni, pianta alberi.
Se pensi in termini di centinaia di anni, insegna alla gente.
- Confucio

If you think in terms of years, plant rice.
If you think in terms of decades, plant trees.
If you think in terms of hundreds of years, teach people.
- Confucio

Alla mia famiglia

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RIASSUNTO

La seconda metà del XIX secolo è stata caratterizzata da un crescente utilizzo di risorse non rinnovabili (petrolio, carbone e gas) dovuto all’esponenziale incremento della popolazione mondiale e alla sempre maggiore richiesta di energia e beni di consumo. Dopo più di mezzo secolo, la produzione di energia e prodotti vari è ancora ampiamente basata sull’utilizzo di risorse fossili. Tuttavia, la consapevolezza sociale degli effetti dello sfruttamento delle risorse fossili sui cambiamenti climatici globali, l'esigenza di diversificare le fonti per le produzioni energetiche e di beni, la spinta ad aumentare l’indipendenza nazionale dalle fonti per le produzioni energetiche e di beni hanno alimentato l'interesse per la ricerca di soluzioni alternative alle risorse fossili. Anche se lentamente, il mondo sta assistendo in questi anni alla progressiva deriva da una economia basata su risorse fossili ad un’economia basata su fonti rinnovabili. Questo aspetto è generalmente considerato come una questione chiave per lo sviluppo di una società industriale sostenibile, per un sistema energetico stabile ed indipendente e per la gestione efficace delle emissioni di gas serra (Ragauskas et al., 2006). Negli ultimi anni, gli onerosi investimenti a livello globale nella ricerca scientifica ed industriale hanno permesso di trovare strade alternative alla via petrolchimica per la produzione di energia e prodotti chimici. Una delle soluzioni proposte e sempre più adottate a livello mondiale è quella delle “bioraffinerie”. Il termine bioraffineria è stato oggetto di dibattito.

L'obiettivo generale dell'approccio alla produzione secondo un processo di bioraffineria è la generazione di una varietà di beni da materie prime quali biomasse mediante una combinazione di tecnologie/processi volti allo sfruttamento di tutti i componenti della biomassa. Una bioraffineria dovrebbe integrare i processi di conversione per produrre uno spettro di vettori energetici e sostanze chimiche dalla biomassa (FitzPatrick et al., 2010). Ad oggi, i processi petrolchimici per la produzione di energia e materie prime sono ancora economicamente favoriti rispetto ai relativamente nuovi processi di bioraffineria, ma nei prossimi anni questa tendenza è destinata a cambiare. Infatti, nei prossimi decenni assisteremo: i) alla progressiva diminuzione delle risorse non rinnovabili ancora a disposizione con il conseguente aumento del loro prezzo; ii) alla riduzione dei costi associati ai processi di bioraffineria (e.g. gestione delle risorse, uso di piante e/o microorganismi geneticamente modificati, sviluppo di nuovi processi, ecc.) rendendoli economicamente vantaggiosi rispetto ai processi petrolchimici; iii) alla consapevolezza da parte delle società sviluppate di riconoscere ai processi eco-sostenibili (quali quelli offerti dalle bioraffinerie) aliquote di costi maggiori pur di preservare la sopravvivenza del sistema ambiente. Oggigiorno, biocarburanti e bioprodotti possono essere ottenuti da processi di bioraffineria a partire da risorse rinnovabili ed abbondanti quali le biomasse. I biocarburanti sono solitamente classificati come biocarburanti di prima, seconda e terza generazione. La produzione di biocarburanti di prima generazione è andata progressivamente fallendo a causa della competizione con le risorse alimentari per l’uomo e gli animali come mais, canna da zucchero, semi di soia, ecc. (Lee e Lavoie, 2014). Negli ultimi decenni l'attenzione si è spostata sui biocarburanti di seconda generazione: utilizzo di bio-risorse non edibili elaborate nell'ambito di bioraffinerie integrate destinate non solo alla produzione di vettori energetici, calore ed energia elettrica, ma anche alla produzione di biomateriali (Stöcker, 2008). I biocarburanti di seconda generazione potrebbero ridurre significativamente la deviazione delle colture alimentari a fini energetici (Gupta, 2014). Negli ultimi anni, molti impianti industriali per la produzione di biocarburanti di seconda

generazione sono stati realizzati in tutto il mondo, per esempio in Norvegia (ChemCell Ethanol), Brasile (GranBio), China (Beta Renewables) e USA (Gevo). I biocarburanti di seconda generazione includono il butanolo. Rispetto all'etanolo, un biocombustibile ben noto, il butanolo presenta diversi vantaggi: è meno volatile, ha una densità energetica simile a quella della benzina, è meno igroscopico (può essere facilmente trasportato nelle attuali infrastrutture per combustibili di natura fossile) (Cascone, 2008). Il butanolo è anche utilizzato come solvente nell'industria cosmetica, come intermedio chimico nella produzione del butilacrilato, come estraente nell'industria farmaceutica. Il butanolo è prodotto dal petrolio mediante il processo Oxo (reazione di alcheni con monossido di carbonio e idrogeno). Il butanolo può anche essere prodotto per via biotecnologica da risorse rinnovabili (biomasse): la fermentazione acetone-butanolo-etanolo (ABE).

Le biomasse lignocellulosiche sono considerate l'equivalente perfetto del petrolio per la produzione di prodotti chimici con emissioni nette di carbonio prossime a zero (Ragauskas et al., 2006). Numerosi polimeri naturali costituenti della biomassa sono potenzialmente utilizzabili per processi produttivi industriali, sia (termo)chimici e sia biotecnologici. I polimeri includono amido, cellulosa, emicellulosa, lignina, proteine e lipidi. I principali prodotti che possono essere ottenuti mediante processi di bioraffineria della matrice polimerica naturale sono: prodotti chimici, acidi organici, polimeri e resine, biomateriali, alimenti e mangimi, fertilizzanti (Cherubini, 2010). Questi materiali possono essere ottenuti dall'estrazione/modifica diretta dei *building block* dalla biomassa (e.g. glicopolimeri) oppure dalla fermentazione degli zuccheri presenti nella biomassa (e.g. acido succinico). In ogni caso, è necessario procedere al pretrattamento della biomassa per rendere disponibili e trasformabili l'insieme dei polimeri richiamati.

La produzione di biocarburanti e biomateriali da biomassa lignocellulosica (produzione di seconda generazione) è più difficile che dall'amido di mais o dalla canna da zucchero perché la biomassa lignocellulosica è recalcitrante alla trasformazione per via fermentativa. Processi addizionali sono necessari - come il pretrattamento e la saccarificazione enzimatica - per un utilizzo efficiente di questa risorsa (Lee et al., 2014). L'obiettivo del processo di pretrattamento è rimuovere la lignina (polimeri reticolati dei monomeri fenolici), ridurre la cristallinità della cellulosa e aumentare la porosità della matrice lignocellulosica. I metodi di pretrattamento possono essere approssimativamente classificati in quattro gruppi: metodi fisici (*milling and grinding*), metodi fisico-chimici (*steam pretreatment/autohydrolysis, hydrothermolysis, and wet oxidation*), metodi chimici (*alkali, dilute acid, oxidizing agents, and organic solvents*), e metodi biologici. Viene anche proposta una combinazione di questi metodi (Kumar et al., 2009). Molti di questi metodi richiedono prodotti e apparecchiature costosi ed energivori. Il pretrattamento alcalino è noto per essere caratterizzato da molti vantaggi rispetto ad altri processi di pretrattamento: alta efficienza per la solubilizzazione della lignina; basso livello di degradazione dei carboidrati e minore formazione di composti furano-derivati; incremento della porosità della biomassa. Quest'ultimo aspetto è di particolare interesse per la successiva fase di idrolisi. Infatti, lo swelling della biomassa ne aumenta l'area superficiale interna della cellulosa e favorisce l'accesso di agenti idrolitici (ad esempio enzimi) che possono raggiungere più facilmente i siti di idrolisi (polisaccaridi). Inoltre, questo metodo di pretrattamento è abbastanza economico ed efficace per molti materiali lignocellulosici.

L'efficacia del processo di pretrattamento alcalino può essere migliorata applicando simultaneamente un campo di ultrasuoni (US). L'applicazione degli ultrasuoni genera fenomeni cavitazionali nel mezzo liquido: microbolle sono prodotte per un breve intervallo di tempo (microsecondi); l'esplosione delle microbolle produce micro-onde, onde d'urto e fenomeni di micro-convezione che generano forti forze di taglio. Questi fenomeni si verificano negli hotspot locali dove si raggiungono alte temperature (fino a 5000 °C) e pressione (fino a 50 MPa): radicali e altre specie chimiche vengono prodotte durante il trattamento a ultrasuoni. L'azione combinata di questi fenomeni danneggia l'integrità strutturale della biomassa lignocellulosica, fornendo la separazione dei polimeri strutturali (cellulosa, emicellulosa e lignina). Inoltre, l'uso di solventi può incrementare la formazione delle sopracitate specie chimiche favorendo il pretrattamento (Luo et al., 2014).

La degradazione della lignina è condotta in natura in condizioni energeticamente blande mediante diversi funghi e loro enzimi. Tuttavia, il processo di pretrattamento/delignificazione biologico è caratterizzato da velocità di conversione molto bassa fondamentalmente dovuta alla bassa velocità di espressione degli enzimi (e.g. laccasi) responsabili della degradazione della biomassa (Kumar et al., 2009). Pertanto, le potenzialità del processo – in termini di biomassa trattata nell'unità di tempo e volume - trae vantaggi dal disaccoppiare la produzione degli enzimi (processo lento) dal loro utilizzo (processo veloce). Di contro, questa strategia è penalizzata dagli elevati costi di produzione degli enzimi, che però attraverso l'applicazione delle sempre più moderne tecniche biotecnologiche potrebbe essere considerevolmente ridotti.

Le laccasi sono tra i più vecchi enzimi descritti in letteratura. Le basse richieste di energia e reagenti – solo ossigeno (aria) per lavorare e produrre acqua come unico *by-product* – e l'elevata efficienza catalitica includono le laccasi tra gli enzimi più ecocompatibili del XXI secolo. Per questi motivi, questi enzimi hanno riscontrato grande interesse nella comunità scientifica. Considerando queste caratteristiche, le laccasi possono essere usate nel pretrattamento di biomasse perché in grado di degradare la frazione di lignina, promuovendo il successivo processo di idrolisi enzimatica (Giacobbe et al., 2018).

La configurazione reattoristica adottata per il processo di bioraffineria è un altro parametro chiave per il successo del processo stesso: diversi tipi di reattore possono determinare risultati diversi. Un tipo di reattore diffuso a livello industriale è la colonna a bolle. Questi reattori sono usati in molti processi chimici di ossidazione, alchilazione, polimerizzazione e processi biochimici come le fermentazioni (Kantarci et al., 2005). Il successo delle colonne a bolle in questi campi di applicazione è dovuto ai vantaggi di questo sistema rispetto ad altri reattori: design semplice, eccellente trasferimento di massa e calore, costi di operazione e manutenzione bassi dovuti all'assenza di parti mobili.

Scopo della tesi. Lo studio svolto durante il DdR ha avuto come scopo l'utilizzo di residui di lavorazione agro-alimentare (agro-food wastes - AFWs) per produrre zuccheri fermentabili e/o bioprodotti a valore aggiunto in accordo al concetto di bioraffineria. Le attività sono state focalizzate su due aspetti: i) promuovere il rilascio di zuccheri (pentosi ed esosi) dalla cellulosa ed emicellulosa da usare in processi fermentativi per produrre biocarburanti e/o biomolecole (e.g. biobutanolo e acido succinico); ii) caratterizzare la presenza di composti inibitori e/o di molecole ad alto valore aggiunto eventualmente prodotti durante i processi di pretrattamento ed idrolisi. Il lavoro è stato condotto presso il Dipartimento di Ingegneria Chimica, dei Materiale e della Produzione Industriale dell'Università degli Studi di Napoli 'Federico II'. Parte delle attività sono state condotte nel corso di un periodo di studio presso la "University of Western Ontario (UWO)" Canada.

Il coffee silverskin come materia prima in bioraffineria

Il coffee silverskin (CSS) è la sottile pellicola residua che avvolge e protegge i chicchi verdi di caffè. È prodotto durante la fase di tostatura dei chicchi verdi. Il CSS essiccato è principalmente composto di carboidrati (40%w/w) e lignina (30% w/w). Tuttavia, la composizione può cambiare perché dipende dalla varietà di caffè, dalle caratteristiche del suolo come nutrienti e disponibilità di acqua, e dalle condizioni meteo. I principali temi riguardanti la fornitura di CSS sono:

- La produzione annuale mondiale di chicchi di caffè è di circa 160 milioni di sacchi da 60 kg;
- Il CSS rappresenta circa il 4.2 %(w/w) dei chicchi di caffè;
- Il processo di tostatura avviene in tutto il mondo.

Questi temi fanno del CSS un potenziale candidato per essere usato come materia prima nelle bioraffinerie.

Lo studio del CSS come feedstock della bioraffinera ha previsto approfondimenti riguardanti il pretrattamento, l'idrolisi enzimatica e la fermentazione.

Il pretrattamento del CSS è stato condotto considerando una combinazione di soluzione alcalina e ultrasuoni (US). Le prove sono state effettuate focalizzandosi su quattro differenti parametri che influenzano questo tipo di processo: la quantità di biomassa, il tempo di sonicazione, la concentrazione alcalina, il tempo di permanenza in autoclave. Gli esperimenti sono stati organizzati ed analizzati in accordo alla *response surface methodology*. Il processo è stato caratterizzato in termini di resa in zuccheri dopo l'idrolisi enzimatica del CSS pretrattato. Le condizioni operative che hanno fornito i migliori risultati di resa in zuccheri sono state selezionate per le successive prove di idrolisi enzimatica e fermentazione.

L'idrolisi enzimatica è stata condotta investigando gli effetti della quantità di biomassa e della quantità di enzima idrolitico sul rilascio di zuccheri dal CSS pretrattato con soluzione alcalina.

L'idrolizzato (ricco in zuccheri) ottenuto dopo il pretrattamento e l'idrolisi enzimatica del CSS è stato integrato con altri nutrienti e usato come terreno di crescita per la fermentazione di *C. acetobutylicum* e *A. succinogenes*, per la produzione di solventi (acetone-butanolo-etanolo) e acido succinico, rispettivamente. Le prove batch sono state effettuate con lo scopo di evidenziare la capacità fermentativa degli zuccheri ottenuti dal CSS con differenti microorganismi per produrre bioprodotti commerciabili.

In definitiva, il CSS si è dimostrato essere un ottimo candidato per le applicazioni in bioraffineria in quanto differenti prodotti commerciabili sono stati ottenuti a partire da questo residuo: zuccheri fermentabili, lignina, solventi (fermentazione ABE), biomolecole (composti fenolici e acido succinico).

Sansa di mele come materia prima per applicazioni di bioraffineria

La mela è un frutto largamente prodotto e fruito in tutto il mondo. In Italia, più di 2.5 milioni di tonnellate di mele sono prodotti ogni anno. A livello mondiale, il 20 % delle mele raccolte è processato in prodotti ad elevato valore aggiunto: succo di mela concentrato (65%), sidro di mele, vino di mele, purea, marmellate e mele essiccate. Il principale residuo di questi processi è la sansa di mele. Questo residuo costituisce il 25-35% della massa secca delle mele. La sansa di mele è un ottimo candidato lignocellulosico per essere utilizzato in processi di bioraffineria, ed è già stato utilizzato come substrato per la produzione di butanolo, etanolo e proteine (o enzimi), acido citrico, bio-idrogeno. Il pretrattamento della sansa di mele è stato effettuato utilizzando l'enzima ligninolitico "laccasi". Lo scopo di questo studio si è basato sul determinare la possibilità di condurre un processo biologico di pretrattamento ed idrolisi della sansa di mele utilizzando una colonna a bolle come bioreattore. Alcuni dei parametri che possono influenzare il processo sono stati ottimizzati tenendo conto della quantità di zuccheri rilasciati durante tutto il processo. In un primo stadio, è stata condotta l'ottimizzazione delle condizioni operative del pretrattamento, cioè quantità di biomassa, quantità di laccasi e velocità di agitazione con aria. Fissate le condizioni operative di pretrattamento, ulteriori indagini hanno riguardato lo stadio di idrolisi enzimatica in colonna studiando gli effetti della quantità di enzima e della velocità di agitazione sulla resa in zuccheri. Tutti gli esperimenti sono stati effettuati utilizzando una colonna a bolle in scala da laboratorio.

L'elevata quantità di zuccheri ottenuta da questo residuo ne fanno uno dei principali candidati per essere usato in processi fermentativi per la produzione di solventi e biomolecole. Inoltre, anche in questo caso un residuo solido di lignina può essere recuperato e riutilizzato alla fine del processo.

Fermentazione ABE presso la University of Western Ontario (UWO) (Canada)

L'attività di ricerca è stata focalizzata sulla fermentazione ABE di un prodotto industriale derivante dal mais: lo sciroppo di mais. Gli obiettivi sono stati: i) *screening* di quattro specie di *Clostridium* per determinare il microorganismo caratterizzato da prestazioni elevate in termini di produzione di solventi, resa e produttività; ii) studiare gli effetti della concentrazione di substrato/prodotto sul processo fermentativo (inibizione); iii) incrementare la produzione di solventi adottando un processo fed-batch. Lo scopo ultimo di questo progetto è stato l'investigare la possibilità di usare lo sciroppo di mais per produrre solventi per mezzo della fermentazione ABE. I dettagli riguardanti tali attività sono riportati nella sezione *Appendix*. Riguardo al mio progetto di tesi, lo studio condotto presso la UWO ha avuto un duplice scopo: 1) migliorare le mie competenze sulla fermentazione anaerobica condotta con differenti specie di *Clostridium*, in accordo con lo scopo finale del progetto europeo Waste2Fuels, cioè la produzione di bio-butanolo da residui agro-industriali; 2) confrontare le capacità fermentative di questi microorganismi su prodotti agro-industriali caratterizzati da vari livelli di difficoltà di fermentazione (in particolare sciroppo di mais e coffee silverskin).

SUMMARY

The study carried out during the present Ph.D. program aimed at investigating the use of agro-food processing wastes (AFWs) to produce fermentable sugars and/or value-added bioproducts according to the biorefinery approach. The work was carried out at the Dipartimento di Ingegneria Chimica, dei Materiale e della Produzione Industriale of the Università degli Studi di Napoli 'Federico II'. The activities were focused on the release of sugars (pentoses and hexoses) from the AFWs investigated through different pretreatment processes, followed by enzymatic hydrolysis step. The fermentation of the produced sugar solutions was also carried out to produce biobutanol and succinic acid. Two AFWs were investigated: coffee silverskin and apple pomace.

Coffee silverskin as feedstock for biorefinery applications

Coffee silverskin (CSS) are the residual thin teguments wrapping and protecting the external layer of green coffee beans. It is produced in large amount during the roasting phase of green coffee beans. It is mainly composed of carbohydrates (40% w/w) and lignin (30% w/w). The study of CSS was aimed at investigating the pretreatment, enzymatic hydrolysis and fermentation of this residue. A combination of mild alkaline solution and ultrasound (US) was applied to pretreat CSS. Biomass loading, sonication time, alkali concentration, residence time in autoclave were the parameters investigated and analysed according to a response surface methodology approach. The process was characterized in terms of sugar yield after the enzymatic hydrolysis of pretreated CSS. Alkali concentration and residence time in autoclave were the most significant parameters affecting the pretreatment process according to the statistical analysis. Under optimal operating conditions the maximum sugar yield was of 60% g/g. Moreover, the phenolic content assessed in the supernatant after CSS pretreatment was (25 mg_{GAE}/g_{raw_CSS}) larger than that reported in the literature for similar works on CSS.

The study about enzymatic hydrolysis of alkali-pretreated CSS highlighted that both biomass loading and cellulolytic enzymes loading affect sugars release. A sugar yield higher than 90% g/g was obtained with 10% w/v biomass loading and 80 $FPU/g_{cellulose}$ enzyme loading. The rich-in-sugars hydrolysate obtained after pretreatment and enzymatic hydrolysis of CSS was supplemented with some other nutrients and used as media for fermentation of *C. acetobutylicum* (ABE production) and *A. succinogenes* (succinic acid production). Concentration of 3.2 g/L ABE and 20.8 g/L succinic acid was obtained, at the end of each fermentation, respectively.

With the proposed process (pretreatment, hydrolysis and fermentation), we were able to obtain fermentable sugars, lignin, solvents (ABE fermentation) and biochemicals (phenolic compounds and succinic acid) from CSS, stressing the potentialities of this residue to be used in biorefinery processes.

Apple pomace as feedstock for biorefinery applications

Apple is a fruit widely produced and eaten all over the world. In Italy, more than 2.5 million tons of apple are produced every year. Worldwide, 20 % of fresh apple is processed into value added products: apple juice concentrate (65%), apple cider, wine, vermouth, purees, jams and dried apple products. The main residue of these processes is the apple pomace. This residue accounts for 25-35% of the dry mass of apple. The apple pomace is a good lignocellulosic candidate to be used in a biorefinery process, and it was already used as feedstock for production of butanol, ethanol and crude protein (or enzymes), citric acid, microbial colours, bio-hydrogen.

Pretreatment of apple pomace was carried out by means of the ligninolytic enzyme "laccase". The aim of this study was to investigate the possibility to carry out

biological pretreatment and hydrolysis of apple pomace in a bubble column bioreactor. The proposed pretreatment/hydrolysis system aimed at the optimization of some parameters affecting the process in terms of amount of sugars released during the whole process. The enzymatic delignification was carried out using fungal laccases from *Pleurotus ostreatus*. Then the cellulose in the pretreated biomass was hydrolysed using Cellic CTec2® (Novozymes). The optimization of enzymatic pretreatment/hydrolysis process indicated that all the parameters investigated (biomass loading, laccase loading, gas (air) flow rate, cellulase loading) affect the performances of the process in terms of biomass composition and sugars release during the enzymatic hydrolysis. 15 %w/v biomass loading, 60 nL/h air flow rate, 30 $U/g_{biomass}$ laccase concentration, and 20 $FPU/g_{cellulose}$ cellulase concentration were the optimal conditions for the sequential enzymatic pretreatment and hydrolysis process in the bubble column. All the experiments were carried out in a lab-scale bubble column. Under the operating conditions investigated, a maximum sugar yield of 0.34 $g_{sugars}/g_{raw\ biomass}$ was obtained, corresponding to 61% ($g_{sugars}/g_{sugars\ available}$) of the theoretical sugar yield obtainable from raw apple pomace.

The high amount of sugars obtained, make AP one of the main feedstocks to be used in fermentative processes to produce solvents and biochemicals. Moreover, a solid residue of lignin that can be recovered and reused was obtained at the end of the process.

ABE fermentation at University of Western Ontario (UWO) (Canada)

The reasearch activity was focused on the ABE fermentation of an industrial product derived from corn: corn syrup. The objectives were: i) to screen four Clostridium species to identify the microorganism characterized by the best fermentative performance in terms of solvents production, yield and productivity; ii) to study the effects of substrate/product concentration on fermentation process (substrate/product inhibition effects); iii) to increase solvents production by means of a fed-batch process.

The final aim of this project was to investigate the possibility of using corn syrup to produce solvents through ABE fermentation. The details regarding this activity are reported in the section *Appendix*.

Regarding the main topic of my Ph.D. project, the work carried out at UWO have had a double purpose: 1) to improve my skills in anaerobic fermentative processes carried out by different *Clostridium* species in accordance to the final aim of the European project Waste2Fuels that is the bio-butanol production starting from agro-industrial residues; 2) to compare the fermentative performances of *Clostridium spp.* on agro-industrial products with various degrees of difficulty to ferment (in particular corn syrup and coffee silverskin).

1. INTRODUCTION

1.1 Biorefinery concept

Today's world population is achieving about 8 billion persons, and it is estimated to grow up to 11 billion by 2100 (United Nations Prospects, 2017). The demographic features of population (size, urbanization, age) are the main factors affecting the global climate change and greenhouse gas (GHG) emissions (O'Neill et al., 2012). Indeed, the population growth - related to the modern development of the depressed regions - causes a high increase of demand for energy and commodities. To meet these requests, different energy sources have been utilized. Among these, fossil fuels (oil, coal and gas) are the main resources exploited for energy and material purposes, reaching a global energetic coverage of 87 % (Figure 1.1). No bio-based renewable resources participate to this demand by only the 5 %, while energy produced starting from bio-based material (bioenergy) account for 8 % of total global energy coverage (Kircher, 2015). Bioenergy contribution to global energy supply is expected to grow up to 20 % by 2020 (IRENA, 2014) and 40 % by 2050 (Kircher, 2012).

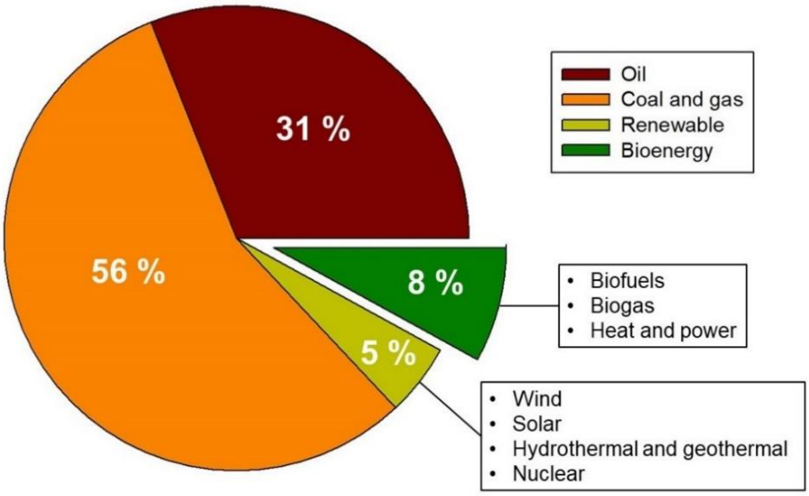


Figure 1.1. *Global energetic sources. Adapted from Kircher (2015)*

The predicted increase of bioenergy production and, in general, of renewable energies in the next future is mainly due to the environmental pollution produced by use of fossil resources. Indeed, electrical and/or transportation purposes are obtained by combustion of fossil fuels that generate carbon dioxide (CO_2) as main product. CO_2 is a GHG internationally recognized as leading factor influencing the climate change we are experiencing in the last decades (Anderson et al., 2016). Moreover, worldwide crude oil depletion is waited after 2050 even though the real extension of natural reservoir is still an open question (Tsoskounoglou et al., 2008). Besides fuels production, fossil resources are also employed for the production of chemicals. Indeed, 4% of total oil is used all over the world for chemicals and plastic production (Cherubini, 2010). These data highlight how strong is the human bond with fossil resources, created after many years of uncontrolled exploitation of these limited resources. However, the increasing demand and price of crude oil, (long-term) depletion of oil reserves, environmental issues related to the use of fossil-derived products (i.e. pollution and climate changes), social pressure related to the use of regional resources for energy and material necessities, are shifting the attention of scientific/industrial community towards new sources to use in both energy and non-energy (chemicals and materials) sectors. Renewable energy sources were proposed in the last years, mainly solar, wind, nuclear. All of them are excellent options for energetic purposes, but this is both an advantage and a drawback of this kind of resources because they can be used only for electricity production. Conversely, the use of biomass allows to fulfil both requests of energy and products (Cherubini, 2010). The shift of society's dependence from fossil-based to renewable biomass-based resources is generally viewed as a key issue for the development of a sustainable industrial society, energy independence and stability, and for the effective management of greenhouse gas emissions (Ragauskas et al., 2006). However, the economic success of the bio-based production of commodities and energy vectors typically asks for the exploitation of the biomass by valorisation of all high-value components according to the biorefinery concept. Several definitions of biorefinery were proposed in the recent years, but the most exhaustive still remains that proposed by the IEA Bioenergy Task 42 ‘‘Biorefineries”: ‘‘Biorefining is the sustainable processing of biomass into a spectrum of marketable bio-products and bio-energy” (Cherubini, 2010). The overall goal of the biorefinery production approach is the generation of a portfolio of goods from biomass feedstocks by means of a combination of technology/processes aimed at the exploitation of all components of the biomass. Ideally, a biorefinery should integrate different processes to produce fuels, power, materials, and chemicals from renewable biomass (FitzPatrick et al., 2010). A biorefinery approach involves multi-step processes. After feedstock selection, the first step typically involves treating the biomass to make it more amenable for further processing. This step is conventionally referred as pretreatment. Provided the pretreatment, the biomass components are subject to a combination of biological and/or chemical treatments. The outputs of this step are chemicals and/or reducing sugars that could be converted into chemical building blocks for further processing uses. The conversion to specialty polymers ready for market use, to a fuel/energy source, or use in composite materials are possible processing options. The main steps characterizing a biorefinery process are shown in Fig. 1.2.

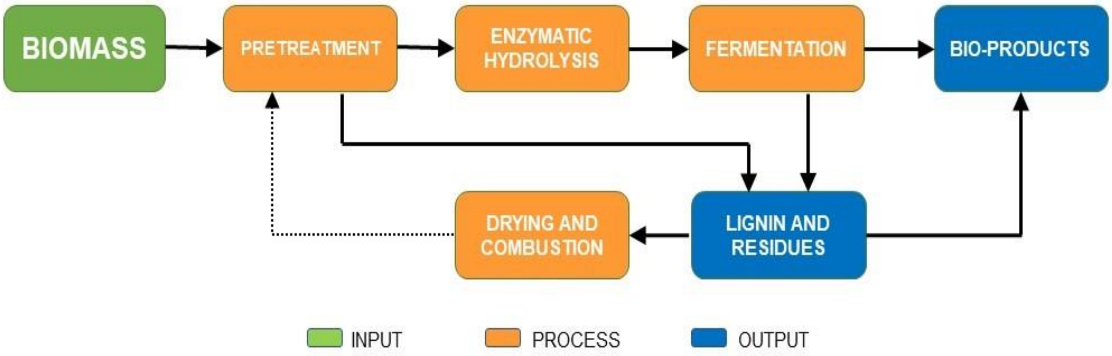


Figure 1.2. *Main steps of a biorefinery process.*

Biorefinery concept is based on the wide idea of “sustainable development”. This issue was first expressed by Brundtland Commission in its landmark publication Our Common Future (WCED, 1987). The first paragraph of the Brundtland definition (WCED, 198, p. 43) reads: “Sustainable development is development that meets the needs of the present without compromising the ability of future generations to meet their own needs”. From this definition it is clear that each human generation must use

Earth's resources in a very careful way. The concept of "overshoot" is a valuable example to explain how the "not-sustainable development" of the last decades is causing an always faster increase use of global resources. The "Earth overshoot day" marks the date when all of humanity have used more from nature than our planet can renew in the entire year. We are using 1.7 Earths. We use more ecological resources and services than nature can regenerate through overfishing, overharvesting forests, and emitting more CO_2 into the atmosphere than ecosystems can absorb (https://www.overshootday.org/). Workable solutions to invert this negative trend were proposed and are proposed everyday by scientists worldwide. From an environmental point of view, bioenergy is continuing gain momentum as valuable option for energetic and materials production in a sustainable world.

1.2 Bio-based products

Biorefinery processes allow to exploit all the components of the biomass that is being processed. When different products are obtained starting from the same biomass, the whole process can be defined as multiproduct biorefinery (Ulonska et al., 2018). Usually, at industrial scale, two main product streams are generated from pretreatment and enzymatic hydrolysis of lignocellulosic biomass: a fermentable sugars solution and a solid residue mainly composed of unhydrolyzed lignin. Sugars solution can be commercialized or directly fermented in the plant to produce biofuels and/or biochemicals. In the same way, lignin can be commercialized and processed to produce polyphenols-based chemicals or solid biofuel. In many cases, lignin residue is directly used in the plant as solid biofuel to provide energy to the process and make it more economically sustainable. Examples of multiproduct biorefinery are Renmatix Inc. (Pennsylvania, USA) and Sekab (Domsjö, Sweden). More detailed information about biofuels and biochemicals production are reported in the following.

1.2.1 Biofuels

Biofuels are fuels produced starting from biological material. The carbon balance of biofuels is close to neutral because the amount of CO_2 produced by combustion of these fuels is almost the same that the plant captured from atmosphere during its growth. For this reason, bio-based fuels are a more environmentally friendly option when compared to fossil-based fuels (Lee and Lavoie, 2014). Biofuels are directly generated from plants and microorganisms. In the last decades, the various kinds developed and produced biofuels have been divided into three generations: first, second and third generation (Rodionova et al., 2017).

First-generation biofuels are produced from sugar, starch, vegetable oil or animal fats. Seeds and grains such as wheat, corn and rapeseed are the main basic feedstocks. The most common first-generation biofuels are bioethanol, biodiesel and starch-derived biogas. The high sugar or oil content of the raw materials is the main advantage of first-generation biofuels, favouring their easy conversion into biofuel. The main drawback of first-generation biofuels is the feedstock competition with food and feed industries for the use of water and agricultural land. For these reasons, these biofuels created ethical, political and environmental concerns (Cherubini, 2010). To overcome these issues, second-generation biofuels were developed.

Second-generation biofuels are defined as fuels produced from a wide spectrum of feedstock not in competition with food and/or feed industries. The feedstock used to produce these fuels are not-edible lignocellulosic biomass. These biomass are usually separated in three categories: homogeneous (e.g. wood chips); quasi- homogeneous (e.g. agricultural and forest residues); and non-homogeneous (e.g. municipal solid wastes) (Lee and Lavoie, 2014). Contrarily to first-generation, second generation biofuels have several advantages: price for this biomass is significantly less than the price for vegetable oil, corn, and sugarcane; not only seeds and grains, but the whole plant can be used for bioenergy purposes; higher land-use efficiency and environmental performance; different bioproducts can be generated starting from the same feedstock (Cherubini, 2010; Lee and Lavoie, 2014). The main drawback of second-generation biofuels is the more complex structure of lignocellulosic feedstock that causes an increase of the conversion process cost. Indeed, the only pretreatment area may represent between 30% and 50% of the total equipment cost for an industrial scale plant (Valdivia et al., 2016).

Second generation biofuels include butanol. Butanol is a simple four-carbon alcohol considered a superior biofuel candidate with respect to ethanol (Lu et al., 2012). Indeed, butanol has interesting features compared to ethanol: higher energy content, lower vapor pressure and a similar air-to-fuel ratio to gasoline (Lu et al., 2012; Jin et al., 2011). A comparison of physical/chemical properties of butanol, ethanol and gasoline is reported in Table 1.1.

Table 1.1. *Comparison of chemical properties of butanol, ethanol and gasoline (Jin et al., 2011).*

Properties	**n-butanol**	**Ethanol**	**Gasoline**
Density (g/L) at 20 °C	0.808	0.790	0.72-0.78
Octane number	96	108	80-99
Heating value (MJ/kg)	33.1	26.8	42.7
Oxygen content (%w)	21.6	34.8	<2.7
Autoignition temperature (°C)	385	434	300

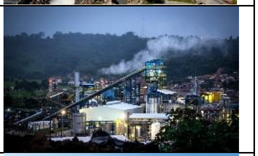
Butanol can be blended with either gasoline or diesel at any fraction and is compatible with the current engines without any retrofitting (Cascone, 2008; Bharathiraja et al., 2017). Butanol is conventionally produced from the petroleum according to the Oxo process (reaction of alkenes with carbon monoxide and hydrogen), but it can be also produced starting from bio-based materials (biobutanol) through the biotechnological route defined ABE fermentation. ABE fermentation is typically carried out by solventogenic *Clostridium* species under appropriate operating conditions (Raganati et al., 2015). Clostridium species are saccharolytic butyric acid-producing bacteria able to ferment pentoses, hexoses, mono-, di- and polysaccharides (Bharathiraja et al., 2017; Raganati et al., 2015). *Clostridium spp*. can ferment both simple and complex carbon sources, but the fermentation substrate directly affects the cost of the process to produce bio-butanol. The entire process could be made more economically feasible if cheaper and rich-in-sugars substrates like lignocellulosic biomass were used (Ezeji et al., 2007a).

Third-generation biofuels are fuels produced from lipids produced by algal biomass. Lipids obtained from algae can be processed via transesterification process or can be submitted to hydrogenolysis to produce kerosene grade alkane suitable for use as drop-in aviation fuels (Lee and Lavoie, 2014).

1.2.1.1 Second-generation biofuels: commercial status

During the last years, numerous projects have been founded all over the world for the development of second-generation biofuels production processes. As an example, the Horizon 2020 European project “Waste2Fuel” (Grant Agreement no. 654623) aims to develop next generation biofuel technologies capable of converting agrofood-waste (AFW) (lignocellulosic biomass) streams into high quality biobutanol. This project, carried out by 21 partners, is almost to start the process scale-up. Other biofuel producing projects already reached a pilot plant level. Among these, the “BioTFuel” project, launched by Total S.A. and other five partners, aims to produce biofuels (second-generation biodiesel and biojet fuel) from lignocellulosic biomass such as straw, forest waste, dedicated energy crops via thermochemical conversion. Besides these starting bench/pilot scale projects, plants for the production of second-generation biofuels at industrial scale are already running worldwide. Since the number of these plants is increasing, a 50 per cent growth in the second-generation biofuels market has been forecast between 2014 - 2020 and its value in 2020 has been estimated to amount to US$ 23.9 billion (Allied Market Research, 2014). Supportive regulations and governmental incentives are important stimulating factors towards biofuels production. Indeed, the countries where these regulations are applied represent the economies that are dominating the biofuels’ market. United States, China, Canada, European Union and Brazil are the countries ruling the global second-generation biofuels’ market (UNCTAD, 2016). Table 1.2 reports some of the main industrial plants for the production of second-generation biofuels based in the above-mentioned countries.

Table 1.2. *Examples of second-generation biofuels industrial plants (UNCTAD, 2016).*

Company	Biofuel	Location	Feedstock	Production capacity*	Start year	
Abengoa Bioenergy Biomass of Kansas LLC	Ethanol	Hugoton, USA	Corn Stover/Straw/ Switchgrass	94.64	2014	
Gevo	Ethanol, Biobutanol	Luverne, MN, USA	Lignocellulosic crops or residues	68.58	2006	
Borregaard Industries AS - ChemCell Ethanol	Ethanol	Sarpsborg, Norway	Wood pulping residues	20.00	1938 (pulping site)	
GranBio / Beta Renewables/ Chemtex	Ethanol	Sao Miguel, AL, Brazil	Sugarcane bagasse and straw	82.38	2014	
Beta Renewables - Fuyang Bioproject	Ethanol	Fuyang, China	Wheat straw, corn stover, poplar residues	253.49	2016	
Enerkem Alberta Biofuels LP	Ethanol	Edmonton, Canada	Post-sorted municipal solid waste	38.02	2014	

* million litres per year

1.2.2 Biochemicals

Despite their decreasing availability and frightening environmental effects, fossil resources are still the starting point for the industrial production of a wide spectrum of chemicals and synthetic polymers (Isikgor and Becer, 2015). According to biorefinery concept, the shift towards the use of lignocellulosic biomass to produce bio-based chemicals could help to decrease our dependence on fossil resources. Indeed, the exploitation of renewable resources allow to produce chemical products based on the idea of sustainability (Cherubini, 2010). The biological production of chemicals is not a modern technology. Several commodity products were produced by fermentation in the first half of the 20th century, including acetic acid, citric acid, lactic acid, and itaconic acid (FitzPatrick et al., 2010). With the development of petrochemical refineries, the use of biomass to produce bio-products was un-competitive. However, today the questionable competitive price of fossil fuels and environmental/social issues have increased a new interest in bio-based products (Isikgor and Becer, 2015). Biomass is considered to be the perfect equivalent to petroleum for the production of fine chemicals at quasi-zero carbon emission (Ragauskas et al., 2006). Numerous natural polymers present in biomass have the potential for physical and chemical modifications. Among these, starches, cellulose, hemicellulose, lignin, proteins and lipids are the main candidate for the exploitation.

Natural polymers can replace fossil derived polymers like plastics and textiles (Cherubini, 2010). The most important chemical and material products which can be produced in biorefineries are: chemicals, organic acids, polymers and resins, biomaterials, food and animal feed, fertilizers (Cherubini, 2010). Lignocellulosic biomass feedstocks provide sugars (from cellulose and hemicellulose) and aromatic compounds (from lignin) that can be directly or indirectly utilized to produce chemicals. The direct use of sugars and/or lignin consists of chemical reactions (e.g. Fischer–Tropsch reactions, condensation polymerization) of these compounds to produce valuable market products. An example are glycopolymers, synthetic macromolecules with pendant carbohydrate moieties employed to design novel drugs and gene delivery systems (Isikgor and Becer, 2015). The indirect use of lignocellulosic biomass consists of fermentation of biomass-derived C5 and C6 sugars. These fermentable sugars are used to feed yeasts or bacteria able to produce bio-compounds of industrial interest. A lot of biochemicals are produced worldwide via fermentation at industrial scale, for example succinic acid, 1,3-propanediol, itaconic acid, lactic acid, levulinic acid, and their market is continuously growing. Succinic acid production via fermentation is a mature technology (Cherubini, 2010). Myriant, BioAmber, Succinity and Reverdia are the main producers of bio-based succinic acid. Succinc acid is a four-carbon aliphatic bicarboxylic acid that finds applications in many different market sectors: from chemical (surfactant, detergent, extender and foaming agent) to food (acidulant and pH modifiers, flavouring agent, anti-microbial agents) and pharmaceutical (cosmetic, pharmaceuticals, antibiotics, amino acids, and vitamins) (Zeikus et al., 1999). Various poly-amides, polyesters, and poly(ester-amide)s can be produced via the condensation reaction of succinic acid or succinic acid diesters with diamines or diols (Isikgor and Becer, 2015). 1,3-Propanediol is a key building block for polypropylene terephthalate, which is not available from petrochemical sources, in addition to being used as fibre in the apparel and carpet industries. In nature, 1,3-propanediol is produced through the fermentation of glycerol, but Genencor and DuPont have developed a low-cost route of producing 1,3-propanediol by modifying natural routes through metabolic engineering of a *E. coli* K12 strain (Nakamura and Whited, 2003). Itaconic acid is one of the top twelve building blocks according to the Department of Energy of United States. It is produced via fermentation of carbohydrates by fungi and find applications in production of fibers, coatings, adhesives, thickeners and binders. Itaconic acid is considered as a biofriendly substitute for acrylic and methacrylic acid in polymers and in styrene–butadiene systems (Cherubini, 2010). Lactic acid is the most widely occurring carboxylic acid in nature. Owing to its biofunctionality, it can be converted into a variety of reaction compounds such as acetaldehyde, acrylic acid, propanoic acid, 2,3-pentanedione and dilactide. Polylactic acid (PLA), the homopolymers from lactic acid, is a biodegradable substitute of polyethylene terephthalates (Isikgor and Becer, 2015). Large scale production of levulinic acid from lignocellulosic biomass has already been achieved. This acid is precursor of many pharmaceuticals, plasticizers and additives (Isikgor and Becer, 2015).

1.3 Biorefinery process

The process of biomass conversion into bio-based products is characterized by five major steps:

- Feedstock selection
- Pretreatment process
- Enzymatic hydrolysis

- Sugars fermentation
- Downstream process

Altogether, these steps allow to exploit the potentialities of biomass feedstock in the fields of bioenergy and bio-commodities.

1.3.1 Feedstock selection

The term “feedstock” indicates the raw material used in biorefinery (Cherubini, 2010). The choice of feedstock is a critical issue in biorefinery design. The availability and composition of raw materials determine the potentialities of the biomass to be used in this kind of processes. Nowadays, a vast range of different biomass feedstock like sugar- or starch-rich crops, lignocellulosic biomass and algae, can be used for biorefinery applications. Sugar- and starch-based biomass are the main feedstock used in first-generation biorefinery processes. Due to their composition, these materials are easy to use because no pretreatment steps are required: sugars are already available for fermentation (enzymatic hydrolysis can be necessary only when no saccharolytic microorganisms are employed). The main drawback of these feedstock is their high production cost. Moreover, the use of such biomass in biorefinery processes is also in competition with their food/feed purposes (Suhag and Sharma, 2015). Consequently, scientific attention focused on cheaper and abundant biomass not in competition with human/animal food. Lignocellulosic biomass was elected as new biorefinery feedstock.

Lignocellulosic biomass is the most abundant renewable resource on Earth. This material is continuously naturally produced and is always available in large amount. The worldwide biomass production is estimated in 170-200 x 10^9 tons per year (Pauly and Keegstra, 2008). Currently, only 2% of this amount is used in human activities (e.g. heat production, paper industry, textile industry) (Pauly and Keegstra, 2008). Their great abundance, along with their no-edibility, makes lignocellulosic material the perfect candidate to substitute starchy-based feedstock in development of second-generation biorefinery.

Lignocellulosics are mostly composed of three major units: cellulose, hemicellulose and lignin. In nature cellulose fibres are embedded in a matrix of hemicellulose and lignin (Menon and Rao, 2012). Cellulose, hemicellulose and lignin constitute more than 75% of the vegetal material. Organic polymers of high molecular weight are the components of the three major units. The three-dimensional structural organization of these components is shown in Fig. 1.3.

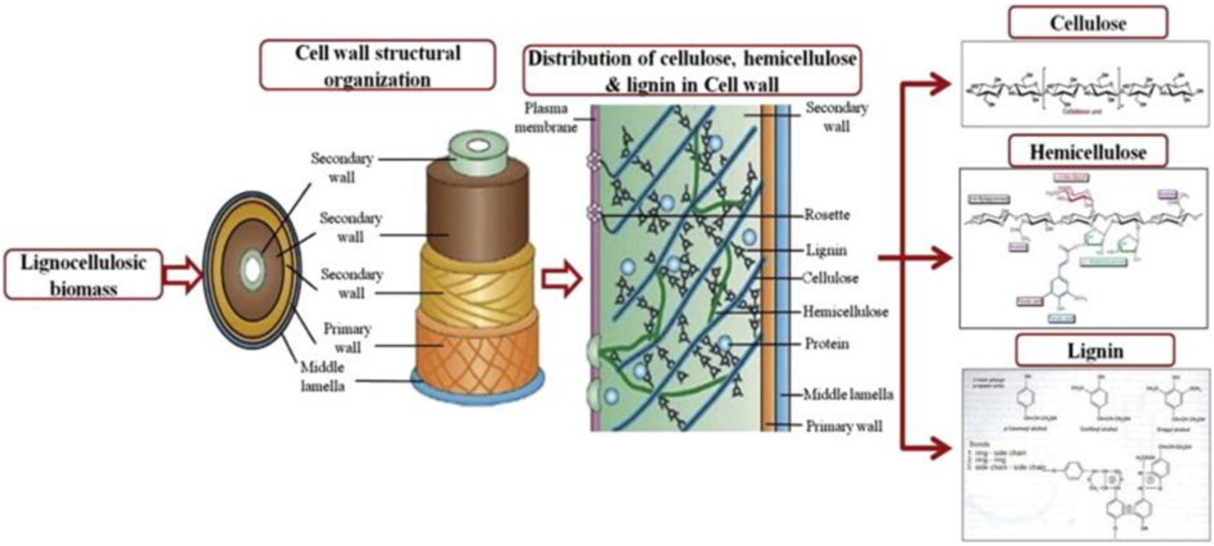


Figure 1.3. *Diagrammatic illustration of the framework of lignocellulose (from Menon and Rao, 2012).*

Cellulose is a deeply stable polymer. It is composed of glucose monomers, attached by covalent bonds to create linear chains up to 12,000 residues. (1,4)-D-glucopyranose units are the major constituents, which are linked by β-1,4 linkages. The average molecular weight of each polymer is of around 100,000 (Himmel et al., 2007). Biomass from plants contains 40-50% of cellulose molecules. Inter- and intramolecular hydrogen bonds in native state held together the cellulose polymers, increasing the rigidity of cellulose. This chemical structure makes cellulose highly insoluble and highly resistant to most organic solvents. Naturally cellulose chains are packed into micro-fibrils which are stabilized by hydrogen bonds. The fibrils order creates crystalline and amorphous regions (Iqbal et al., 2011). The chemical formula of cellulose is $(C_6H_{10}O_5)_n$ and the structure of the polymer's chain is presented in Fig. 1.3.

Hemicellulose is the second most important carbohydrate fraction after cellulose in wood. Hemicellulose is bounded to cellulose and lignin by covalent and non-covalent bonds in the cell wall (Saha et al., 2003). The main difference of hemicellulose with respect to cellulose is that hemicellulose contains both homopolymers and branched heteropolymers with short lateral chains consisting of different sugars. This fraction varies from one vegetal species to another. The sugars that can be find in hemicellulosic fraction are: pentoses (xylose, rhamnose, and arabinose), hexoses (glucose, mannose, and galactose), and uronic acids (e.g. 4-omethylglucuronic, D-glucuronic, and D-galactouronic acids). Based on these sugars, hemicellulose can be classified as xylans, heteroxylans, galactomannan etc. Usually, the branches are linked to the backbone by β-(1,4)-glycosidic bonds and β-(1,3)-glycosidic bonds. Hemicelluloses can also have some degree of acetylation (e.g. in heteroxylan). Branched structure of hemicellulose id showed in Fig. 1.3. Conversely to cellulose, hemicellulosic polymers are characterized by low crystallinity index and can be more easily hydrolysate (Kumar et al., 2009). Lignocellulosic biomass usually contains 25-30% of hemicellulose (Mennon and Rao, 2012), with an average molecular weight of <30,000 (Anwar et al., 2014). Hemicellulose binds tightly to cellulose through non-covalent interactions on the surface of cellulose microfibrils (Anwar et al., 2014).

Lignin is a complex structure that usually represents the smallest fraction (about 15-20% of the biomass by weight) among the three major polymers in lignocellulosic material (Menon and Rao, 2012). Lignin is a long-chain, heterogeneous, polyphenolic polymer produced from enzymatic dehydrogenative polymerization of three phenyl-propanoid units: trans-coniferyl alcohol, trans-sinapyl alcohol and trans-p-coumaryl alcohol (Kumar et al., 2009). These units are commonly linked by ether bonds. Besides the three main units, lignin is also composed of methoxy groups and other polyphenolics, that are responsible of binding the cell walls components together (Anwar et al., 2014). Lignin is present in all plant biomass. The lignin purpose is two-fold: acting as a glue between and around carbohydrates polymers and make call walls very rigid structures. Even if ether bonds (C-O-C) are the majority, there are other strong covalent chemical bonds characterizing lignin structure, such as carbon-carbon bonds (C-C) and ester bonds (C-O-O-C). The presence of such strong bonds is responsible of the high resistance of lignin to structural/chemical modifications. Lignin structure was developed by plants for another important purpose: mechanism of defence against microbial attacks. Indeed, lignin not only gives rigidity to lignocellulose structure, but it is also a barrier that protects the carbohydrates from the hydrolytic action of cellulases released by hydrolytic microorganisms. Lignin directly interacts with carbohydrates (mainly hemicellulose) to create the lignin-carbohydrate complex (LCC). The covalent linkages between lignin and carbohydrates are mainly benzyl ether type, benzyl ester type and phenyl glycoside type (Anwar et al., 2014). Due to these bonds, more than 50% of lignin is covalently linked to carbohydrates. This complex limits many industrial applications of plants for instance hindering efficient enzymatic hydrolysis of biomass for biorefinery applications (You et al., 2015).

In general, the composition of lignocellulose highly depends on its source. Lignocellulosic biomass denotes wood and non-wood resources which differ in terms of their physical properties and chemical composition. The wood fibres often refer to hardwood (lignin content 18-25% DW) and softwood (lignin content 25-35% DW) (Kumar et al., 2009), and wood lignocellulosic biomass is physically larger, denser and structurally stronger than non-wood fibres. Moreover, the wood fibres can be harvested all-year round, which alleviates long-term storage issues. Chemically, even though it contains a large amount of lignin, its relatively low pentose (or xylose) content is an advantage for biofuel production from cellulose (Tye et al., 2016). Lignocellulosic biomass is produced from a variety of non-food crops, that include forestry (woody substrates), dedicated crops, municipal solid and other industrial wastes, agricultural residues.

Woody materials can be produced by forest or agriculture land. These materials are usually differentiated in hardwood and softwood. Hardwood is characterized by high density (heavy material) and slow growth rate. Conversely, softwood is a low-density material (lighter than hardwood) with a fast growth rate. Moreover, softwood are always evergreen trees; while hardwood are mostly deciduous trees (FAOSTAT). The main difference is the lignin content, that anyways is never less than 20% DW. The high lignin content is responsible for the strength of the trees, allowing them to reach important heights. A large issues spectrum should be considered for the selection of woody materials for biorefinery applications. The main issues are: land availability; growth time; competition of tree species with other purposes; transport costs; chemical/physical characteristics.

Perennial crops include grasses and tree characterized by fast growth rate and especially cultivated for bioenergy production. Example of perennial crops are giant reed (*Arundo donax*), miscanthus and switchgrass. These biomass are mainly cultivated in places like hilly areas, where the yield of food/feed crops (e.g. cereals) are very low and issues related to soil erosion can occur (Fagnano et al., 2015). These crops are able to reduce the soil erosion (Fagnano et al., 2015). Moreover, other lands not suitable for food/feed crops cultivation like polluted areas, are becoming interesting alternatives for cultivation of perennial crops since they act as phyto-remediating agents (Fiorentino et al., 2013).

Municipal solid and other industrial wastes. The composition of these feedstocks varies greatly according to the source. Papers and organic fractions are good examples of these biomass (Liguori et al., 2013). Great availability and low price are the main factors involving these biomass in the biorefinery processes. Moreover, their utilization as renewable resources will help to optimize the current improper management of these residues, reducing the environmental pollution.

Agricultural residues are waste materials obtained from the annual crops such as corn, wheat, rice and other crops grown for food and fiber. Stems and leaves of the plant are the main constituent of these residues. Examples are corn stover, wheat straw etc. Usually, these wastes are subjected to incineration or disposal to the soil or landfill, causing environmental concern. Seasonal production, great availability, chemical composition and low price are the main features of these biomass. Seasonal production is a drawback because it makes the availability of the biomass not constant over the year. However, this drawback could be balanced by the low costs of pretreatment (Guragain et al., 2011), making the whole biorefinery process more economically feasible.

Another variety of non-food biomass are **Agriculture food processing wastes (AFWs)**. These materials are lignocellulosic biomass produced during the processing of agro-raw materials for commercialization of agricultural products. AFWs could be valorised for recovery of compounds with potential application in biorefinery processes (Manara et al., 2015); indeed, they are composed of large amounts of organic valuable materials such as carbohydrate polymers (starch, cellulose and hemicellulose), proteins, lipids, lignin and organic acids (Ezejiofor et al., 2014). In recent years, AFWs gained importance thanks to novel characteristics that include renewability, recyclability and sustainability (Arevalo-Gallegos et al., 2017). This kind of bio-resource is identified as a viable alternative to the biomass investigated until now (and described above) for biorefinery applications. One of the main advantages of these materials is their origin sector. Indeed, agro-food sector is stable, resilient and robust. In 2014, the production of the sector was higher than any previous annual production since 2008. The EU food and drink industry turnover exceeded € 1.2 trillion in 2013 and value added reached € 206 billion in 2012. Table 1.3 reports the main features for EU agro-food sector.

Table 1.3. *Main features of EU agro-food sector (European Food and Drink Industry 2014-2015)*

TURNOVER	€ 1,244 billion
EMPLOYMENT	4.2 million people
EXPORTS	€ 91.7 billion
IMPORTS	€ 64.1 billion
EU MARKET SHARE OF GLOBAL EXPORTS	18%
NUMBER OF COMPANIES	289.000
VALUE ADDED	1.8% of EU gross value added
R&D EXPENDITURE	€ 2.8 billion

The economical data about EU agro-food sector reflect the high amount of raw agriculture products that are annually processed. Unfortunately, the commercialization of this products is based only on a portion of the entire raw material: this implies that a huge amount of wastes/residues are generated by raw material processing. For example, it is estimated that fruits and vegetable processing industries release 50% of the weight of raw materials as waste products (Ezejiofor et al., 2014). This means that AFWs are a remarkable fraction of lignocellulosic biomass and experimental evidences proved that they may be a viable option to produce marketable chemicals and/or biofuels, being economic and eco-friendly due to their unique chemical composition, great availability, sustainability and low costs (Sud et al., 2008). Due to their agriculture origin, the composition of AFWs depends on the nature of the products and environmental conditions of growth. Moreover, the composition of the same AFW could be different from one year to another. Processing technique can also affect the composition of AFWs, especially their moisture content. This feature is important to consider because higher the moisture content faster the decay of material with loss of nutrients (Ezejiofor et al., 2014). The food industry produces both solid and liquid wastes. An example of liquid waste is the "black water" from olives processing for oil production. This waste is rich in antioxidants and proteins. The managing of this kind of waste is of particular attention due to its potential environmental hazard (Al-Khatib et al., 2009). For these reasons, the exploitation of such a waste in biorefinery processes can produce a double benefit: production of bio-based chemicals and reduction of environmental pollution.

The majority of AFWs is solid. Cereals, fruits, vegetables and nuts processing generate solid residues in form of husks, hulls, chaffs, stalks, skins, peels, stones, fibres, piths, shells, coir (Ezejiofor et al., 2014). The current disposal management of these wastes is carried out in different ways: transportation to landfill; incineration; animal feed; used as soil conditioner and fertiliser. In many cases AFWs disposal is very costly. For example, it is estimated that in the USA are annually spent $10 million for disposal of apple processing residues (Shalini and Gupta, 2010). In these cases, the use of these residues to produce biofuels and/or value-added products in a biorefinery process is preferable (Dyk et al., 2013). Examples of AFWs are: exhausted pressed pulps (usually referred as "pomace") from juices production (e.g. apple, grapefruit, lemon, orange, etc.); peels (e.g. potatoes, tomatoes, banana, carrot, etc.); skins (e.g. onion); shells (e.g. nuts); processing waters (e.g. olive "black water"). As reported above, great seasonal availability and low costs are among the main features of these residues. Table 1.4 reports a description of some AFWs available in Europe, with the respective composition, availability and cost.

Table 1.4. *Summary of composition, market price and available amount of various AFWs in the European Union in 2016*

AFW	**Starch**[a]	**Cellulose**[a]	**Hemicellulose**[a]	**Lignin**[a]	**EU availability**[b]	**Market price at the factory**[c]
Brewers' spent grain	6-10	23-25	30-35	7-8	8	30-45
Potato peel	27	22	10	4	0.60	0
Spent coffee ground	0	9-12	37-39	24-32	1.6	0-30
Coffee silverskin	0	18-24	13-17	29-30	0.1	0
Vegetables	0	15-30	11-46	4-6	1.34	0-30
Fruits	0	7-27	2-12	0.8-3	0.10	0-30

[a] Dry weight percentage; [b] Mt / year; [c] € / wet ton

The very low price of AFWs (Table 1.4) is a pushing factor towards the use of this kind of residues to produce both energy and commodities. The price of the feedstock and the biorefinery concept are key factors in the new rising world of circular economy. Conversely to the linear fossil-based economy, circular economy is based on the 3Rs approach – reduce, reuse, recycling of wastes – aimed at development of a closed-loop system to recover as much as possible from the waste at the end-of-life (Liguori and Faraco, 2016). The circular economy model allows to prevent, minimize and valorise wastes (AFWs included), helping to solve the problems related to environmental pollution, landfills filling and fossil resources shortage (Liguori and Faraco, 2016). In the last years, the economic issue was a limitation for biorefinery platform development. Many technologies have been developed for the conversion of renewable resources, allowing to reduce the production costs of bio-based products. Today, the world is benefiting of these efforts; indeed, the final cost of biofuels (e.g. ethanol and biodiesel) is comparable (or only slightly higher) to the cost of fossil-based fuels (U.S. DoE, 2018). The major of these processes is based on the use of rich in sugars feedstock (first-generation biofuels). However, more studies are ongoing (Giacobbe et al., 2018; Procentese et al., 2018a) to develop efficient and profitable technologies to process the new class of AFWs, making them the bioresource to bet on for the next future.

1.3.2. Pretreatment processes

The development of an economically viable technology for bioconversion of cellulose into bio-products is made more difficult by different impediments such as association with lignin and hemicellulose, crystallinity, degree of polymerization and available surface area. Lignocellulosic biomass developed their chemical composition and structure to prevent microbial attacks: the result is that biomass can survive the attack of cellulolytic enzymes during biocatalytic conversion. A remarkable amount of these enzymes interacts with the residual biomass through non-productive bonds, decreasing their activity on the substrate. Lignin is one of the major components of most potential cellulosic substrates for bioconversion. Lignin removal or modification by effective and economically viable processes is the keystone for bioconversion of cellulose hidden in the complex lignocellulosic matrix.

Pretreatment processes represent the crucial step in the production of biofuels from lignocellulosic biomass (Menon and Rao, 2012). According to the biorefinery concept, pretreatment step is necessary to extract fermentable sugars from lignocellulosic biomass and convert them in final bioproducts. The goal of the pretreatment process is to remove lignin and hemicellulose, reduce the crystallinity of cellulose, and increase the porosity of the lignocellulosic materials (Kumar et al., 2009). Choice of pretreatment depends on raw material characteristics and process integration. Some attributes must be considered in order that pretreatment results balance against their impact cost on downstream processing steps and operational, capital and biomass costs. These attributes are: energy performance, operating environment, presence of inhibitors, a cost effectiveness, process integration and intensification (Bhutto et al., 2017). Various technologies are available for the pretreatment of biomass. These processes strongly affect performances of the successive enzymatic hydrolysis and microbial conversions. Some treatments can result in the formation of microbial inhibitors: furfural, hydroxymethyl furfural (HMF), and acetic, ferulic, glucuronic, ρ-coumaric acids (Ezeji et al., 2007b; Toquero and Bolado, 2014) that negatively affect the fermentation process. For this reason, attention must be paid at the inhibitor concentration at the end of the pretreatment step. Pretreatment methods can be divided into different categories: mechanical (milling and grinding), physicochemical (steam pretreatment/autohydrolysis, hydrothermolysis), chemical (alkali, dilute acid, oxidizing agents, and organic solvents), biological, or a combination of these (Kumar et al., 2009). Some of the most investigated pretreatment technologies are described in the following.

Mechanical pretreatments generally aim at modification of physical features of biomass. Combination of chipping, grinding, and/or milling can be applied to reduce the particle size of lignocellulosic material: the result is an increase of available surface area for enzymatic hydrolysis and decrease of cellulose crystallinity (Kumar et al., 2009).

Physicochemical pretreatments are carried out to disrupt the three-dimensional matrix of lignocellulosic biomass.

Steam explosion is the most exploited physicochemical pretreatment. In this method, biomass is treated with high-pressure saturated steam; then, the pressure is rapidly reduced to cause an explosive decompression that allows to damage the complex lignocellulosic matrix. Usually, the temperature range is of 160-260 °C (corresponding to a pressure range of 0.69-4.83 MPa), held for seconds or few minutes. The main result of the process is the hemicellulose solubilization. Lignin is only partly affected because reactions of depolymerization/polymerization can occur, redistributing the phenolic polymers on the surface of cellulose fibres. One of the main drawbacks of this process is the formation of inhibitors produced from sugars and lignin degradation occurring at high temperatures (Garcia-Aparicio et al., 2006; Kumar et al. 2009).

The *hydrothermal pretreatment* is carried out by using liquid water at high temperature and pressure. Hemicellulose solubilization and cellulose digestibility increase are the main results. Temperature of about 200 °C and pretreatment time of 15 min are the main features of the process. Remarkable amount of energy is required because of high pressures. Moreover, large amounts of water are consumed during the process.

Ammonia fibres explosion (AFEX) and *carbon dioxide explosion* are other physicochemical technologies with mechanism of action on lignocellulosic material similar to steam explosion. For both processes, the main advantage with respect to steam explosion is the lower processing temperature, even if longer pretreatment time are usually required (Kumar et al., 2009; Bhutto et al., 2017).

Chemical pretreatments are carried out by means of chemicals that allow to break down the chemical bonds of lignocellulosic material. Acidic or alkaline reagents are usually used in this type of process. Dilute sulfuric acid, dilute nitric acid, dilute hydrochloric acid, dilute phosphoric acid and peracetic acid have been employed for *acidic hydrolysis*. Dilute sulphuric acid pretreatment is one of the main used methods to improve lignocellulose hydrolysis. Dilute-acid pretreatment is typically carried out at temperatures higher than 160 °C with continuous-flow process for low solids loadings and temperatures lower than 160 °C with batch process for high solids loadings (Kumar et al., 2009). The main result of this pretreatment is the hydrolysis of hemicellulose to xylose and other sugars. Drawbacks related to this technology are: inhibitors production (e.g. furfural and HMF) and corrosive nature of the reagent that causes an increase of reactor maintenance costs (Bhutto et al., 2017). The main reagents used for *alkali pretreatment* are sodium hydroxide, ammonia, and calcium hydroxide. Concentrated solution of sodium hydroxide is widely used in the soda pulping and kraft pulping processes for the delignification of wood. Dilute NaOH treatment was shown to cause swelling, removal of lignin and hemicellulose, increase in surface area and decrease in the degree of polymerization. The alkaline hydrolysis mechanism is based on saponification of intermolecular ester bonds crosslinking xylan hemicelluloses and other components such as lignin. The mild operating conditions produce a high lignin solubility, especially for biomass with a low lignin content such as softwood and grasses. Furthermore, limited degradation of sugars to furfural, HMF and organic acids is founded (Kumar et al., 2009; Bhutto et al., 2017). Many organic solvents (ethanol, methanol, acetone, and ethylene glycol) have been used in *organosolv pretreatment* to dissolve the lignin from the biomass. The organosolv process allows to increase the enzymatic hydrolysis through delignification, but these solvents are costly and high-pressure equipment is also required. One of the advantages of this process is the possibility of solvent recycling, that is important for reduction of total cost and environmental impact. Solvent from the pretreated biomass must be removed to avoid possible solvent inhibition during enzymatic hydrolysis and fermentation (Anwar et al., 2014).

Biological pretreatments have attracted the attention of scientists thanks to their eco-friendly advantages: low-energy demand; no release of toxic compounds to environment and no effluent and fermentation inhibitors generation during the process (Bhutto et al., 2017). Microorganisms like brown, white and soft rot fungi are used in biological pretreatment to degrade lignin and hemicellulose polymers (Sindhu et al., 2016a). Fungi breakdown lignin anaerobically using family of extracellular enzymes collectively termed “lignases”. Important process parameters can affect the effectiveness of biological pretreatment: biomass type; incubation temperature (Millat et al., 2011); incubation time (Du et al., 2011); moisture content (Reid, 1989); type of microorganism (Sindhu et al., 2016a); aeration (Couto et al., 2002); inoculum concentration and particle size (van Kuijk et al., 2015). The main disadvantage of biological pretreatment is that it requires long incubation time for effective delignification. Moreover, enzyme yield is low if the process is not well optimized (Sindhu et al., 2016a). These issues can be overcome by using only the ligninolytic enzymes instead of the whole cell. The main advantages of using enzymes over whole cells include the possibility of using higher temperatures that could be not optimal for the growth of the microorganisms and high catalytic efficiencies of enzymes. However, there are some disadvantages which include high enzyme production costs despite the possibility of recombinant production of laccases and missed opportunity of using a combination of enzymes available in whole cells (Kudanga and Le Roes-Hill, 2014). Four major types of extracellular ligninolytic enzymes, namely laccase, manganese peroxidase, lignin peroxidase, and versatile peroxidase, are responsible for lignin degradation and lignin modification. Peroxidases are heme-containing glycoprotein which requires hydrogen peroxide as oxidant (manganese peroxidase requires also Mn^{2+}) (Masran et al., 2016). Unlike peroxidases, no oxidant agents are required by laccase, as they use molecular oxygen to catalyse the reaction reducing it to water, and water is the only by-product of the reaction (Piscitelli et al., 2011). Laccase (benzenediol oxygen oxidoreductase, EC 1.10.3.2) is a copper-containing phenol oxidase, which can oxidize electron-rich substrates of phenolic and non-phenolic origin with a concomitant reduction of oxygen to water through a radical-catalysed reaction mechanism. The broad substrate range of laccase allows the enzyme to be applied in many industries. Currently, the main applications are in the pulp and paper industry, textile industry and in bioremediation while application potential has been demonstrated for the food industry, pharmaceutical industry, organic synthesis, lignocellulose modification, biofuels production and diagnostic purposes (Kudanga and Le Roes-Hill, 2014).

Ultrasound-assisted pretreatment was also investigated in the last years. This method generates cavitational phenomena in the liquid medium: microbubbles are produced for a short time interval (microseconds); the collapse of microbubbles produces microjets, shockwaves and micro-convection that generate strong shear forces. These phenomena occur in local hotspots where high temperature (as high as 5000 °C) and pressure (up to 50 MPa) are achieved: radicals and others chemical species are produced during ultrasound treatment. All together, these phenomena damage the structural integrity of lignocellulosic biomass, providing the separation of structural polymers (cellulose, hemicelluloses and lignin). Moreover, the use of solvents can enhance the chemical effects of agents (Luo et al., 2014). The advantages (reduction of processing time, reduction of chemical loading, low energy requirements) and effects of ultrasound have attracted the attention of scientists to use this process for the pretreatment of lignocellulosic biomass (Bussemaker and Zhang, 2013).

1.3.3. Enzymatic hydrolysis

At present, two major ways are employed to hydrolyse the carbohydrates in lignocellulosic biomass (i.e. cellulose and hemicellulose) into simple fermentable sugars: chemical or enzymatic saccharification. The hydrolysis catalysed by acid reagents such as sulphuric acid, is a fast reaction requiring low residence time. Although a very fast reaction rate is achieved during chemical catalysed hydrolysis, many drawbacks are related to the use of this method: hydrolysis equipment corrosion, high operating temperature (energy consuming), non-recyclability of reagents (causing economic and environmental issue), fermentation inhibitors production (Maitan-Alfenas et al., 2015). Nowadays, biological conversion of lignocellulose is the main hydrolysis methods employed at industrial level because it allows to overcome the problems caused by chemical catalysed hydrolysis. Indeed, enzymatic hydrolysis does not require expensive corrosion resistant equipment; fermentation inhibitors production is almost completely avoided; mild operating conditions are required (es. temperature of 50 °C), decreasing the energetic cost of the process. The entire process of enzymatic hydrolysis depends on variety of factors, e.g. porosity (accessible surface area) of the biomass materials, crystallinity, pH, time, temperature and enzyme activities (Anwar et al., 2014). The presence of lignin and hemicellulose makes the accessibility of cellulase enzymes to cellulose more difficult, thus reducing the efficiency of the hydrolysis process. Pretreatment is required to alter the size and structure of the biomass, as well as its chemical composition, so that the hydrolysis of the carbohydrate fraction to monomeric sugars can be achieved rapidly and with greater yields. In the hydrolysis process, the sugars are released by breaking down the carbohydrate chains, before they are fermented.

The structure and configuration of lignocellulosic materials makes decomposition of cellulose and hemicellulose very difficult by action of an individual enzyme. The efficient degradation of biomass polysaccharides requires action of different proteins: cellulolytic and hemicellulolytic enzymes act in synergy to hydrolyse carbohydrate polymers of biomass into sugars (Menon and Rao, 2012). Synergism between enzymes reflects the degree of cooperation that two or more enzymes display in the degradation of a substrate (Van Dyk et al., 2013). Cellulases and xylanases are the main actors of carbohydrates depolymerization during enzymatic hydrolysis. Cellulases are the enzymes that hydrolyse β-1,4 linkages in cellulose chains. They are produced by fungi, bacteria, protozoans, plants, and animals. Cellulases contain non-catalytic carbohydrate-binding domain (CBD) to interact with the polymer. In nature, complete cellulose hydrolysis is mediated by a combination of three main types of cellulases: (1) endoglucanases (EC 3.2.1.4), (2) exoglucanases, including cellobiohydrolases (EC 3.2.1.91), and (3) β-glucosidase (EC 3.2.1.21). The action of all these enzymes under relatively mild conditions (50 °C and pH ~5) allows to release monomeric fermentable glucose from cellulose polymers in lignocellulosic biomass (Zhang and Zhang, 2013). Since hemicellulose is a polysaccharide composed of different sugars depending on the biomass source, a great number of different xylanases could be requested to completely hydrolyse the polymer. Some of these enzymes are here reported: endo-xylanases (EC 3.2.1.8), β-xylosidases (EC 3.2.1.37), mannanase (EC 3.2.1.78), xyloglucan hydrolases (EC 3.2.1.150, 151, 155), pectin lyases (EC 4.2.2.2, 6, 9, 10), alpha-L-arabinofuranosidases (EC 3.2.1.55), alpha-galactosidases (EC 3.2.1.22), alpha-glucuronidases (EC 3.2.1.139). High conversion degree, less environmental impact, cheaper utility costs and possibility to recover and reuse the enzymes (Mesa et al., 2015) are the factors making enzymatic hydrolysis a promising saccharification methods compared to chemical catalysed hydrolysis. However, 20% of the cost of the overall process of biomass bio-conversion through enzymatic hydrolysis is linked to the high cost of the enzymes employed (Stephen, Mabee and Saddler, 2012), that represents the main limitation to produce bio-based chemicals via enzymatic catalysis from lignocellulose at industrial scale.

1.3.4. Fermentation strategies

Carbohydrates of lignocellulosic biomass are hydrolysed to monomeric fermentable sugars by enzymatic cocktails during enzymatic hydrolysis step. C-6 (glucose, galactose, mannose) and/or C-5 (xylose, arabinose) sugars are produced. The fermentation of these sugars allows to produce biofuels (e.g. ethanol, biobutanol) and/or biochemicals (e.g. succinic acid) (see § 1.2. for description of bio-based products). Usually, different fermentation strategies can be applied. In *Separate Hydrolysis and Fermentation (SHF)* process, carbohydrates hydrolysis and fermentation occur in separate vessels: this strategy allows to carry out each process at their respective optimal conditions (e.g. pH and temperature). In *Simultaneous Saccharification and Fermentation (SSF)* process, the two steps of enzymatic hydrolysis and fermentation are performed in one reactor. In this case, the operating conditions in both type of reaction are comparable. Avoidance of product inhibition for cellulase, low cellulolytic enzymes requirement, and reduction of downtime are some of the advantages of SSF. The simultaneous fermentation of C-6 and C-5 sugars can be achieved through *Simultaneous Saccharification and Co-Fermentation (SSCF)* process: a single microorganism is employed to metabolize both types of sugar at the same time. With this strategy all sugars from enzymatic hydrolysis can be converted in valuable products, without any sugar waste. In the last years, a new strategy called *Consolidated BioProcessing (CBP)* is gaining attention. With this process, enzyme production, enzymatic hydrolysis and fermentation steps, are performed by a single microorganism or group of microorganisms operating at the same conditions in one reactor. No external enzyme additions are required. Usually, genetically modified microorganisms (GMOs) are used. Reduction of enzyme costs, microbial contamination and downtime are the main advantages of this process.

Enzymatic hydrolysis and fermentation processes are also affected by the bioreactor system configuration adopted. Many studies focused on the application of different bioreactors in a biorefinery process both for SHF and for SSF. Stirred tank bioreactor was employed for enzymatic hydrolysis of *Arundo donax* (Palmqvist and Lidén, 2012; Kadić et al. 2014) and corn stover (Kadam et al., 2004); continuous twin-screw extruder was used for pretreating *Miscanthus* (Han et al., 2014); rotary drum reactor was used for SSF of cogon grass (Lin and Lee, 2011) and sugarcane bagasse (Lin et al., 2013). Reactors used at industrial scale include bubble columns. Bubble column reactors belong to the general class of multiphase reactors. A bubble column reactor is basically a cylindrical vessel with a gas distributor at the bottom. The bubbles of gas are sparged into either a liquid phase or a liquid–solid suspension; when a solid phase exists, these reactors are generally defined as slurry bubble column reactors (Shaikh and Al-Dahhan, 2013). These reactors are used in many chemical processes involving reactions such as oxidation, alkylation, polymerization, synthesis of fuels by gas conversion and biochemical processes as fermentation (Kantarci et al., 2005). The use of bubble columns as bioreactors was investigated in many fermentative/enzymatic processes to produce a wide spectrum of by-products, e.g. acetic acid (Sun and Furusaki, 1990), taxol (Son et al., 2000), ethanol (Ogbonna et al., 2001), gluconic acid (Ramezani et al., 2013). Recently, Mulakhudair et al. (2016) investigated the application of bubble column for the pretreatment of lignocellulosic biomass. The success of bubble column reactor in several fields is due to the advantages of this system with respect to other reactors: simple design, excellent heat and mass transfer, low operating costs because the absence of moving parts (Kantarci et al., 2005).

2. AIM OF THE THESIS

The research carried out during the Ph.D. program aimed at investigating the use of agro-food wastes (AFWs) to produce fermentable sugars and/or value-added bioproducts according to the biorefinery approach. The activities were focused on two aspects: i) to promote the release of sugars (pentoses and hexoses) from cellulose and hemicellulose to be used in fermentative processes for biofuels and/or biochemicals production (e.g. biobutanol and succinic acid); ii) to investigate the presence of inhibitory compounds and/or value-added bioproducts possibly produced during pretreatment/hydrolysis steps. The work was carried out at the Dipartimento di Ingegneria Chimica, dei Materiale e della Produzione Industriale of the Università degli Studi di Napoli *Federico II* and at the "University of Western Ontario (UWO)" Canada.

During the PhD period the activities regarded:

- optimization of the pretreatment assisted by ultrasounds or lignocellulosic enzymes;
- the optimization of a lab-scale bubble column reactor to combine pretreatment and hydrolysis of selected biomass in the same unit;
- the application of the investigated technologies with reference to two agro-food wastes (coffee silverskin and apple pomace) arranging the study within the circular economy concept;
- the production of two fermentation products (butanol and succinic acid) as reference products of the biorefinery.

Coffee silverskin as feedstock for biorefinery applications

Coffee silverskin (CSS) are the residual thin teguments wrapping and protecting the external layer of green coffee beans. It is produced during the roasting phase of green coffee beans. Dried CSS is mainly composed of carbohydrates (40% w/w) and lignin (30% w/w). However, the composition can change because it is affected by the coffee plant species, soil characteristics like nutrients and water availability, and weather conditions. The main issues regarding the CSS supplying are:

- the global production of coffee beans is about 160 million of 60 kg bags;
- the CSS is about 4.2 % (w/w) of the coffee beans;
- the roasting factories are spread in the world.

These issues make CSS - a process residue - a potential candidate to be used as feedstock for biorefineries.

The study of CSS was aimed at investigating pretreatment, enzymatic hydrolysis and fermentation of this residue.

A combination of mild alkaline solution and ultrasound (US) was applied to pretreat CSS. Four parameters were investigated: biomass loading, sonication time, alkali concentration, residence time in autoclave. The response surface methodology approach was adopted to set the operating conditions. The process was characterized in terms of sugar yield after the enzymatic hydrolysis of pretreated CSS. The operating conditions that gave the best results of sugar yield were selected for the successive tests of enzymatic hydrolysis and fermentation.

The enzymatic hydrolysis investigation was addressed to investigate the effects of biomass loading and cellulolytic enzymes loading on sugars released from alkali-pretreated CSS. The rich-in-sugars hydrolysate obtained after pretreatment and enzymatic hydrolysis of CSS was supplemented with nutrients and used as media for fermentation of *C. acetobutylicum* and *A. succinogenes*, for the production of solvents (acetone-butanol-ethanol) and succinic acid, respectively. Batch tests were carried out with the aim to highlight the capability of sugars from CSS to be fermented by different microorganisms to produce marketable bio-products.

Apple pomace as feedstock for biorefinery applications

Apple is a fruit widely produced and eaten all over the world. In Italy, more than 2.5 million tons of apple are produced every year. Worldwide, 20 % of fresh apple is processed into value added products: apple juice concentrate (65%), apple cider, wine, vermouth, purees, jams and dried apple products. The main residue of these processes is the apple pomace. This residue accounts for 25-35% of the dry mass of apple. The apple pomace is a good lignocellulosic candidate to be used in a biorefinery process, and it was already used as feedstock for production of butanol, ethanol and crude protein (or enzymes), citric acid, microbial colours, bio-hydrogen.

The study of apple pomace was aimed at investigating the possibility to carry aout pretreatment - by means of the ligninolytic enzyme “laccase” – and enzymatic hydrolysis in the same multi-phase reactor.

The proposed pretreatment/hydrolysis system aimed at the optimization of operating conditions affecting the process in terms of amount of sugars released during the whole process. The first stage regarded the optimization of pretreatment parameters (i.e. biomass loading, laccase loading and gas (air) flow rate). The second stage regarded operating conditions to optimize the effect of cellulolytic enzyme loading and gas (air) flow rate on the enzymatic hydrolysis.

A lab-scale bubble column was selected as reactor.

Acetone-Butanol-Ethanol (ABE) fermentation at University of Western Ontario (UWO) (Canada)

The research activity was focused on the ABE fermentation of a corn industrial product: corn syrup. The objectives were: i) to screen four *Clostridium* species to identify the microorganism characterized by the best fermentative performance in terms of solvents production, yield and productivity; ii) to study the effects of substrate/product concentration on fermentation process (substrate/product inhibition effects); iii) to increase solvents production by means of a fed-batch process.

The final aim of this project was to investigate the possibility of using corn syrup to produce solvents by ABE fermentation. Regarding the main topic of my Ph.D. project, the work carried out at UWO have had a double purpose: 1) to improve expertise in anaerobic fermentative processes of different *Clostridium* species; 2) to compare the performance of *Clostridium spp*. using agro-industrial products as sugar-carbon source. Two case studies have been selected: corn syrup (sugar-rich stream from food industry) and coffee silverskin (lignocellulosic waste from food industry). The critical issue about the fermentation of these two feedstock regards their fermentability: corn syrup is a sugars mixture produced by simply hydrolysing corn; coffee silverskin is a lignocellulosic biomass and requires pretreatment before the enzymatic hydrolysis because the presence of lignin polymers. As a matter of fact, the latter feedstock is more recalcitrant than corn to fermentation.

Organization of the result sections of the PhD Thesis.

The section 3 reports on the pretreatment and exploitation of CSS. The focus was mainly on the biomass pretreatment methods and fermentation of hydrolysed sugars.

The section 4 reports on the pretreatment and exploitation of apple pomace. The focus was mainly on biomass pretreatment strategy, enzymatic pretreatment/hydrolysis and fermentation of hydrolysed sugars.

The appendix reports the results regarding the research carried out at the University of Western Ontario (UWO) (Canada).

3. COFFEE SILVERSKIN AS BIOREFINERY FEEDSTOCK

This section focused on the use of coffee silverskin (CSS) as new feedstock for biorefinery developments. The first part (§ 3.1) reports on the work carried out about the optimization of combined alkaline-ultrasound pretreatment of CSS. The study was aimed at characterizing the effect of the operating conditions of pretreatment on sugar yield, phenolics release and inhibitors production. The second part (§ 3.2) focused on maximixation of sugars recovery through the enzymatic hydrolysis of akali pretreated CSS and their subsequent fermentation to produce solvents and biochemicals.

3.1 Combined pretreatments of coffee silverskin to enhance fermentable sugar yield

Saverio Niglio[1], Alessandra Procentese[2], Maria Elena Russo[2], Giovanni Sannia[3], Antonio Marzocchella[1]

[1] Dipartimento di Ingegneria Chimica dei Materiali e della Produzione Industriale, Università degli Studi di Napoli Federico II, P.le V. Tecchio 80, 80125 Napoli
[2] Istituto di Ricerche sulla Combustione - Consiglio Nazionale delle Ricerche, P.le V. Tecchio 80, 80125 Napoli
[3] Dipartimento di Scienze Chimiche, Università degli Studi di Napoli Federico II, Complesso Universitario di Monte Sant'Angelo, Via Cinthia, 80126 Napoli

Abstract

Lignocellulosic biomass is the most abundant natural and renewable resource on the Earth. A huge amount of lignocellulosic biomass is produced as waste by agricultural practices and by agro-industry processes. Agro-food processing wastes (AFWs) are a potential feedstock for biorefinery: the exploitation of the several biomass components. Pretreatment methods are required to promote the enzymatic hydrolysis of AFWs for the recovery of monomeric fermentable sugars. AFWs include coffee silverskin (CSS): a residue produced during the coffee beans roasting process. CSS is recalcitrant to enzymatic and microbial attacks, reducing the interest of CSS as feedstock in biorefinery applications. This study reports the characterization of a pretreatment process based on the combination of ultrasound and mild alkaline solutions. For the effective separation of lignin and cellulose. The effects of sonication time, biomass loading, sodium hydroxide concentration and residence time in the autoclave were investigated. The operating conditions were selected according to the Response Surface Methodology. The operating conditions providing the maximum reducing sugar yield (0.6 g/g) were: 5 min sonication, 11% w/v biomass loading, 5% w/v NaOH, and 75 min autoclave. Analysis of the liquid phase after pretreatment pointed out that fermentation inhibitors – e.g. furfural, HMF, ferulic and p-coumaric acid - were absent or present at non-toxic concentrations for various *Clostridium* sp. The phenolic content was 25 mg_{GAE}/g_{raw_CSS}. SEM and XRD analysis provided the changes in biomass structural features after the pretreatment process.

Keywords Biorefinery, Lignocellulose, Coffee silverskin, Ultrasound, Alkaline pretreatment, Phenolic compounds.

3.2 Coffee silverskin as feedstock for butanol and succinic acid production

Saverio Niglio[a], Alessandra Procentese[b], Maria Elena Russo[b], Giovanni Sannia[c], Antonio Marzocchella[a]

[a] Dipartimento di Ingegneria Chimica dei Materiali e della Produzione Industriale, Università degli Studi di Napoli Federico II, P.le V. Tecchio 80, 80125 Napoli
[b] Istituto di Ricerche sulla Combustione - Consiglio Nazionale delle Ricerche, P.le V. Tecchio 80, 80125 Napoli
[c] Dipartimento di Scienze Chimiche, Università degli Studi di Napoli Federico II, Complesso Universitario di Monte Sant'Angelo, Via Cinthia, 80126 Napoli

Abstract

Coffee silverskin is an agro-food waste obtained from the roasting phase of green coffee beans. It is the only by-product produced in the roasting process. Very few studies focused on the use of CSS as biorefinery feedstock. In this work, biomass loading and cellulase concentration were the operating conditions investigated to maximize the sugar release during enzymatic hydrolysis of alkali-pretreated coffee silverskin. 10 %w/v biomass loading gave the best results in terms of sugar concentration and sugar yield. Curve fitting of experimental data was performed: increasing of hydrolysis rate and sugar concentration was observed increasing cellulase concentration. 10 %w/v biomass loading and 80 FPU/$g_{cellulose}$ enzyme loading were applied to produce the hydrolysate to use as the medium for fermentation tests: the sugars from coffee silverskin were the only carbon source. 2.2 g/L butanol and 20.8 g/L succinic acid were produced by two different microorganisms.

Keywords: Biorefinery, coffee silverskin, enzymatic hydrolysis, fermentation, biofuels, biochemicals.

4. APPLE POMACE AS BIOREFINERY FEEDSTOCK

This section focused on the enzymatic pretreatment and hydrolysis of apple pomace. A bubble column bioreactor system was adopted to carry out these activities. The main operating conditions (biomass loading, enzyme loading, air flow rate) that affect the process were investigated. A sequential pretreatment/hydrolysis process of apple pomace in the bubble column was proposed.

4.1 Integrated enzymatic pretreatment and hydrolysis of apple pomace in a bubble column bioreactor

Saverio Niglio[a], Alessandra Procentese[b], Maria Elena Russo[b], Alessandra Piscitelli[c,d], Antonio Marzocchella[a]

[a] Dipartimento di Ingegneria Chimica dei Materiali e della Produzione Industriale, Università degli Studi di Napoli Federico II, P.le V. Tecchio 80, 80125 Napoli, Italy
[b] Istituto di Ricerche sulla Combustione - Consiglio Nazionale delle Ricerche, P.le V. Tecchio 80, 80125 Napoli, Italy
[c] Dipartimento di Scienze Chimiche, Università degli Studi di Napoli Federico II, Complesso Universitario di Monte Sant'Angelo, Via Cinthia, 80126 Napoli, Italy
[d] Biopox srl, Via Salita Arenella 9, 80129 Napoli, Italy

Abstract

Waste biomass form agro-food industry can be transformed in different products according to the sugar-based biorefinery concept. The present work is focused on the use of apple pomace as possible renewable feedstock for the production of second-generation biofuels and bio-commodities. The aim of this work is the optimization of the enzymatic pretreatment and hydrolysis of apple pomace in a bubble column bioreactor using recombinant laccase as biocatalyst for the delignification step and commercial cocktail of cellulases for the hydrolysis step. To this aim, a recombinant laccase from *Pleurotus ostreatus* was adopted. Both the enzymatic processes (delignification and cellulose hydrolysis) were investigated in order to select the optimal operating conditions in terms of biomass loading, air flow rate, laccase concentration, and cellulase concentration. Results showed that 15 %w/v biomass loading, 60 nL/h air flow rate, 30 U/$g_{biomass}$ laccase activity loading, and 20 FPU/$g_{cellulose}$ cellulase activity loading were the optimal conditions for the maximization of glucose yields of the sequential enzymatic pretreatment and hydrolysis process in the bubble column. Moreover, the selected operating conditions were applied to a long term test of the sequential pretreatment/hydrolysis in the bubble column. The test resulted in a maximum sugar yield of 0.34 $g_{sugars}/g_{Raw\ biomass}$ that is 61% ($g_{sugars}/g_{sugars\ available}$) of the theoretical sugar yield obtainable from the raw apple pomace. Results discussion highlighted advantages and drawback of pneumatic mixing and sequential pretreatment/hydrolysis approach applied to enzymatic delignification and cellulose hydrolysis.

5. DISCUSSION

Petroleum has been used for many years as the esclusive source to produce fuels, chemicals and materials. Nowadays, the global demand for the petroleum-based products is still increasing due to the world population growth. Even if the petrochemical route to produce energy and commodities is very competitive from economic and efficient point of views, the research on alternative renewable resources is mandatory. Indeed, the awareness about the long-term depletion of fossil resources, the opportunity to develeop an economy based on regional resources and the global climate changes caused by CO_2 emissions have been responsible for the shift of the scientific community attention from a fossil-based to a bio-based world. Bio-based processes are still in a developing phase and they require innovative solutions to support the success of the biorefinery route.

Various biofuels and chemicals can be produced via biotechnological processes using biological material as feedstock (biomass) and microorganisms as biocatalysts. The innovative solutions to produce bio-based compounds regard the optimization of the whole process, from the selection of the feedstock to the downstream process.

The selection of the feedstock is a key issue in a biorefinery process. In the last years, different feedstock were proposed to produce both biofuels and biochemicals. Biomass like corn and sugarcane were highly employed in the processes of the “first generation biorefinery”, but the competition of these resources with human/animal food forced the scientists to focus on a different bio-resource: lignocellulosic biomass. The composition of these biomass allows to produce both sugars (from carbohydrates) and chemicals (from lignin or sugar fermentation) by processing the feedstock according to the biorefinery concept. Among lignocellulosic biomass, agro-food wastes (AFWs) seem to be a valuable option to produce both biofuels and biochemicals. AFWs are: available in large amount (seasonal availability), sustainable and cheaper than other biomass (e.g. corn). The negative aspect of using these resources for biorefinery applications is the need of pretreatment processes to modify and/or remove the lignin fraction and make the carbohydrates more accessible for the successive step of enzymatic hydrolysis.

This study focused on the use of AFWs to support biorefinery developments. Coffee silverskin and apple pomace were the feedstock subjected to the pretreatment and enzymatic hydrolysis processes to extract both sugars (to use in fermentative processes) and value-added molecules.

5.1 Pretreatment of coffee silverskin for biorefinery applications

The production of second-generation biofuels and biochemicals depends on the exploitation of all the components of lignocellulosic biomass. These materials have been extensively investigated for biorefinery applications (Isikgor and Becer, 2015). In the last years, agro-food wastes (AFWs) - a class of lignocellulosic biomass – have attracted the international attention as a new feedstock for bioproducts production (Lin et al., 2013).

Coffee silverskin (CSS) is an AFWs obtained during the roasting phase of green coffee beans. These residues have no special use and they are usually delivered to disposal or burned. The main components of this residue are carbohydrates (holocellulose) and lignin (Ballesteros et al., 2014). As a lignocellulosic biomass, pretreatment processes are required to make the carbohydrates more available to the enzymatic hydrolysis. Since this feedstock is relatively new, only few works on pretreatment of this residue are available in literature. Procentese et al. (2018a) carried out pretreatment of CSS using deep eutectic solvents (DES) to remove the lignin fraction and to recovery antissoidants. A lignin content reduction from 30 %w/w (raw biomass) to 19 %w/w was observed when CSS was pretreated at high temperatures (150 °C); while cellulose content was increased from 17.5 to 33 %w/w. Giacobbe et al. (2018) carried out pretreatment of CSS using ligninolytic enzyme laccases. They obtained 48% lignin reduction after pretreatment (24 h) with a laccase-mediator system (LMS) using vanillin as natural mediator.

Results regarding pretreatment of CSS by combining ultrasound and mild alkaline solution (US-AA) have been reported in the present PhD thesis (§ 3.1).

The operating condistions under which to carry out tests were set according the Response Surface Methodology (RSM) taking into account the industrial potential conditions. The strategy was also addressed to identify the operating conditions charcaterized by the best process performance in terms of sugar yield. The lignin content reduction from 30.5 to 27.9 %w/w and the cellulose content increase from 20.9 to 28 %w/w was measured under the best operating conditions assessed. These results would suggest that the US-AA pretreatment is not characterized by the excellent performance assessed by using DES and laccases. Although the differences in the compositional analysis were marked, the difference in sugar yield assessed after enzymatic hydrolysis of pretreated CSS was not marked. The saccharification yield with US-AA pretreatment was of 61% g/g while that for laccases and DES pretreatment were 73 and 88% g/g, respectively (the yield of DES pretreatment only refers to glucose). This result become more significant when the operating conditions of the pretreatments are compared. The pretreatment proposed by Giacobbe et al. (2018) took very long time (24 h) and required the supplement of a mediator (vanillin). The pretreatment proposed by Procentese et al. (2018a) took just 3 h but a high amount of solvents and high temperature (150 °C) was required. The US-AA pretreatment was carried out at room temperature for 5 min (sonication step) and at 120 °C for 1h (alkaline hydrolysis step). Procentese et al. (2018a) also tested the effects of temperatures lower than 150 °C, but in this case the biomass composition obtained was similar to that reported for the US-AA pretreatment. Moreover, a lower sugar yield (35% g/g) was obtained carrying out DES pretreatment at 115 °C with respect to that reported in this Ph.D. thesis (§ 3.1).

CSS contains polyphenols that can be valuable resources for application in food, cosmetic, and pharmaceutical areas (Conde and Mussatto, 2016). Some publications are available in literature about the extraction of polyphenols from CSS. Machado et al. (2012) used different fungal strains to release phenolic compounds from CSS under solid state cultivation conditions. They were able to obtain 3.47 mg/g as maximum amount of phenolics. Conde and Mussatto (2016) carried out hydrothermal pretreatment of CSS (120 °C for 20 min) and obtained 19.17 mg/g of polyphenols. In both cases, the amount of polyphenols obtained was lower than that extracted by US-AA pretreatment described in this thesis: 25 mg/g total phenolic compunds (§ 3.1) were assayed. Higher concentration of phenolic compounds was reported by Narita and Inouye (2012) but for an energivor hydrothermal pretreatment (210 °C for 10 min).

Considering both the results of sugar yield and polyphenols extraction, the US-AA pretreatment proposed in this thesis seems to be a viable option to process CSS according to a biorefinery concept. Moreover, the comparison with research in the same field suggests that the results reported in the present investigation are encouraging when the operating conditions are taken in account.

From an industrial point of view, the scalability of the ultrasound process is not easy. Considering that the contribution of the US to the increment of final sugar yield and phenolics concentration is not remarkable, it could be intriguing to stop the CSS pretreatment just at the alkaline hydrolysis.

5.2 Enzymatic hydrolysis and fermentation of coffee silverskin

Enzymatic hydrolysis and fermentation are key steps of a biorefinery process along with pretreatment process. The present study (§ 3.2) shows the results of sugar concentration maximization and fermentation of alkali-pretreated CSS. As expected, both biomass loading and enzyme loading affected the final sugar concentration and sugar yield. At the end of enzymatic hydrolysis, 36.7 g/L of total sugar were obtained with a biomass and enzyme loading of 10 %w/w and 80 FPU/$g_{cellulose}$, respectively. Mussatto et al. (2012) and Procentese et al. (2018b) reported results for hydrolysis of CSS carried out according to different hydrolysis strategies: 20 g/L of total sugar and 20 g/L of glucose were reported, respectively. Mussatto et al. (2012) carried out acid hydrolysis of raw CSS; Procentese et al. (2018b) carried out enzymatic hydrolysis of alkali-pretreated CSS. As a result of the comparison, the highest sugar concentration was provided by applying the strategy proposed in the present thesis (§ 3.2). This promising result was due to the alkaline pretreatment and of hydrolysis strategies adopted. In the present work, the enzyme amount for unit of biomass weight was set quite high (80 FPU/$g_{cellulose}$) to extract as much sugars as possible to improve the subsequent fermentation step. On the contrary, Procentese et al. (2018b) set the amount of enzyme for unit of biomass weight quite low (1 FPU/$g_{cellulose}$) because the aim was only to carry out preliminary experiments on the fermentability of the sugars derived from CSS; Mussatto et al. (2012) carried out acid hydrolysis without a pretreatment step to reduce operating costs by producing a degradation of available sugars for the very harsh conditions of hydrolysis.

The sugars of the CSS hydrolysate produced in the present work were used as carbon source for two fermentation process: ABE fermentation by using *C. acetobutylicum* and succinic acid fermentation by using *A. succinogenes*. The results highlighted that CSS hydrolysate was successful in succinic acid fermentation: a final concentration of 20.8 g/L of the acid was obtained, corresponding to a yield of 84 % g/g, that is among the highest yield reported in literature for succinic acid production from biomass hydrolysates. ABE fermentation was less performing than succinic acid production. Indeed, only 3.2 g/L ABE were produced. The relatively low concentration of solvents obtained was probably due to the specific microorganism used and to the use of a not optimized medium. In these experiments, *A. succinogenes* was able to carry out a better fermentative performance with respect to *C. acetobutylicum* even without using an optimized medium.

Only few CSS fermentations are reported in literature. Mussatto et al. (2012) used CSS hydrolysate from acid hydrolysis to carry out fermentation with different yeast strains able to produce ethanol: 12 g/L ethanol were produced by *S. cerevisiae* from a 20 g/L (total sugars) hydrolysed. Procentese et al. (2018b) investigated the potentiality of CSS hydrolysates as carbon source for production of butanol and isopropanol through fermentation carried out by *Clostridium beijerinckii* DSM 6423. Butanol and isopropanol concentration of 4.4 and 2.2 g/L was obtained, respectively.

The fermentation evidences support that the sugars contained in CSS hydrolysates may be a good option to substitute or integrate the synthetic media used in fermentation processes to produce both biofuels (e.g. butanol and ethanol) and biochemicals (e.g. succinic acid). In particular, the results reported in § 3.2 about the succinic acid production from CSS hydrolysates are very promising and are the basis for further investigations and improvements.

Altogether, CSS proposes as a sustainable option for biorefinery applications. Indeed, a portfolio of molecules can be recovered/produced from this residue: fermentable sugars, lignin, solvents (e.g. butanol, ethanol, isopropanol) and biochemicals (phenolic compounds and succinic acid).

5.3 Pretreatment and enzymatic hydrolysis of apple pomace

The great availability and the chemical composition of apple pomace make this residue an interesting feedstock for biorefinery processes. Apple pomace is the by-product obtained from the processes aimed at producing apple juice, apple cider, wine, purees, jams from raw apples. This AFW is mainly composed of carbohydrates and soluble sugars (< 50 %w/w), with a relative low amount of lignin (17 %w/w). The use of apple pomace for biorefinery applications has been reported in the literature. In particular, it was used in processe for the production of: bio-butanol (Voget et al., 1985; Hijosa-Valsero et al., 2017; Maiti et al., 2018); ethanol and crude protein (or enzymes), citric acid, microbial colours (Joshi and Attri, 2006); bio-hydrogen (Feng et al., 2010); polyhydroxyalkanoate (Urbina et al., 2018). In the present study (§ 4.1), enzymatic pretreatment of apple pomace by means of the ligninolytic enzyme laccases was carried out. Both enzymatic pretreatment and hydrolysis were carried out in a bubble column bioreactor. The success of bubble column reactors is due to sevral advantages: simple design, excellent heat and mass transfer, low operating costs because the absence of moving parts (Kantarci et al., 2005).

The whole process of enzymatic pretreatment and subsequent hydrolysis in the bubble column was successful. A lignin reduction of 16% was obtained after 24 h pretreatment. The combined process of enzymatic pretreatment and hydrolysis of apple pomace in a bubble column allowed to produce a hydrolysate characterized by a sugar concentration of about 52 g/L. The lignin reduction is comparable to that reported by Giacobbe et al. (2018) by carrying out enzymatic pretreatment of apple pomace in shaken flasks. Surprisingly, a lower amount of laccases for unit of biomass weight was required by the pretreatment in bubble column with respect to the process proposed by Giacobbe et al. (2018). Procentese et al. (2018a) carried out pretreatment of apple pomace by means of deep eutectic solvents: the lignin content decreased from 18.5 %w/w to 7 %w/w after three hours of pretreatment at 150 °C. Although the delignification reported by Procentese et al. (2018a) was higher than that obtained in the present study, the energy/biomass request of the proposed processe is expected to be low: low temperature (28 °C) was set for the pretreatment in the bubble column, the fraction of the biomass recovered after the pretreatment step was larger in the bubble column.

No fermentation inhibitors (furfural and HMF) were detected a rich-in-sugars solutions recovered after the pretreatment/hydrolysis process in the bubble columns. Threfore, the produced hydrolysates could be a valid source for fermentative processes. Indeed, the overall process pretreatment-hydrolysis-fermentation may be considered environmentally friendly when compared with processes that requires high amount of energy and/or chemicals.

The main advantage of the combined process is the opportunity to carry out all steps in the bubble column reactor.

- No moving parts are present and the mixing is provided by the bubble flow.
- The temperature required to maximize laccases activity is about 28 he and may easily controlled.
- The water consumption is drastically reduced because the same buffer solution (pH 5) can be used for both processes of enzymatic pretreatment and hydrolysis. No washing step is required for the biomass at the end of the pretreatment.
- The fermentation step may be potentially carried out in the same reactor provided the change of the atmosphere.

The low energy requirements, the absence of chemicals, and the water saving are key feature to promote this process as a “green process”.

5.4 General considerations

The development of the biorefinery approach to produce energy and commodities is experiencing an exponential phase since the beginning of this decade. Nowadays, it is well known that the chemical components of biomass can be used to produce biofuels and biochemicals derived from fossil resources. The processes based on the exploitation of biomass vs. fossil resources are the symbol of the future on which humanity must bet. This is the main strategy to reduce our negative impact on Earth and many efforts are ongoing worldwide to accelerate the shift from a petroleum-based economy in favour of a bio-based economy. AFWs are emerging as a potential option to be used in biorefinery processes. These residues are available all the year and they are not in competition with the cultivated land and fresh water dedicated to food/feed production. Another advantage of AFWs is their very low price. The cost of substrate is a key feature for the economic success of an industrial process. In many cases, companies must pay to dispose AFWs: the recycle through a biorefinery plant could reduce the disposal costs, favouring the development of a bio-based circular economy. Although the main steps of biorefinery processes are still under investigation to be optimized, the literature is full of examples of pretreatment, hydrolysis and fermentation of lignocellulosic biomass carried out at industrial scale.

The present study proposed a lab-scale biorefinery process for two AFWs: coffee silverskin and apple pomace. Coffee silverskin was pretreated coupling ultrasound and mild alkaline pretreatment; apple pomace was pretreated by ligninolytic enzymes (laccases). Both biomass were subjected to enzymatic hydrolysis after the pretreatment step. The results showed that for both AFWs a rich-in-sugar hydrolysate to be used in fermentative processes was obtained. A large amount of polyphenols was extracted from CSS during the pretreatment step. Moreover, the fermentation of CSS hydrolysate allowed to produce more than 20 g/L of succinic acid.

The innovative aspect of the pretreatment of apple pomace was the use of a bubble column as bioreactor to carry out both pretreatment and enzymatic hydrolysis processes and potentially the fermentation step. This was one of the first attempts aimed at carrying out a biorefinery process in bubble column. The positive results obtained should encourage to follow and optimize this “green” strategy for the biorefinery of others different biomass.

It is important to highlight that the solid residue at the end of the enzymatic hydrolysis was mainly composed of lignin (in particular when CSS was used). According to the biorefinery approach, this residue is a resource that could be recovered and processed to produce chemicals or solid fuel (in this last case the lignin fraction should be used to produce energy for the industrial plant). This strategy is already applied at industrial level (e.g. Renmatix Inc. in USA; Sekab in Sweden).

The results reported in the Appendix section regarding the anaerobic fermentation of corn syrup allow to carry out a partial comparison among the fermentative performances of *Clostridium spp.* on media composed by sugars derived from agro-products with various degrees of difficulty to ferment. *C. acetobutylicum* was used to ferment corn syrup and CSS hydrolysate. This microorganism showed better results in ABE production when the CSS hydrolysate was used as only carbon source for the fermentation process (Chapter 3.2), even if its sugars concentration was less than sugars concentration in corn syrup media (Annex 2). This result could be due to the different mix of sugars that characterize the two media. While other authors already reported that *C. acetobytylicum* is not a good choice to ferment molasses (Shaheen et al., 2000), CSS hydrolysate was mainly composed of glucose that is quickly metabolized by *C. acetobutylicum* (Raganati et al., 2015). Moreover, being the sugars concentration of CSS hydrolyzate less than that in corn syrup media, the possible inhibitory effect was avoided. At the same time, other species of *Clostridium*, gave good ABE results from fermentation of corn syrup media. This means that other *Clostridium spp.* could be tested on CSS hydrolysate to improve ABE production because, as reported in Chapter 3.2 and Annex 2, different microbial species can give different fermentative results using the same colture media.

Regarding apple pomace, no fermentative data are still available from the work reported in this thesis. However, other authors reported about the anaerobic fermentation of apple pomace. Hijosa-Valsero et al. (2017) carried out fermentation of apple pomace hydrolysate by *C. beijerinckii*. The sugars concentration of the apple pomace hydrolyzate was similar to that of corn syrup media (Annex 2) (42 g/L and 44 g/L, respectively) and similar ABE concentration and sugar consumption were obtained (about 12 g/L and > 90%, respectively). This comparison highlights the great potentialities of apple pomace to be used in biorefinery processes. This kind of feedstock (CSS and apple pomace) should be preferred for biorefinery developments because its use as second-generation feedstock implies various advantages with respect to the use of first-generation feedstock (e.g. corn) as extensively described in the Chapter 1 of this thesis.

Altogether, results reported in this thesis underline the potentialities of AFWs to guide the shift from a fossil-based to a greener bio-based world.

The main aim of this Ph.D. thesis was to maximize the sugar recovery from the AFWs investigated by means of a spectrum of processes without any economic ottimization. The delivered scenario of yields as a function of the process is also a base data collecetion for scale-up studies. However, the base data collection must be integrated with data regarding the process-required power (not included in the present study) to support a scale-up investigation. Indeed, the process investigation at lab-scale does not allow to correctly address the resources needed (i.e. water, enzymes, solvent/chemicals, energy) whose demand is fundamental fro a correct analysis of the process from an economic and sustainable point of view. The investigation at pilot-scale of selected processes will allow to collect data to support the tecno-economic scale-up at low undetermined assessment.

The scale-up of a production process based on the fermentation of biomass must include investigation regrading the three main section/steps: pretreatment-hydroliysis of the biomass, fermentation, product recovery. Although the three steps are typicaly investigated stand alone, they are strongly connected each-other. Indeed, the product concentration and its pureness in the fermentation broth affect the efficiency and the energy required of the downstream process.

The chapter 3.2 highlights how different biochemicals (i.e. ABE and succini acid) can be produced by fermentation of AFW hydrolizates. However, the recovery of these bio-products is not an easy task. As an example, although different strategies have been developed to recover solvents from the fermentation broth - i.e. distillation, liquid-liquid extraction, adsorption and pervaporation (Bharathiraja et al., 2017) – the process performances depend on the solvents. As a rule of thumb, the performances of downstream processes increase with the concentration of the bio-product in the fermentation broth. To fulfil this best practice, a suitable strategy of pretreatment and hydrolysis of the biomass must be applied to produce a rich-in-sugars solution at the end of the enzymatic hydrolysis to increase the fermentative performances. Indeed, the downstream process performance depends on the efficiency of the pretreatment/hydrolysis process.

All the three main section/steps demand for key resources like water and energy. Threfore, downstream processes and pretreatmetn/hydrolysis processes that save water and energy are to be encouraged. From this point of view, the bubble column strategy reported in this thesis is a promising starting point.

6. CONCLUSION AND FUTURE PERSPECTIVES

The present Ph.D. project was aimed at the valorization of selected lignocellulosic residues derived from the industrial processing of agricultural products according to procedure typical of the biorefinery approach. Coffee silverskin (CSS) and apple pomace (AP) were considered as potential sources of fermentable sugars and biochemicals. The activities followed the three main steps of a biorefinery process: pretreatment, enzymatic hydrolysis, and fermentation. The characterization of some products was also carried out. The research activity was carried out at Dipartimento di Ingegneria Chimica, dei Materiali e della Produzione Industriale of the Università degli Studi di Napoli 'Federico II'.

CSS was successfully pretreated by combining alkaline and ultrasound pretreatments. A maximum sugar yield of 0.6 g/g was obtained applying the following operating conditions for the pretreatment step: 5 min sonication, 11% w/v biomass loading, 5% w/v NaOH, and 75 min autoclave. This result was achieved using the *Response Surface Methodology* approach. Beside the fermentable sugars obtained after the enzymatic hydrolysis of pretreated CSS, the whole process allowed to release a significant amount of phenolic compounds (25 mg/g_{rawCSS}) from the raw CSS. Moreover, at the end of enzymatic hydrolysis, the solid residue not hydrolysed was mainly composed of lignin, that can be considered as a bioproduct because it can be utilized as solid biofuel or as feedstock to produce biochemicals.

To maximize sugars recovery with reduced energy request CSS was alkali-pretreated (no ultrasound was applied in this case). The effects of biomass loading and enzyme loading on the performances of enzymatic hydrolysis were investigated. At 10 %w/v biomass loading and 80 $FPU/g_{cellulose}$, sugars concentration of 35 g/L and sugar yield of 93% were obtained in 72 h. As expected, the increase of enzyme loading corrisponded to the increase of sugars release from pretreated CSS. The enzyme loading of 80 $FPU/g_{cellulose}$ also gave the highest hydrolysis rate (in the range investigated). The obtained sugars were used as carbon source for the fermentations of *C. acetobutylicum* and *A. succinogenes*: 2.2 $g_{butanol}/L$ and 20.8 $g_{succinic\ acid}/L$ were produced by these microorganisms, respectively. These results suggest that the sugars recovered from CSS can be used in fermentative processes.

The work carried out with CSS highlighted the great potentialities of these residue for biorefinery applications. With the proposed process, we were able to obtain fermentable sugars, lignin, solvents (ABE fermentation) and biochemicals (phenolic compounds and succinic acid).

For the pretreatment of AP a new configuration system for the pretreatment and hydrolysis was investigated. The enzymatic pretreatment and hydrolysis of this residue was carried out in a bubble column bioreactor. Laccases were the ligninolytic enzyme selected for the pretreatment step. Biomass loading, enzyme loading and aeration rate were the operating conditions optimized: 15 %w/v biomass loading, 30 $U/g_{biomass}$ laccase concentration and 60 nL/h air flow rate were the condition characterized by the highest sugars concentration. A final sugars concentration of 51.8 g/L corresponding to a sugar yield of 0.61 $g_{sugar}/g_{sugar\ available}$ (0.34 $g_{sugar}/g_{raw\ AP}$) was obtained. The high amount of sugars obtained and the absence of inhibitory compounds make this relatively new process very promising for further investigations on the AP. It is important to stress the fact that with the proposed process no filtration and washing steps are required because the entire process (pretreatment and hydrolysis) is carried out in the same bubble column bioreactor. This is a great advantage of the process because the processing time is reduced. Moreover, the absence of chemicals, the water saving, and the low energy requirements of this bioreactor configuration well agree with the modern concept of green process, being more environmentally friendly.

The present study highlighted that the use of AFWs is a key issue to unlock the shift from a fossil-based economy to a bio-based economy through the development of biorefinery processes that start from organic materials, easy to harvest and reuse. This study shows how even a recalcitrant biomass like CSS can be used in biorefinery processes to produce biochemicals through the steps of ptretreatment, enzymatic hydrolysis and fermentation. At the same time, the novel pretreatment/hydrolysis proposed process of apple pomace - based on the laccase pretreatment in a bubble column bioreactor – is a proof of a new and green process to pretreat and hydrolise lignocellulosic biomass. Indeed, other different AFWs could be tested for their possible use in biorefinery processes based on the strategies described in this thesis.

The results reported in the present Ph.D. thesis are satisfying and put the basis for possible future investigations. The sugars obtained from CSS could be used to carry out fermentations with other different microorganisms to produce a spectrum of biochemicals, or the medium could be optimized for a specific microorganism. As an example, *A. succinogenes* gave great performances that could be further improved through the medium optimization. The same target can be applied to the hydrolyzates from AP. Moreover, the enzymatic pretreatment and hydrolysis in the bubble column proposed for AP could be also applied to different biomass. Since no inhibitors were detected for AP, the bubble column system could be used to carry out all the main steps of the biorefinery process, including the fermentation process. A similar approach would drastically reduce the downtime of the whole process.

References

Adney, B., Baker, J., 2008. Measurement of Cellulase Activities, Laboratory Analytical Procedure (LAP); NREL; TP 510 42628.

Al-Khatib, A., Aqra, F., Al-Jabari, M., Yaghi, N., Basheer, S., Sabbah, I., Al-Hayek, B., Mosa, M. 2009. Environmental pollution resulting from olive oil production. Bulgarian Journal of Agricultural Science 15(6):544-551.

Allied Market Research (2014) World Second Generation Biofuels (Advanced Biofuels) Market - Opportunities and Forecasts, 2013 - 2020.

Anderson, T. R., Hawkins, E., Jones, P. D. 2016. CO2, the greenhouse effect and global warming: from the pioneering work of Arrhenius and Callendar to today's Earth System Models. Endeavour 40(3). https://doi.org/10.1016/j.endeavour.2016.07.002

Anwar, Z., Gulfraz, M., Irshad, M. 2014. Agro-industrial lignocellulosic biomass a key to unlock the future bio-energy: A brief review. Journal of Radiation Research and Applied Sciences 7(2):163-173. https://doi.org/10.1016/j.jrras.2014.02.003

Arevalo-gallegos, A., Ahmad, Z., Asgher, M., Parra-saldivar, R., Iqbal, H.M.N. 2017. Lignocellulose: A sustainable material to produce value-added products with a zero waste approach — A review. International Journal of Biological Macromolecules 99:308–18. http://dx.doi.org/10.1016/j.ijbiomac.2017.02.097

Avanthi A., Banerjee, R. 2016. A strategic laccase mediated lignin degradation of lignocellulosic feedstocks for ethanol production, Ind. Crops Prod. 92, 174–185.

Bak, J.S., Ko, J.K., Han, Y.H., Lee, B.C., Choi, I.G., Kim, K.H., 2009. Improved enzymatic hydrolysis yield of rice straw using electron beam irradiation pretreatment. Bioresour. Technol. 100, 1285–1290. https://doi.org/10.1016/j.biortech.2008.09.010

Bala, A., Singh, B. 2019. Development of an environmental-benign process for efficient pretreatment and saccharification of Saccharum biomasses for bioethanol production, Renewable Energy, 130, 12-24.

Balasundram, N., Sundram, K., Samman, S., 2006. Phenolic compounds in plants and agri-industrial by-products : Antioxidant activity, occurrence, and potential uses. FOOD Chem. 99, 191–203. https://doi.org/10.1016/j.foodchem.2005.07.042

Ballesteros, L.F., Teixeira, J.A., Mussatto, S.I. 2014. Chemical, Functional, and Structural Properties of Spent Coffee Grounds and Coffee Silverskin. Food Bioprocess Technol 7(12):3493–3503. https://doi.org/10.1007/s11947-014-1349-z

Ballesteros, L.F., Teixeira, J.A., Mussatto, S.I., 2014. Chemical, Functional, and Structural Properties of Spent Coffee Grounds and Coffee Silverskin. Food Bioprocess Technol. 7, 3493–3503. https://doi.org/10.1007/s11947-014-1349-z

Bharathiraja, B., Jayamuthunagai, J., Sudharsanaa, T., Bharghavi, A., Praveenkumar, R., Chakravarthy, M., Yuvaraj, D. 2017. Biobutanol – An impending biofuel for future: A review on upstream and downstream processing techniques. Renewable and Sustainable Energy Reviews, 68:788–807. http://dx.doi.org/10.1016/j.rser.2016.10.017

Bhutto, A.W., Qureshi, K., Harijan, K., Abro, R., Abbas, T., Bazmi, A.A., Karim, S., Yu, G. 2017. Insight into progress in pre-treatment of lignocellulosic biomass. Energy 122:724-745. http://dx.doi.org/10.1016/j.energy.2017.01.005

Bussemaker, M., J., Zhang, D. 2013. Effect of ultrasound on lignocellulosic biomass as a pretreatment for biorefinery and biofuel applications. Ind. Eng. Chem. Res. 52:3563−3580. https://doi.org/10.1021/ie3022785

Cara C, Moya M, Ballesteros I, Negro MJ, González A, Ruiz E. Influence of solid loading on enzymatic hydrolysis of steam exploded or liquid hot water pretreated olive tree biomass. Process Biochem 2007;42:1003–9. doi:10.1016/j.procbio.2007.03.012.

Carvalheiro, F., Duarte, L.C., Gírio, F.M., 2008. Hemicellulose biorefineries: A review on biomass pretreatments. J. Sci. Ind. Res. (India). 67, 849–864. https://doi.org/10.1016/j.talanta.2015.06.045

Cascone, R. 2008. Biobutanol - a replacement for bioethanol? Chem. Eng. Prog. S4-S9.

Chen, Y.;Stevens, M.A.;Zhu, Y.;Holmes, J.;Xu, H., 2013. Understanding of alkaline preteatment parameters for corn stover enzymatic saccharification. Biotechnol. Biofuels 6, 1–10.

Cherubini, F. 2010. The biorefinery concept: Using biomass instead of oil for producing energy and chemicals. Energy Conversion and Management 51:1412–1421. http://dx.doi.org/10.1016/j.enconman.2010.01.015

Chiang, P., Lee, D., Whiteley, C.G., Huang, C., 2017. Antioxidant phenolic compounds from Pinus morrisconicola using compressional-puffing pretreatment and water – ethanol extraction : Optimization of extraction parameters. J. Taiwan Inst. Chem. Eng. 70, 7–14.

https://doi.org/10.1016/j.jtice.2016.10.010

Childs, B.R.E., Bardsley, W.G., 1975. The Steady-State Kinetics of Peroxidase with 2,2'-Azino-di-(3-ethylbenzthiazoline- 6-sulphonic acid) as Chromogen. Biochem. J. 145, 93–103. https://doi.org/10.1042/bj1450093

Conde, T., Mussatto, S. 2016. Isolation of polyphenols from spent coffee grounds and silverskin by mild hydrothermal pretreatment. Preparative Biochemistry and Biotechnology 46(4):406-409. https://doi.org/10.1080/10826068.2015.1084514

Couto, S.R., Barreiro, M., Rivela, I., Longo, M.A., Sanroman, A. 2002. Performance of a solid-state immersion bioreactor for lignolytic enzyme production: evaluation of different operational variables. Process Biochem. 38:219-227. https://doi.org/10.1016/S0032-9592(02)00083-3

Demirbas, A., 2007. Progress and recent trends in biofuels. Prog. Energy Combust. Sci. 33, 1–18. https://doi.org/10.1016/j.pecs.2006.06.001

Desai, R.B., Kolhatkar, R. V., Joshi, J.B., Ranade, V. V., Mashelkar, R.A., 1995. Turbulence structure in bubble disengagement zone: Role of polymer addition. AIChE J. 41, 1329–1332. https://doi.org/10.1002/aic.690410529

Didanna, H.L., 2014. A critical review on feed value of coffee waste for livestock feeding. World J. Biol. Biol. Sci. 2, 72–86.

Diedericks, D., Van Rensburg, E., Görgens, J.F., 2012. Fractionation of sugarcane bagasse using a combined process of dilute acid and ionic liquid treatments. Appl. Biochem. Biotechnol. 167, 1921–1937. https://doi.org/10.1007/s12010-012-9742-4

Doig, S.D., Ortiz-Ochoa, K., Ward, J.M., Baganz, F., 2005. Characterization of oxygen transfer in miniature and lab-scale bubble column bioreactors and comparison of microbial growth performance based on constant kLa. Biotechnol. Prog. 21, 1175–1182. https://doi.org/10.1021/bp050064j

Du, W., Yu, H., Song, L., Zhang, J., Weng, C., Ma, F., Zhang, X. 2011. The promising effects of by-products from *Irpex lacteus* on subsequent enzymatic hydrolysis of bio-pretreated corn stalks. Biotechnol. Biofuels 4, 37. https://doi.org/10.1186/1754-6834-4-37

Duret, X., Fredon, E., Masson, E., Desharnais, L., Gerardin, P., 2013. Optimization of Acid Pretreatment in order to Increase the Phenolic Content of Picea abies Bark by Surface Response Methodology. BioResources 8, 1258–1273.

Eblaghi, M., Niakousari, M., Sarshar, M., Mesbahi, G.R., 2016. Combining Ultrasound with Mild Alkaline Solutions as an Effective Pretreatment to Boost the Release of Sugar Trapped in Sugarcane Bagasse for Bioethanol Production. J. Food Process Eng. 39, 273–282. https://doi.org/10.1111/jfpe.12220

European Food and Drink Industry 2014-2015. http://www.fooddrinkeurope.eu/uploads/publications_documents/Data_and_Trends_2014-20151.pdf

Ezeji, T., C., Qureshi, N., Blaschek, H., P. 2007a. Production of acetone butanol (AB) from liqueWed corn starch, a commercial substrate, using *Clostridium beijerinckii* coupled with product recovery by gas stripping. J Ind Microbiol Biotechnol, 34:771–777. https://doi.org/10.1007/s10295-007-0253-1

Ezeji, T., Qureshi, N., Blaschek, H. 2007b. Butanol production from agricultural residues: impact of degradation products on *Clostridium beijerinckii* growth and butanol fermentation. Biotechnol. Bioeng., 97:1460-1469. https://doi.org/10.1002/bit.21373

Ezeji, T.C., Blaschek, H.P., 2008. Fermentation of dried distillers' grains and solubles (DDGS) hydrolysates to solvents and value-added products by solventogenic clostridia. Bioresour. Technol. 99, 5232–42. https://doi.org/10.1016/j.biortech.2007.09.032

Ezejiofor, T.I.N.; Enebaku, U., E.; Ogueke, C. 2014. Waste to Wealth- Value Recovery from Agrofood Processing Wastes Using Biotechnology: A Review. British Biotechnology Journal, 4(4):418-481. https://doi.org/10.9734/BBJ/2014/7017

Fagnano, M., Impagliazzo, A., Mori, M. and Fiorentino N. 2015. Agronomic and environmental impacts of giant reed (*Arundo donax L.*): results from a long-term field experiment in hilly areas subject to soil erosion. Bioenergy Res. 8: 415–422. https://doi.org/10.1007/s12155-014-9532-7

Falls, M., Sierra-Ramirez, R., Holtzapple, M.T., 2011. Oxidative lime pretreatment of dacotah switchgrass. Appl. Biochem. Biotechnol. 165, 243–259. https://doi.org/10.1007/s12010-011-9247-6

FAOSTAT- Forestry database: https://www.FAOSTAT3.FAO.org.

Fava F, Totaro G, Diels L, Reis M, Duarte J, Carioca OB, et al. 2015. Biowaste biorefinery in Europe: Opportunities and research & development needs. N Biotechnol 32:100–8. doi:10.1016/j.nbt.2013.11.003.

Feng, X., Wang, H., Wang, Y., Wang, X., Huang, J. 2010. Biohydrogen production from apple pomace by anaerobic fermentation with river sludge. Int. J. Hydrogen Energy 35:3058–3064. https://doi.org/10.1016/j.ijhydene.2009.07.015

Ferone M, Raganati F, Olivieri G, Salatino P, Marzocchella A. Biosuccinic Acid from Lignocellulosic-Based Hexoses and Pentoses by Actinobacillus succinogenes: Characterization of the Conversion Process. Appl Biochem Biotechnol 2017. doi:10.1007/s12010-017-2514-4.

Fiorentino, N., Fagnano, M., Adamo, P., Impagliazzo, A., Mori, M., Pepe, O., Ventorino, V., Zoina, A. 2013. Assisted phytoextraction of heavy metals: compost and *Trichoderma* effects on giant reed (*Arundo donax L*) uptake and soil N-cycle microflora. Ital. J. Agron. 8:244-254. https://doi.org/10.4081/ija.2013.e29

FitzPatrick, M., Champagne, P., Cunningham, M., F., Whitney, R., A. 2010. A biorefinery processing perspective: Treatment of lignocellulosic materials for the production of value-added products. Bioresource Technology 101:8915–8922. http://dx.doi.org/10.1016/j.biortech.2010.06.125

Florence, T.M., 1980. Degradation of protein disulphide bonds in dilute alkali. Biochem. J. 189, 507–520. https://doi.org/10.1042/bj1890507

Friedl, A., 2012. Lignocellulosic biorefinery. Environ. Eng. Manag. J. 11, 75–79.

Gallezot, P., 2007. Process options for converting renewable feedstocks to bioproducts. Green Chem. 9, 295. https://doi.org/10.1039/b615413a

Ganesh, K., Joshi, J.B., Sawant, S.B., 2000. Cellulase deactivation in a stirred reactor. Biochem. Eng. J. 4, 137–141. https://doi.org/10.1016/S1369-703X(99)00045-5

Gao K, Boiano S, Marzocchella A, Rehmann L. 2014a. Cellulosic butanol production from alkali-pretreated switchgrass (*Panicum virgatum*) and phragmites (*Phragmites australis*). Bioresour Technol 174:176–81. doi:10.1016/j.biortech.2014.09.152.

Gao K, Rehmann L. ABE fermentation from enzymatic hydrolysate of NaOH-pretreated corncobs. 2014. Biomass and Bioenergy 66:110–5. doi:10.1016/j.biombioe.2014.03.002.

Garcia-Aparicio, M.P., I. Ballesteros, et al. 2006. Effect of inhibitors released during steam- explosion pretreatment of barley straw on enzymatic hydrolysis. Applied Biochemistry and Biotechnology 129(1-3):278-288. https://doi.org/10.1385/ABAB:129:1:278

Ghadge, R.S., Sawant, S.B., Joshi, J.B., 2003. Enzyme deactivation in a bubble column, a stirred vessel and an inclined plane. Chem. Eng. Sci. 58, 5125–5134. https://doi.org/10.1016/j.ces.2003.08.008

Ghose TK. Measurement of cellulase activities. Pure Appl Chem 1987;59. doi:10.1351/pac198759020257.

Giacobbe, S., Pezzella, C., Lettera, V., Sannia, G., Piscitelli, A. 2018. Laccase pretreatment for agrofood wastes valorization. Bioresource Technology 265:59–65. https://doi.org/10.1016/j.biortech.2018.05.108

Gullón, B., Falqué, E., Alonso, J.L., Parajó, J., 2007. Evaluation of Apple Pomace as a Raw Material forAlternative Applications in Food Industries. Food Technol. Biotechnol. 45, 426–433. https://doi.org/10.1021/jf070442v

Gupta, C., Sarethy, I.P. 2014. Biofuels as alternative energy sources. Indo Global Journal of Pharmaceutical Sciences, Vol. 4 No. 3 pp. 206.

Guragain, Y., Coninck, N., Husson, J., Durand, F., Rakshit, A., Kumar, S. 2011. Comparison of some new pretreatment methods for second generation bioethanol production from wheat straw and water hyacinth. Bioresour. Technol. 102:4416-4424. https://doi.org/10.1016/j.biortech.2010.11.125

Gusakov, A. V., Nadjemy, B., Sinitsyn, A. P. and Klyosov, A. A. 1984. Kinetics of the enzymatic hydrolysis of cellulose: 1. A mathematical model for a batch reactor process, Pri. Biochim. MicrobioL., 20, 55-63.

Han, M., Moon, S.K., Choi, G.W. 2014. Pretreatment solution recycling and high-concentration output for economical production of bioethanol. Bioprocess Biosyst Eng 37:2205–2213. http://dx.doi:10.1007/s00449-014- 1198-1

He Q, Chen H. Improved efficiency of butanol production by absorbent fermentation with a renewable carrier. Biotechnol Biofuels 2013;6:1–9. doi:10.1186/1754-6834-6-121.

Hijosa-Valsero, M., Paniagua-Garcia, A.I., Diez-Antolinez, R. 2017. Biobutanol production from apple pomace: the importance of pretreatment methods on the fermentability of lignocellulosic agro-food wastes. Applied Microbiology and Biotechnology 101(21):8041-8052. https://doi.org/10.1007/s00253-017-8522-z

Himmel, M.E., Ding, S.Y., Johnson, D.K., Adney, W.S., Nimlos, M.R., Brady, J.W., et al. 2007. Biomass recalcitrance: engineering plants and enzymes for biofuel production. Science, 315:804-807. https://doi.org/10.1126/science.1137016

ICO. International Coffee Organization 2017:2017 – https://www.ico.org.

Ioelovich M, Morag E. Study of enzymatic hydrolysis of pretreated biomass at increased solids loading. BioResources 2012;7:4672–82. doi:10.15376/biores.7.4.4672-4682.

Iqbal, H.M.N., Ahmed, I., Zia, M.A., & Irfan, M. 2011. Purification and characterization of the kinetic parameters of cellulase produced from wheat straw by Trichoderma viride under SSF and its detergent compatibility. Advances in Bioscience and Biotechnology, 2(3):149-156. https://doi.org/10.4236/abb.2011.23024

IRENA: REmap 2030 — a renewable energy roadmap. 2014:124-127.

Isikgor, F.H., Becer, C.R. 2015. Lignocellulosic biomass: a sustainable platform for the production of bio-based chemicals and polymers. Polym. Chem. 6:4497. https://doi.org/10.1039/c5py00263j

Jeoh T, Cardona MJ, Karuna N, Mudinoor AR, Nill J. 2017. Mechanistic kinetic models of enzymatic cellulose hydrolysis—A review. Biotechnol Bioeng 114:1369–85. doi:10.1002/bit.26277.

Jiang M, Xu R, Xi Y-L, Zhang J-H, Dai W-Y, Wan Y-J, et al. 2013. Succinic acid production from cellobiose by Actinobacillus succinogenes. Bioresour Technol 135:469–74. doi:10.1016/j.biortech.2012.10.019.

Jin, C., Yao, M., Liu, H., Lee, C.-f., F., Ji, J. 2011. Progress in the production and application of n-butanol as a biofuel. Renewable and Sustainable Energy Reviews, 15:4080–4106. https://doi.org/10.1016/j.rser.2011.06.001

Jonsson, L.J., Alriksson, B., Nilvebrant, N.O., 2013. Bioconversion of lignocellulose: Inhibitors and detoxification. Biotechnol. Biofuels 6, 1–10. https://doi.org/10.1186/1754-6834-6-16

Joshi, V.K., Attri, D. 2006. Solid state fermentation of apple pomace for the production of value added products. Indian J. Nat. Prod. Resour. 5:289–296.

Kadam, K. L., Rydholm, E. C., McMillan, J. D. 2004. Development and validation of a kinetic model for enzymatic saccharification of lignocellulosic biomass. Biotechnol. Prog. 20:698−705. http://dx.doi:10.1021/bp034316x

Kadic, A., Palmqvist, B., Lidén, G. 2014. Effects of agitation on particle-size distribution and enzymatic hydrolysis of pretreated spruce and giant reed. Biotechnology for Biofuels, 7:77. http://dx.doi:10.1186/1754-6834-7-77

Kantarci, N., Borak, F., Ulgen, K., O. 2005. Bubble column reactor. Process Biochemistry 40:2263-2283. https://doi.org/10.1016/j.procbio.2004.10.004

Kharazipour, A., Huettermann, A., Luedemann, H.D., 1997. Enzymatic activation of wood fibres as a means for the production of wood composites. J. Adhes. Sci. Technol. 11, 419–427. https://doi.org/10.1163/156856197X00796

Kim, I., Han, J., 2012. Optimization of alkaline pretreatment conditions for enhancing glucose yield of rice straw by response surface methodology. Biomass and Bioenergy 46, 210–217. https://doi.org/10.1016/j.biombioe.2012.08.024

Kim, S., Holtzapple, M.T., 2006. Effect of structural features on enzyme digestibility of corn stover. Bioresour. Technol. 97, 583–591. https://doi.org/10.1016/j.biortech.2005.03.040

Kircher, M. 2012. The transition to a bio-economy: emerging from the oil age. Biofpr, 6:369-375. https://doi.org/10.1002/bbb.1352

Kircher, M. 2015. Sustainability of biofuels and renewable chemicals production from biomass. Current Opinion in Chemical Biology 29:26–31. https://doi.org/10.1016/j.cbpa.2015.07.010

Klein-Marcuschamer D, Oleskowicz-Popiel P, Simmons BA, Blanch HW. 2012. The challenge of enzyme cost in the production of lignocellulosic biofuels. Biotechnol Bioeng 109:1083–7. doi:10.1002/bit.24370.

Krishna, R., Van Baten, J.M., 2003. Mass transfer in bubble columns. Catal. Today 79–80, 67–75. https://doi.org/10.1016/S0920-5861(03)00046-4

Kruczek, M., Drygaś, B., Habryka, C., 2016. Pomace in fruit industry and their contemporary potential application 48, 259–265.

Kudanga, T., Le Roes-Hill, M. 2014. Laccase applications in biofuels production: current status and future prospects. Appl Microbiol Biotechnol 98:6525-6542. https://doi.org/10.1007/s00253-014-5810-8

Kumar, P., Barrett, D., Delwiche, M.J., Stroeve, P. 2009. Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production. Ind. Eng. Chem. Res. 48(8):3713–3729. https://doi.org/10.1021/ie801542g

Lee, D.S., Wi, S.G., Lee, S.J., Lee, Y.G., Kim, Y.S., Bae, H.J. 2014. Rapid saccharification for production of cellulosic biofuels. Bioresource Technology 158:239–247. https://doi.org/10.1016/j.biortech.2014.02.039

Lee, R.A., Lavoie, J.M. 2014. From first- to third-generation biofuels: Challenges of producing a commodity from a biomass of increasing complexity. Animal Frontiers 3(2):6-11. https://doi.org/10.2527/af.2013-0010

Li Q, Yang M, Wang D, Li W, Wu Y, Zhang Y, et al. 2010. Efficient conversion of crop stalk wastes into succinic acid production by Actinobacillus succinogenes. Bioresour Technol 101:3292–4. doi:10.1016/j.biortech.2009.12.064.

Li, H., Prakash, A., 2000. Influence of slurry concentrations on bubble population and their rise velocities in a three-phase slurry bubble column. Powder Technol. 113, 158–167. https://doi.org/10.1016/S0032-5910(00)00228-X

Liguori, R., Amore, A., Faraco, V. 2013. Waste valorisation by biotechnological conversion into added value products. Appl. Microbiol. Biotechnol. 97:6129-6147. https://doi.org/10.1007/s00253-013-5014-7

Liguori, R., Faraco, V. 2016. Biological processes for advancing lignocellulosic waste biorefinery by advocating circular economy. Bioresource Technology 215:13–20. http://dx.doi.org/10.1016/j.biortech.2016.04.054

Lin, C.S.K., Pfaltzgraf, L.A., Herrero-Davila, L., et al. 2013. Food waste as a valuable resource for the production of chemicals, materials and fuels. Current situation and global perspective. Energy Environ. Sci. 6(2):426-464. https://doi.org/10.1039/c2ee23440h

Lin, Y.S., Lee, W.C. 2011. Simultaneous saccharification and fermentation of alkaline pretreated cogongrass for bioethanol production. BioResources 6:2744–2756.

Lin, Y.S., Lee, W.C., Duan, K.J., Lin, Y.H. 2013. Ethanol production by simultaneous saccharification and fermentation in rotary drum reactor using thermotolerant *Kluyveromyces marxianus*. Appl Energy 105:389–394. https://doi.org/10.1016/j.apenergy.2012.12.020

Liu G, Zhang J, Bao J. 2016. Cost evaluation of cellulase enzyme for industrial-scale cellulosic ethanol production based on rigorous Aspen Plus modeling. Bioprocess Biosyst Eng 39:133–40. doi:10.1007/s00449-015-1497-1.

Lu, C., Zhao, J., Yang, S.-T., Wei, D. 2012. Fed-batch fermentation for n-butanol production from cassava bagasse hydrolysate in a fibrous bed bioreactor with continuous gas stripping. Bioresource Technology 104:380–387. https://doi.org/10.1016/j.biortech.2011.10.089

Luo, J., Fang, Z., Smith, R.L. 2014. Ultrasound-enhanced conversion of biomass to biofuels. Progress in Energy and Combustion Science 41(1):1-38. https://doi.org/10.1016/j.pecs.2013.11.001

Machado, E.M.S., Rodriguez-Jasso, R.M., Teixeira, J.A., Mussatto, S.I., 2012. Growth of fungal strains on coffee industry residues with removal of polyphenolic compounds. Biochem. Eng. J. 60, 87–90. https://doi.org/10.1016/j.bej.2011.10.007

Maitan-Alfenas, G.P., Visser, E., Guimaraes, V. 2015. Enzymatic hydrolysis of lignocellulosic biomass: Converting food waste in valuable products. Current Opinion in Food Science 1, 1:44-49. http://dx.doi.org/10.1016/j.cofs.2014.10.001

Maiti, S., Gallastegiu, G., Suresh, G., et al. 2018. Hydrolytic pre-treatment methods for enhanced biobutanol production from agro-industrial wastes. Bioresource Technology 249:673-683. https://doi.org/10.1016/j.biortech.2017.09.132

Manara, P., Vamvuka, V., Sfakiotakis, S., Vanderghem, C., Richel, A., ZabanotiouA. 2015. Mediterranean agri-food processing wastes pyrolysis after pre-treatment and recovery ofprecursor materials: A TGA-based kineticmodeling study. Food Research International 73:44–51. https://doi.org/10.1016/j.foodres.2014.11.033

Martin, C., Klinke, H.B., Marcet, M., Garcia, L., Ena, H., Thomsen, A.B., 2014. Study of the phenolic compounds formed during pretreatment of sugarcane bagasse by wet oxidation and steam explosion Study of the phenolic compounds formed during pretreatment of sugarcane bagasse by wet oxidation and. Holzforschung 61, 483–487. https://doi.org/10.1515/HF.2007.106

Masran, R., Zanirun, Z., Bahrin, E.K., Ibrahim, M.F., Lai Yee, P., Abd-Aziz, S., 2016. Harnessing the potential of ligninolytic enzymes for lignocellulosic biomass pretreatment. Appl. Microbiol. Biotechnol. 100, 5231–5246. https://doi.org/10.1007/s00253-016-7545-1

McIntosh, S., Vancov, T., 2011. Optimisation of dilute alkaline pretreatment for enzymatic saccharification of wheat straw. Biomass and Bioenergy 35, 3094–3103. https://doi.org/10.1016/j.biombioe.2011.04.018

McKinlay JB, Laivenieks M, Schindler BD, McKinlay A a, Siddaramappa S, Challacombe JF, et al. 2010. A genomic perspective on the potential of Actinobacillus succinogenes for industrial succinate production. BMC Genomics 11:680. doi:10.1186/1471-2164-11-680.

Menon, V., Rao, M., 2012. Trends in bioconversion of lignocellulose: Biofuels, platform chemicals & biorefinery concept. Prog. Energy Combust. Sci. https://doi.org/10.1016/j.pecs.2012.02.002

Mesa, L., Gonzalez, E., Cara, C., et al. 2015. An approach to cellulase recovery from enzymatic hydrolysis of pretreated sugarcane bagasse with high lignin content. Biocatalysis and Biotransformation 33, 5-6:287-297. http://dx.doi.org/10.3109/10242422.2016.1168816

Millat T, Janssen H, Bahl H, Fischer R, Wolkenhauer O. 2011. The pH-induced Metabolic Shift from

Acidogenesis to Solventogenesis in Clostridium acetobutylicum – From Experiments to Models. Beilstein-Institut 33–54.

Millati, I.R., Syamsiah, S., Nikalasson, C., Cahyanto, M.N., Lundquist, K., Taherzadeh, M.J. 2011. Biological pretreatment of lignocelluloses with white rot fungi and its applications: a review. Bioresources 6:1-36.

Moilanen, U., Kellock, M., Galkin, S., Viikari, L. 2011. The laccase-catalyzed modification of lignin for enzymatic hydrolysis. Enzyme and Microbial Technology 49:492-498. https://doi.org/10.1016/j.enzmictec.2011.09.012

Monschein, M., Reisinger, C., Nidetzky, B., 2013. Enzymatic hydrolysis of microcrystalline cellulose and pretreated wheat straw: a detailed comparison using convenient kinetic analysis. Bioresour. Technol. 128, 679–87. https://doi.org/10.1016/j.biortech.2012.10.129

Morales, A., Gullón, B., Dávila, I., Eibes, G., Labidi, J., Gullón, P., 2018. Optimization of alkaline pretreatment for the co-production of biopolymer lignin and bioethanol from chestnut shells following a biorefinery approach. Ind. Crops Prod. 124, 582–592. https://doi.org/10.1016/j.indcrop.2018.08.032

Masran, R., Zanirun, Z., Bahrin, E.K., Ibrahim, M.F., Yee, P.L., Abd-Aziz, S. 2016. Harnessing the potential of ligninolytic enzymes for lignocellulosic biomass pretreatment. Appl Microbiol Biotechnol 100:5231-5246. http://dx.doi.org/10.1007/s00253-016-7545-1

Mulakhudair, A.R., Hanotu, J., Zimmerman, W., 2016. Exploiting microbubble-microbe synergy for biomass processing: Application in lignocellulosic biomass pretreatment. Biomass and Bioenergy 93: 187–193. https://doi.org/10.1016/j.biombioe.2016.07.014

Mussatto SI, Ballesteros LF, Martins S, Maltos DAF, Aguilar CN, Teixeira JA. 2013. Maximization of Fructooligosaccharides and β-Fructofuranosidase Production by Aspergillus japonicus under Solid-State Fermentation Conditions. Food Bioprocess Technol 6:2128–34. doi:10.1007/s11947-012-0873-y.

Mussatto SI, Dragone G, Fernandes M, Milagres AMF, Roberto IC. 2008. The effect of agitation speed, enzyme loading and substrate concentration on enzymatic hydrolysis of cellulose from brewer’s spent grain. Cellulose 15:711–21. doi:10.1007/s10570-008-9215-7.

Mussatto SI, Machado EMS, Carneiro LM, Teixeira JA. 2012. Sugars metabolism and ethanol production by different yeast strains from coffee industry wastes hydrolysates. Appl Energy 92:763–8. doi:10.1016/j.apenergy.2011.08.020.

Nakamura, C.E., Whited, G.M. 2003. Metabolic engineering for the microbial production of 1,3-propanediol. Curr. Opin. Biotechnol. 14, 454–459. https://doi.org/10.1016/j.copbio.2003.08.005

Nanda S, Golemi-Kotra D, McDermott JC, Dalai AK, Gökalp I, Kozinski JA. 2017. Fermentative production of butanol: Perspectives on synthetic biology. N Biotechnol 37:210–21. doi:10.1016/j.nbt.2017.02.006.

Narita Y, Inouye K. 2014. Review on utilization and composition of coffee silverskin. Food Res Int 61:16–22. doi:10.1016/j.foodres.2014.01.023.

Narita, Y., Inouye, K., 2012. High antioxidant activity of coffee silverskin extracts obtained by the treatment of coffee silverskin with subcritical water. Food Chem. 135, 943–949. https://doi.org/10.1016/j.foodchem.2012.05.078

Niglio, S., Procentese, A., Russo, M.E., Sannia, G., Marzocchella, A., 2017. Ultrasound-assisted dilute acid pretreatment of coffee silverskin for biorefinery applications. Chem. Eng. Trans. 57, 109–114. https://doi.org/10.3303/CET1757019

O’Neill, B. C., Liddle, B., Jiang, L., Smith, K. R., Pachauri, S., Dalton, M., Fuchs, R. 2012. Demographic change and carbon dioxide emissions. The Lancet 380:157–64. https://doi.org/10.1016/S0140-6736(12)60958-1

Ogbonna, J.C., Mashima, H., Tanaka, H. 2001. Scale up for fuel production rom sugar beet juice using a loofa sponge immobilized bioreactor. Bioresource Technology, 76:1-8. https://doi.org/10.1016/S0960-8524(00)00084-5

Oliva-Taravilla, A., Tomàs-Pejò, E., Demuez, M., Gonzàlez-Fernàndez, C., Ballesteros, M., 2015. Inhibition of cellulose enzymatic hydrolysis by laccase-derived compounds from phenols. Biotechnol. Prog. 31, 700–706. https://doi.org/10.1002/btpr.2068

Olofsson, K., Bertilsson, M., Lidén, G. 2008. A short review on SSF - an interesting process option for ethanol production from lignocellulosic feedstocks. Biotechnol Biofuels. 1-7. http://dx.doi:10.1186/1754-6834-1-7

Overshootday: https://www.overshootday.org/

Palmqvist, B., Lidén, G. 2012. Torque measurements reveal large process differences between materials during high solid enzymatic hydrolysis of pretreated lignocellulose. Biotechnology for Biofuels, 5:57. http://dx.doi:10.1186/1754-6834-5-57

Pan X. 2005. Strategies to Enhance the Enzymatic Hydrolysis of Pretreated Softwood with High Residual Lignin Content. Appl Biochem Biotechnol 121:1069–79. doi:10.1385/ABAB:124:1-3:1069.

Pandiyan, K., Singh, A., Singh, S:, Kumar Saxena, A., Nain, L. 2019. Technological interventions for utilization of crop residues and weedy biomass for second generation bio-ethanol production, Renewable Energy, 132, 723-741.

Pauly, M., Keegstra, K. 2008. Cell-wall carbohydrates and their modification as a resource for biofuels. Plant Journal 54, 4:559-568.

Pezzella, C., Giacobelli, V.G., Lettera, V., Olivieri, G., Cicatiello, P., Sannia, G., Piscitelli, A., 2017. A step forward in laccase exploitation: Recombinant production and evaluation of techno-economic feasibility of the process. J. Biotechnol. 259, 175–181. https://doi.org/10.1016/j.jbiotec.2017.07.022

Piscitelli, A., Del Vecchio, C., Faraco, V., Giardina, P., Macellaro, G., Miele, A., Pezzella, C., Sannia, G. 2011. Fungal laccases: Versatile tools for lignocellulose transformation. C. R. Biologies 334:789-794. doi:10.1016/j.crvi.2011.06.007

Polidoro S, Scapin E, Lazzari E, Nunes A. 2018. Journal of Analytical and Applied Pyrolysis Valorization of coffee silverskin industrial waste by pyrolysis : From optimization of bio-oil production to chemical characterization by GC × GC / qMS. J Anal Appl Pyrolysis 129:43–52. doi:10.1016/j.jaap.2017.12.005.

Prakash, A., Margaritis, A., Li, H., Bergougnou, M.A., 2001. Hydrodynamics and local heat transfer measurements in a bubble column with suspension of yeast. Biochem. Eng. J. 9, 155–163. https://doi.org/10.1016/S1369-703X(01)00137-1

Pratto, B., Beraldo, R., Souza, A. De, 2016. Enzymatic Hydrolysis of Pretreated Sugarcane Straw : Kinetic Study and Semi-Mechanistic Modeling. Appl. Biochem. Biotechnol. 1430–1444. https://doi.org/10.1007/s12010-015-1957-8

Preeti VE, Sandhya SV, Kuttiraja M, Sindhu R, Vani S, Kumar SR, et al. 2012. An evaluation of chemical pretreatment methods for improving enzymatic saccharification of chili postharvest residue. Appl Biochem Biotechnol 167:1489–500. doi:10.1007/s12010-012-9591-1.

Procentese A, Raganati F, Olivieri G, Russo ME, Marzocchella A. 2017a. Pre-treatment and enzymatic hydrolysis of lettuce residues as feedstock for bio-butanol production. Biomass and Bioenergy 96:172–9. doi:10.1016/j.biombioe.2016.11.015.

Procentese A, Raganati F, Olivieri G, Russo ME, Rehmann L, Marzocchella A. 2018a. Deep Eutectic Solvents pretreatment of agro-industrial food waste. Biotechnol Biofuels 11:1–12. doi:10.1186/s13068-018-1034-y.

Procentese, A., Raganati, F., Navarini, L., Olivieri, G., Russo, M.E., Marzocchella, A. 2018b. Coffee Silverskin as a Renewable Resource to Produce Butanol and Isopropanol. Chemical Engineering Transactions 64. https://doi.org/10.3303/CET1864024

Procentese, A., Raganati, F., Olivieri, G., Russo, M.E., de la Feld, M., Marzocchella, A., 2017b. Renewable feedstocks for biobutanol production by fermentation. N. Biotechnol. 39, 135–140. https://doi.org/10.1016/j.nbt.2016.10.010

Qiu, W., Chen, H., 2012. Enhanced the enzymatic hydrolysis efficiency of wheat straw after combined steam explosion and laccase pretreatment. Bioresour. Technol. 118, 8–12. https://doi.org/10.1016/j.biortech.2012.05.033

Raganati, F., Götz, P., Olivieri, G, Marzocchella, A, Salatino, P. 2015. Butanol production from hexoses and pentoses by fermentation of Clostridium Acetobutylicum. Anaerobe 34:146–55. doi:10.3303/CET1227016.

Raganati, F., Procentese, A., Montagnaro, F., Olivieri, G., Marzocchella, A. 2014. Butanol Production from Leftover Beverages and Sport Drinks. BioEnergy Res. doi:10.1007/s12155-014-9531-8.

Raganati, F., Procentese, A., Olivieri, G., Russo, ME, Gotz, P., Marzocchella, A., et al. 2016. Butanol production by Clostridium acetobutylicum in a series of packed bed biofilm reactors. Chem Eng Sci 152:678–88. doi:10.1007/s10295-010-0707-8.

Ragauskas, A.J., Williams, C.K., Davison, B.H., et al. 2006. The Path Forward for Biofuels and Biomaterials. Science 311:484–489. https://doi.org/10.1126/science.1114736

Ramezani, M., Mostoufi, N., Mehrnia, M., R. 2013. Effect of hydrodinamics on kinetics of gluconic acid enzymatic production in bubble column reactor. Chemical Industry & Chemical Engineering Quarterly, 19(3):411−422. https://doi.org/10.2298/CICEQ120407076R

Rattanachitthawat, S., Suwannalert, P., Riengrojpitak, S., Chaiyasut, C., Pantuwatana, S., 2010. Phenolic content and antioxidant activities in red unpolished Thai rice prevents oxidative stress in rats 4, 796–801. https://doi.org/10.5897/JMPR10.067

Rehman, M.S.U., Kim, I., Chisti, Y., Han, J., 2013. Use of ultrasound in the production of bioethanol from lignocellulosic biomass. Energy Educ. Sci. Technol. 30, 1391–1410.

Reid, I.D., 1989. Solid-state fermentations for biological delignification. Enzyme Microb. Technol. 11:786-803. https://doi.org/10.1016/0141-0229(89)90052-5

Rocha-Martín, J., Martínez-Bernal, C., Zamorano, L.S., Reyes-Sosa, F.M., Díez García, B., 2018. Inhibition of enzymatic hydrolysis of pretreated corn stover and sugar cane straw by laccases. Process Biochem. 67, 88–91. https://doi.org/10.1016/j.procbio.2018.01.021.

Rodgers, C.J., Blanford, C.F., Giddens, S.R., Skamnioti, P., Armstrong, F.A., Gurr, S.J., 2010. Designer laccases: a vogue for high-potential fungal enzymes? Trends Biotechnol. 28, 63–72. https://doi.org/10.1016/j.tibtech.2009.11.001

Rodionova, M.V., Poudyal, R.S., Tiwari, I., Voloshin, R.A., Zharmukhamedov, S.K., Nam, H.G., Zayadan, B.K., Bruce, B.D., Hou, H.J.M., Allakhverdien, S.I. 2017. Biofuel production: Challenges and opportunities. International journal of hydrogen energy, 42:8450-8461. http://dx.doi.org/10.1016/j.ijhydene.2016.11.125

Russo M.E., Giardina P., Marzocchella A., Salatino P., Sannia G. 2008. Assessment of anthraquinone-dye conversion by free and immobilized crude laccase mixtures, Enzyme Microb. Technol. 42, 521–530.

Saenger, M., Hartge, E.U., Werther, J., Ogada, T., Siagi, Z., 2001. Combustion of coffee husks. Renew. Energy 23, 103–121. https://doi.org/10.1016/S0960-1481(00)00106-3

Saha, B.C. 2003. Hemicellulose Bioconversion. J Ind Microbiol Biotechnol, 30:279–291. https://doi.org/10.1007/s10295-003-0049-x

Sarkar N, Ghosh SK, Bannerjee S, Aikat K. 2012. Bioethanol production from agricultural wastes: An overview. Renew Energy 37:19–27. doi:10.1016/j.renene.2011.06.045.

Segal, L., Creely, J.J., Martin, A.E., Conrad, C.M., 1959. An empirical method for estimating the degree of crystallinity of native cellulose using X-ray diffractometer. J. Text. Res. 29, 786–794.

Shaheen, R., Shirley, M., Jones, D., T. 2000. Comparative Fermentation Studies of Industrial Strains Belonging to Four Species of Solvent-Producing Clostridia. J. Mol. Microbiol. Biotechnol. 2(1), 115-124.

Shaikh, A., Al-Dahhan, M. 2013. Scale-up of Bubble Column Reactors: A Review of Current State-of the-Art. Ind. Eng. Chem. Res. 52:8091−8108. http://dx.doi.org/10.1021/ie302080m

Shalini, R., Gupta, D.K. 2010. Utilization of pomace from apple processing industries: a review. J Food Sci Technol 47(4):365–371. https://doi.org/10.1007/s13197-010-0061-x

Sindhu, R., Binod, P., Pandey, A. 2016a. Biological pretreatment of lignocellulosic biomass – An overview. Bioresource Technology 199:76-82. https://doi.org/10.1016/j.biortech.2015.08.030

Sindhu, R., Binod, P., Pandey, A., 2016b. A novel sono-assisted acid pretreatment of chili post harvest residue for bioethanol production. Bioresour. Technol. 213, 58–63. https://doi.org/10.1016/j.biortech.2016.02.079

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D. 2012. Determination of Structural Carbohydrates and Lignin in Biomass, Laboratory Analytical Procedure (LAP); NREL/TP-510-42618.

Son, S.H., Choi, S.M., Lee, Y.H., Choi, K.B., Yun, S.R., Kim, J.K., Park, H.J., Kwon, O.W., Noh, E.W., Seon, J.H., Park, Y.G. 2000. Large-scale growth and taxane production in cell cultures of *Taxus cuspidata* (Japanese yew) using a novel bioreactor. Plant Cell Rep. 19, 628–533. https://doi.org/10.1007/s002990050784

Stephen, J.D., Mabee, W.E. & Saddler, J.N. 2012. Will second-generation ethanol be able to compete with first-generation ethanol? Opportunities for cost reduction. Biofuels, Bioprod. Biorefin. 6:159-176. https://doi.org/10.1002/bbb.331

Stöcker, M. 2008. Biofuels and Biomass-To-Liquid Fuels in the Biorefinery: Catalytic Conversion of Lignocellulosic Biomass using Porous Materials. Angew. Chem. Int. Ed. 2008, 47:9200-9211. https://doi.org/10.1002/anie.200801476

Stolarski, M.J., Krzyzaniak, M., Łuczyński, M., Załuski, D., Szczukowski, S., Tworkowski, J., Gołaszewski, J., 2015. Lignocellulosic biomass from short rotation woody crops as a feedstock for second-generation bioethanol production. Ind. Crops Prod. 75, 66–75. https://doi.org/10.1016/j.indcrop.2015.04.025

Sud, D., Mahajan, G., Kaur, M.P., 2008. Agricultural waste material as potential adsorbent for sequestering heavy metal ions from aqueous solutions - A review. Bioresour. Technol. 99, 6017–6027. https://doi.org/10.1016/j.biortech.2007.11.064

Suhag, M., Sharma, H. 2015. Biorefinery Concept: An Overview of Producing Energy, Fuels and Materials from Biomass Feedstocks. Iarjset 2(12):103-109. https://doi.org/10.17148/IARJSET.2015.21219

Sun, Y., Furusaki, S. 1990. Effects of product inhibition on continuous acetic acid production by immobilized *Acetobacter aceti*: theoretical calculations. J Ferment Bioeng, 70:196-198. https://doi.org/10.1016/0922-338X(90)90187-2

Tejirian, A., Xu, F., 2011. Inhibition of enzymatic cellulolysis by phenolic compounds. Enzyme Microb. Technol. 48, 239–247. https://doi.org/10.1016/j.enzmictec.2010.11.004

Toquero, C., Bolado, S. 2014. Effect of four pretreatments on enzymatic hydrolysis and ethanol fermentation of wheat straw. Influence of inhibitors and washing. Bioresource Technology 157:68–76. http://dx.doi.org/10.1016/j.biortech.2014.01.090

Trinh, L.T.P., Choi, Y.S., Bae, H.J., 2018. Production of phenolic compounds and biosugars from flower resources via several extraction processes. Ind. Crops Prod. 125, 261–268. https://doi.org/10.1016/j.indcrop.2018.09.008

Tsoskounoglou, M., Ayerides, G., Tritopoulou, E. 2008. The end of cheap oil: Current status and prospects. Energy Policy 36: 3797– 3806. https://doi.org/10.1016/j.enpol.2008.05.011

Tye, Y.Y., Lee, K.T., Abdullah, W.N.W., Leh, C.P. 2016. The world availability of non-wood lignocellulosic biomass for the production of cellulosic ethanol and potential pretreatments for the enhancement of enzymatic saccharification. Renewable and Sustainable Energy Reviews 60:155-172. http://dx.doi.org/10.1016/j.rser.2016.01.072

U.S. Department of Energy, https://www.afdc.energy.gov/uploads/publication/alternative_fuel_price_report_april_2018.pdf

Ulonska, K., König, A., Klatt, M., Mitsos, A., Viell, J. 2018. Optimization of Multiproduct Biorefinery Processes under Consideration of Biomass Supply Chain Management and Market Developments. Industrial and Engineering Chemistry Research 57(20): 6980-6991. https://doi.org/10.1021/acs.iecr.8b00245

UNCTAD (United Nations Conference on Trade and Development). 2016. Second-generation biofuel markets: state of play, trade and developing countries perspectives.

United Nations Prospects. World Population Prospects 2017. https://esa.un.org/unpd/wpp/

Urbina, L., Wongsirichot, P., Corcuera, M.A., et al. 2018. Application of cider by-products for medium chain length polyhydroxyalkanoate production by Pseudomonas putida KT2440. European Polymer Journal 108:1-9. https://doi.org/10.1016/j.eurpolymj.2018.08.020

Valdivia, M., Galan, J.L., Laffarga, J., Ramos, J.-L. 2016. Biofuels 2020: Biorefineries based on lignocellulosic materials. Microbial Biotechnology, 9(5):585–594. https://doi.org/10.1111/1751-7915.12387

Van Dyk, J.S., Gama, R., Morrison, D., Swart, S., Pletschke, B.I., 2013. Food processing waste: Problems, current management and prospects for utilisation of the lignocellulose component through enzyme synergistic degradation. Renew. Sustain. Energy Rev. 26, 521–531. https://doi.org/10.1016/j.rser.2013.06.016

Van Kuijk, S.J.A., Sonnenberg, A.S.M., Baars, J.J.P., Hendriks, W.H., Cone, J.W. 2015. Fungal treated lignocellulosic biomass as ruminant feed ingredient: a review. Biotechnol. Adv. 33:191-202. https://doi.org/10.1016/j.biotechadv.2014.10.014

Velmurugan, R., Muthukumar, K., 2012. Ultrasound-assisted alkaline pretreatment of sugarcane bagasse for fermentable sugar production: Optimization through response surface methodology. Bioresour. Technol. 112, 293–299. https://doi.org/10.1016/j.biortech.2012.01.168

Voget, C.E., Mignone, C.F., Ertola, R.J., 1985. Butanol production from apple pomace. Biotechnol. Lett. 7, 43–46. https://doi.org/10.1007/BF01032418

Volynets B, Ein-Mozaffari F, Dahman Y. 2017. Biomass processing into ethanol: Pretreatment, enzymatic hydrolysis, fermentation, rheology, and mixing. Green Process Synth 6:1–22. doi:10.1515/gps-2016-0017.

Waheed, A., Qureshi, K., Harijan, K., Abro, R., Abbas, T., Ahmed, A., Karim, S., Yu, G., 2017. Insight into progress in pre-treatment of lignocellulosic biomass 122, 724–745. https://doi.org/10.1016/j.energy.2017.01.005

Wang, F., Shi, S.L., Pei, J.C., Luo, L.Z., 2006. Improving strength and fiber surface properties through laccase catalyzed oxidation. J. Tianjin Universit (Sci. & Technol.) 21, 19–23.

WCED (ed.). 1987. Our Common Future: The World Commission on Environment and Development. Oxford University Press: Oxford.

Wood IP, Elliston A, Ryden P, Bancroft I, Roberts IN, Waldron KW. 2012. Rapid quantification of reducing sugars in biomass hydrolysates: Improving the speed and precision of the dinitrosalicylic acid assay. Biomass and Bioenergy 44:117–21. doi:10.1016/j.biombioe.2012.05.003.

Xu, H., Li, B., Mu, X., 2016. Review of Alkali-Based Pretreatment to Enhance Enzymatic Saccharification for Lignocellulosic Biomass Conversion. Ind. Eng. Chem. Res.

https://doi.org/10.1021/acs.iecr.6b01907

Xue Y, Jameel H, Phillips R, Chang HM. 2012. Split addition of enzymes in enzymatic hydrolysis at high solids concentration to increase sugar concentration for bioethanol production. J Ind Eng Chem 18:707–14. doi:10.1016/j.jiec.2011.11.132.

Yáñez R, Alonso JL, Parajó JC. 2006. Enzymatic saccharification of hydrogen peroxide-treated solids from hydrothermal processing of rice husks. Process Biochem 41:1244–52. doi:10.1016/j.procbio.2005.12.020.

You, T.-T., Zhang, L.-M., Zhou, S.-K., Xu, F. 2015. Structural elucidation of lignin–carbohydrate complex (LCC) preparations and lignin from *Arundo donax* Linn. Industrial Crops and Products 71:65–74. http://dx.doi.org/10.1016/j.indcrop.2015.03.070

Yunus, R., Salleh, S.F., Abdullah, N., Biak, D.R.A., 2010. Effect of ultrasonic pre-treatment on low temperature acid hydrolysis of oil palm empty fruit bunch. Bioresour. Technol. 101, 9792–9796. https://doi.org/10.1016/j.biortech.2010.07.074

Zeikus, J.G., Jain, M.K., Elankovan, P. 1999. Biotechnology of succinic acid production and markets for derived industrial products. Appl. Microbiol. Biotechnol. 51, 545–552. https://doi.org/10.1007/s002530051431

Zhang Q, Wu D, Lin Y, Wang X, Kong H, Tanaka S. 2015. Substrate and product inhibition on yeast performance in ethanol fermentation. Energy and Fuels 29:1019–27. doi:10.1021/ef502349v.

Zhang, X.-Z., Zhang, Y.-H.P. 2013. Cellulase: characteristics, sources, production and applications. Bioprocessing Technologies in Biorefinery for Sustainable Production of Fuels, Chemicals, and Polymers, First Edition. Edited by Shang-Tian Yang, Hesham A. El-Enshasy, and Nuttha Thongchul. http://dx.doi.org/10.1002/9781118642047.ch8

Zheng P, Dong J-J, Sun Z-H, Ni Y, Fang L. 2009. Fermentative production of succinic acid from straw hydrolysate by Actinobacillus succinogenes. Bioresour Technol 100:2425–9. doi:10.1016/j.biortech.2008.11.043.

Zhu S, Wu Y, Yu Z, Liao J, Zhang Y. 2005. Pretreatment by microwave / alkali of rice straw and its enzymic hydrolysis. Process Biochem 40:3082–6. doi:10.1016/j.procbio.2005.03.016.

SCIENTIFIC COMMUNICATION

PAPERS

Niglio, **S.**, Procentese, A., Russo, M., E., Sannia, G., Marzocchella, A., "Ultrasound-assisted Dilute Acid Pretreatment of Coffee Silverskin for Biorefinery Applications" *Chemical Engineering Transactions* 2017, Vol. 57, DOI: 10.3303/CET1757019.

Niglio, S., Procentese, A., Russo, M. E., Sannia, G., Marzocchella, A., "Combined pretreatments of coffee silverskin to enhance fermentable sugar yield". Submitted to New Biotechnology (Elsevier).

Niglio, S., Procentese, A., Russo, M. E., Sannia, G., Marzocchella, A., "Coffee silverskin as feedstock for butanol and succinic acid production". Submitted to BioEnergy Research (Springer).

Niglio, S., Procentese, A., Russo, M., E., Piscitelli, A., Marzocchella, A., "Integrated enzymatic pretreatment and hydrolysis of apple pomace in a bubble column bioreactor". Submitted to Journal of Bioscience and Bioengineering (Elsevier).

Niglio, S., Marzocchella, A., Rehmann, L., "Clostridial conversion of corn syrup to Acetone-Butanol-Ethanol (ABE) via batch and fed-batch fermentation". Submitted to Heliyon (Elsevier).

CONFERENCE PRESENTATIONS

- **Niglio**, **S.**, Procentese, A., Russo, M., E., Sannia, G., Marzocchella, A., "Lignin removal for biomass biorefinery: ultrasound-assisted dilute acid pretreatment of coffee silverskin" XXXIX Meeting of the Italian Section of the Combustion Institute, July 4-6th 2016, Napoli (Italy).

- Carpine, R., Ferone, M., Gifuni, I., **Niglio**, **S.**, Peirce, S., Procentese, A., Raganati, F., Salemme, L., Olivieri, G., Russo, M., E., Salatino, P., Marzocchella, A., Intensification systems in bioconversion processes. Convegno GRICU 2016 "Gli orizzonti 2020dell'Ingegneria Chimica", September 12-14th 2016, Anacapri (NA) (Italy).

- **Niglio**, **S.**, Procentese, A., Russo, M., E., Sannia, G., Marzocchella, A., "Ultrasound-assisted Dilute Acid Pretreatment of Coffee Silverskin for Biorefinery Applications". ICheaP13, May 28-31st 2017, Milano (Italy).

- **Niglio, S.**, Procentese, A., Russo, M. E., Sannia, G., Marzocchella, A., "Pretreatment of coffee silverskin with ultrasound and mild alkaline solution for enhancement of sugar yield". 18th European Congress on Biotechnology – ECB 2018, July 1-4th 2018, Geneva (Switzerland).

- **Niglio, S.**, Procentese, A., Russo, M., E., Piscitelli, A., Marzocchella, A., “Integrated enzymatic pretreatment and hydrolysis of apple pomace in a bubble column bioreactor”. 12th Symposium of the European Society of Biochemical Engineering Sciences – ESBES 2018, September 9-12th 2018, Lisbon (Portugal).

COLLABORATION WITH FOREIGN RESEARCH INSTITUTION

October 2017 – March 2018: Research activity at the Department of Chemical and Biochemical Engineering, University of Western Ontario (UWO), London (Ontario, Canada) on the issue: “**Acetone-Butanol-Ethanol (ABE) fermentation of corn industrial products**”. Supervisor: Prof. Lars Rehmann.

COURSES AND SEMINARS

Attended courses

- “Nanostructures and Nanotechnologies” – Prof. De Rosa, Department of Chemical Sciences, University of Naples “Federico II”, September 21-23rd 2016, Napoli (Italy).

- “Le imprese e la ricerca: Gestione strategica dell’innovazione” – Prof. F. Bellucci, Department of chemical engineering; Dr. M. Frizzarin and Dr. A. Paliani (Ernst & Young), University of Naples “Federico II” – Scuola Politecnica e delle Scienze di Base, February 28th and March 1st-2nd 2017, Napoli (Italy).

- “Developing, enhancing and producing new enzymes” – Dr. A. Piscitelli, Department of Chemical Sciences, University of Naples “Federico II”, June 15-16th 2017, Napoli, (Italy).

- “Green Economy and Management in Engineering Projects and new product development” - Prof. L. Iandoli and Dr. G. Ferruzzi, Department of Industrial Engineering, University of Naples “Federico II”, June 6-27th 2018, Napoli, Italy.

Seminaries

1. **Thierry Tron** - “Functionalized and artificial enzymes: new bio-derived catalysts”, 14/01/2016.
2. **Angharad M. R. Gatehouse** - “Biopesticides which target voltage-gated ion channels: efficacy and biosafety”, 14/01/2016.
3. **Mario Nicodemi** - “The spatial organisation of chromatin in the nucleus of cells”, 22/03/2016.
4. **Lars Rehmann** - “Fermentative butanol production from unconventional resources”, 14/07/2016.
5. **Angelo Fontana** - “Research and exploitation of marine genetic resources from ecopysiology to biotech”, 13/07/2016.
6. **Bogdan Bjola** and **Michele Maremonti** - “La protezione brevettuale: opportunità, procedure, casi studio”, 30/09/16.

7. **Tomaso Zambelli** - “Development of th FluidFM and its applications for 2D patterning as well as 3D microprinting”, 17/10/2016.
8. **Tomaso Zambelli** - “FluidFM for single cell manipulation”, 18/10/2016.
9. **Donatella De Pascale** - “The development of a new marine biodiscovery pipeline”, 9/11/2016.
10. **Raffaele Scoccianti** - “Effective communication in industry (and tips for building a strong CV)”, 23/11/2016.
11. **Tomas Morosinotto** - “Algae metabolic engineering for the sustainable production of bio-commodities”, 6/12/2016.
12. **Leopoldo Staiano** - “Spatiotemporal regulation of phosphoinositides: OCRL and the control of lysosomal function and autophagy”, 12/01/2017.
13. **Daniela Montesarchio** - “Modified nucleosides and oligonucleotides for biomedical applications”, 18/01/2017.
14. **Jean-Bernard Michel** - “Perspectives for new bioenergy technologies in the robotic era”, 3/02/2017.
15. **Sergio Caserta** - “How “smart” a cell can be? Do transport phenomena play a role in dynamic evolution of cell system?”, 8/02/2017.
16. **Sasha Lucchini** - “From the farm to the gut: mechanisms of bacterial adaptation”, 22/02/2017.
17. **Giovanni Scopece** - “The role of pollination mechanisms in plant evolution”, 4/04/2017.
18. **Ece Ummu Deveci** - “Fungal membrane bioreactor and photocatalytic processes in industrial wastewater”, 24/05/2017.
19. **Alessandro Weisz** - “Genomic view of the oncogenic and oncosuppressor activities of ERalpha and ERbeta in breast cancer”, 6/06/2017
20. **Gabriel Luna-Barcenas** - “Nanocomposites for biochemical and environmental applications”, 5/07/2017.
21. **Thomas Kolb** - “The BIOLIQ-BTL-Process line: from basic research to pilot plant operation”, 26/07/2017.
22. **Giuseppe Zollo** and **Luca Iandoli** - “The collapse of complex societies”, 06/06/2018.
23. **Gabriella Ferruzzi** and **Pierluigi Rippa** - “Smart cities in a smart world”, 08/06/2018.
24. **Giuseppe Bruno** and **Carmela Piccolo** - “Energy efficient operations: best practice for improving organizational processes”, 18/06/2018.
25. **Thierry Tron** - “En route to synthetic biology: discussion from several examples”, 22/06/2018.
26. **Ivana Quinto** - “Green supply chain management”, 26/06/2018.
27. **Alessio Cimmino** - “Bioactive compounds: extraction and purification methods”, 26/06/2018.
28. **Brindusa Mihaela Sluser** - “Environmental impact and risk assessment: conventional versus integrated approach”, 27/06/2018.
29. **Carmen Teodosiu** - “Sustainability challenges in wastewater treatment and management”, 27/06/2018.

University activities

Teaching experience

- Mass balance exercises for students of the course “Tecnologia degli impianti dell’industria farmaceutica” (Prof. Antonio Marzocchella).
- Exercises on product recovery techniques (filtration, sedimentation, centrifugation, extraction) for students of the course “Tecnologia degli impianti dell’industria farmaceutica” (Prof. Antonio Marzocchella).

Other activities

- Participation to the “Open Day” of Department of Chemical Sciences for the orientation of high school students – Complesso universitario Monte Sant’Angelo, via Cinthia, Napoli, 23/02/2016.
- Participation to the “Open Day” of Department of Chemical Sciences for the orientation of high school students – Complesso universitario Monte Sant’Angelo, via Cinthia, Napoli,14/02/2017.
- Participation to “Futuro Remoto” at the stand of “Istituto di Ricerche sulla Combustione (IRC-CNR)” – Piazza Plebiscito, Napoli, 25-26/05/2017.

APPENDIX

The section A.1 reports on the results of preliminary tests about the pretreatment of CSS. The pretreatment based on the combination of acid solution and ultrasound field was applied to carry this preliminary study. This work was carried out at Dipartimento di di Ingegneria Chimica, dei Materiali e della Produzione Industriale of the Università degli Studi di Napoli 'Federico II'.

The section A.2 reports on the activity of clostridial fermentation of corn syrup to produce solvents (ABE fermentation) carried out at Department of Chemical and Biochemical Engineering, University of Western Ontario (UWO), London, (Canada) under the supervision of Prof. Lars Rehmann.

A.1 Ultrasound-assisted Dilute Acid Pretreatment of Coffee Silverskin for Biorefinery Applications

Saverio Niglio[a], Alessandra Procentese[b], Maria Elena Russo[b], Giovanni Sannia[c], Antonio Marzocchella[a]

[a] Dipartimento di Ingegneria Chimica dei Materiali e della Produzione Industriale, Università degli Studi di Napoli Federico II, P.le V. Tecchio 80, 80125 Napoli
[b] Istituto di Ricerche sulla Combustione - Consiglio Nazionale delle Ricerche, P.le V. Tecchio 80, 80125 Napoli
[c] Dipartimento di Scienze Chimiche, Università degli Studi di Napoli Federico II, Complesso Universitario di Monte Sant'Angelo, Via Cinthia, 80126 Napoli

Abstract

Coffee silverskin (CSS) is an agro-food processing waste (AFW) largely produced in roasting factories at almost constant rate over the year. The CSS can be used as a source of fermentable sugars within a biorefinery approach for its exploitation. Pretreatment process and enzymatic hydrolysis are required to efficiently recovery monomeric fermentable sugars from AFWs such as CSS. Current research is focused on the selection of novel processes for biomass pretreatments able to provide effective lignin removal, to minimize the production of by-products that may inhibit the fermentation of the produced sugars, and to be cost saving with respect to conventional pretreatments.

The aim of the present study was to investigate the effect of the ultrasound (US) assisted dilute acid pretreatment on the effective separation of lignin and polysaccharides of CSS. The effects of two operating conditions – biomass loading and sulphuric acid concentration - were studied to assess their effects on the sugar yields after enzymatic hydrolysis. The production of typical species that may inhibit the fermentation of the hydrolysate was monitored. Inhibitors like furfural, 5-hydroxymethyl furfural (HMF), ferulic and p-coumaric acid were present at concentrations lower than 1 mg/L after enzymatic hydrolysis of the pretreated CSS.

As a result, the hydrolysate of US dilute acid pretreated CSS may be used for fermentation without detoxification step.

Introduction

The shift from fossil resources to renewable biomass-based resources is generally viewed as the key to the development of a sustainable industrial society and to the effective management of greenhouse gas emissions (Ragauskas et al., 2006). In particular, the recent trend of the development of eco-sustainable processes based on the biomass resources is the exploitation of biomass feedstock according to the biorefinery concept. The overall goal of the biorefinery production concept is the production of a variety of goods (fuels, chemicals, etc.) from biomass feedstocks by means of a combination of technology/processes aimed at the exploitation of all components of the biomass (Fitzpatrick et al., 2010). Biomass feedstocks include agro-food wastes (AFWs). Indeed, recent investigation points out that AFWs are a promising option for the production of marketable chemicals and/or biofuels, being economic and eco-friendly due to their unique chemical composition, large availability, sustainability and low costs (Sud et al., 2008). AFWs are mainly composed of organic materials such as carbohydrate polymers, proteins, lipids, lignin and organic acids (Ezejiofor et al., 2014).

Coffee Silverskin (CSS) may be classified as AFWs. CCS is the residual thin tegument wrapping the green coffee beans and it is produced during beans roasting (Esquivel and Jiménez, 2012). Typical composition of CSS is: 23.77 % cellulose; 16.68 % hemicellulose; 28.58 % lignin (dry matter) (Ballesteros et al., 2014). The global production of coffee beans is about 148 millions of 60 kg bags (ICO) and CSS is about 4.2 % (w/w) of coffee beans (Ballesteros et al., 2014). CCS is produced in roasting factories and it may be easily delivered as renewable residues for biorefinery applications. The first step of CSS exploitation in a biorefinery is its pretreatment.

The pretreatment step is one of the main obstacles for the success of processes based on lignocellulosic biomass (Raganati et al., 2014). The main goals of the pretreatment process are to remove lignin (cross-linked polymers of phenolic monomers), to reduce the crystallinity of cellulose, and to increase the porosity of the lignocellulosic materials to enhance the efficiency of the enzymatic hydrolysis of cellulose and hemicellulose (Kumar et al., 2009). Conventional pretreatment strategies include acid, alkaline solvents, steam explosion, and wet oxidation (Velmurugan and Muthukumar, 2011). Conventional pretreatments are typically carried out at high temperature and produce compounds that may inhibit the fermentation step of hydrolyzed sugars. Recently, alternative pretreatments – e.g. lignin extraction by green solvent (ionic liquids/DES) pretreatment and ultrasound (US) assisted pretreatment - are investigated to increase the fraction of hydrolyzed sugars content and to reduce the inhibitor concentration (Procentese et al., 2015; Bussemaker and Zhang, 2013). US assisted pretreatment exploits high temperature (500−15000 K) and pressure (100−5000 atm) created for a period of microseconds by cavitation phenomena within the liquid phase to enhance physical and chemical disruption of the lignocellulosic biomass providing the separation of structural polymers (cellulose, hemicellulose and lignin) (Subhedar and Gogate, 2013). The use of aqueous solvents can enhance the chemical effects of the process (Luo et al., 2013). Enzymatic hydrolysis of pretreated biomass allows to release fermentable pentose and hexose sugars from carbohydrate polymers that can be used for the production of biofuels (e.g. butanol) (Procentese et al., 2014) and chemicals (e.g. succinic acid) (Ferone et al., 2016).

The aim of the present study was to investigate the application of US irradiation in the dilute acid pretreatment of CSS. Tests were carried out to assess the influence of operating parameters such as biomass loading and sulphuric acid concentration on sugar yield.

Materials and methods

Feedstock

Coffee silverskin was kindly supplied by Illycaffè SPA (Trieste, Italy) (Fig A.1.1A). CSS was oven dried at 50 °C until constant weight was approached. The dry CSS was milled in a cutting mill (MF 10, IKA, Staufen, Germany) to a size lower than 0.5 cm (Figure A.1.1B) and stored under controlled humidity. The raw moisture of CSS was 35%. Cellulose, hemicellulose and lignin content in CSS were assessed according to the US National Renewable Energy Laboratory protocols (Sluiter et al., 2012).

Fig A.1.1*: Raw CSS supplied by Illycaffè SpA (A); dried and milled CSS (B).*

Pretreatment

Pretreatment tests were carried out in a 100 mL glass beaker. CSS powder was mixed in the beaker with a pre-fixed volume (40 mL) of H_2SO_4 solution. A probe type ultrasonic processor (VCX500, 20kHz, Sonics & Materials Inc, USA) equipped with an Epishear^{TM} 6.4 mm probe (Active Motif, USA) was used for the US irradiation of the suspension. The beaker was housed in an ice bath to prevent the temperature increase of the suspension during sonication. The slurry produced after the sonication was washed with deionized water until the pH of supernatant increased up to 7. The biomass solid residue was recovered by centrifugation and it was oven dried at 50°C until a constant weight was reached. The recovered biomass solid was characterized in terms of cellulose, hemicellulose and lignin content.

Enzymatic hydrolysis

The enzymatic hydrolysis of the US pretreated CSS was carried out in 100 mL Pyrex® capped bottles: 1 g of US dilute acid pretreated biomass was incubated in 20 mL 0.1 M sodium citrate buffer pH 4.8 supplemented with 200 μL of 2% sodium azide. Hydrolysis was carried out using 0.015 $g_{enzyme}/g_{cellulose}$ of the cellulase cocktail Cellic CTec2 (Novozymes, Denmark) at 50 °C for 48 h. The bottles were housed on an orbital shaker operated at 180 rpm (SKI 4 Shaker Incubator, ARGO LAB, Italy). The hydrolysed sample was centrifuged (10 min at 10,000 rpm at room temperature) to recover the supernatant. The supernatant was filtered by means of a 0.2 μm filter (Millex, Ireland). The assessment of the reducing sugar in the filtered supernatant was carried out according to the 3,5-dinitrosalicylic acid method (Wood et al., 2012).

The enzymatic hydrolysis of pretreated samples was characterized in terms of ratio between the mass of the recovered sugars and the mass of sugars in the pretreated biomass (Y_S).

Operating conditions

Sample sonication lasted 8 min. The specific sonication power (energy for dry biomass unit) was 300 W/g_{DM}. Tests were carried out to assess the effects of biomass loading and acid concentration on ultrasound-assisted pretreatment of CSS.

The biomass loading (mass of biomass for solution volume unit) ranged between 3 and 10% w/v. The concentrated H_2SO_4 ($96\%_W$) was supplemented to the biomass suspension at dilution ranging between 0.5 and 2% v/v.

The table A.1.1 list the set of operating conditions set in the test campaign. All the tests were carried out in duplicate and the average values were reported.

***Table A.1.1**: Operating conditions of tests*

Trial number	Biomass loading (%w/v)	H_2SO_4 conc. (%v/v)
1	3	2
2	3	0.5
3	10	0.5
4	10	2
Unpretreated	0	0

Inhibitor analysis

The concentrations of furfural, HMF, ferulic acid and p-coumaric acid were measured in the liquid phase of the CSS-pretreated and hydrolysed. The liquid samples were centrifuged, filtered by means of a 0.2 μm cut-off filter (Millex, Ireland), and analysed by means of a HPLC Agilent 1100 system (Agilent Technology, Palo Alto, USA). Inhibitors were separated by means of a Phenomenex Luna C18(2) column (250 mm × 4.6 mm, 5 μm) at room temperature. The mobile phase was 0.1% formic acid aqueous solution (A) and pure methanol (B). The gradient of the mobile phase was: 0-20 min 5% A/95% B-30% A/70% B; 20-40 min 30% A/70% B; 40-45 min 30% A/70% B; after 45 min 5% A/95% B. The volumetric flow rate was: 1.2 mL/min from 0 to 20 min; increase up to 1.5 mL/min between 20 to 21 min; 1.5 mL/min from 21 to 40 min; decrease down to 1.2 mL/min from 40 to 45 min. The injection volume was set at 10 μL. Separated products were detected by means of a photodiode array detector at 276 nm (Agilent Technologies, model G1365B). The concentration of inhibitors was measured with respect to standards.

Results and discussion

Composition of raw and pretreated CSS

Raw CSS and pretreated CSS were analysed according to the procedure reported in “Feedstock” in terms of cellulose, hemicellulose and lignin content. The composition is reported in Table A.1.2. The total fraction of cellulose-hemicellulose-lignin in the pretreated biomass was higher than that assessed in the raw material: the pretreatment reduces the fraction of components such as ashes, proteins, and lipids. The lignin content seems not to be affected by the pretreatment according to potential dissolution and re-polymerization reactions promoted by ultrasound (Bussemaker and Zhang, 2013). The fraction of cellulose/hemicellulose reduced with respect to the lignin for the samples 3 and 4 indicating a likely loss of sugars in the pretreatment solvent.

***Table A.1.2**: Biomass composition after pretreatment*

Trial number	Cellulose (%$_{DM}$)	Hemicellulose (%$_{DM}$)	Lignin (%$_{DM}$)	Tot analysed (%$_{DM}$)
1	25 ± 0.7	8.5 ± 0.8	34 ± 0.3	67.5 ± 2.1
2	24.5 ± 1.4	9 ± 0.7	34 ± 0.7	67.5 ± 2.8
3	24 ± 0.4	9 ± 0.6	35 ± 0.3	68 ± 1.3
4	23.5 ± 0.8	9 ± 1.1	36 ± 1.4	68.5 ± 3.3
Raw dry biomass	23.5 ± 1	7.5 ± 0.7	31 ± 0.8	62 ± 2.5

Hydrolysis of US-assisted pretreated CSS

The hydrolysis of raw CSS was characterized by sugar yield (*Ys*) of about 0.103 g/g. Figure A.1.2 reports the results of sugar yields as a function of the biomass loading (BL) and of the sulphuric acid concentration (Ac). The analysis of the *Ys* suggests that the sugar yield of the pretreated biomass was larger than that assessed for the raw CSS. Although the fraction of lignin increased in the pretreated CSS, the US-assisted acid pretreatment was successful whatever the operating conditions set.

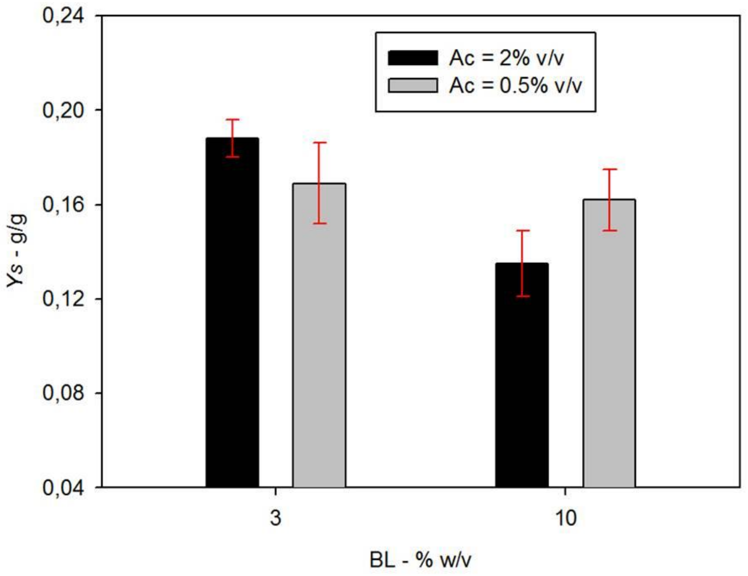


Fig A.1.2*: Sugar yield as function of biomass loading (BL) and acid concentration (Ac).*

The effect of the biomass loading on sugar yield is presented in Figure A.1.2. At the largest acid concentration investigated, the increase of biomass loading adversely affected the sugar yield. At low acid concentration effect of biomass loading on sugar yield seems to be negligible. It is possible to infer that at high biomass loading the accessibility of the biomass to pretreatment agents (acid molecules, radicals and other chemical species produced during cavitation phenomena) is reduced. In heterogeneous systems (slurry), the cavitation activity is enhanced by the presence of the solids but at large concentration of solids the viscosity increases and the mixing reduces with the mass transfer and heat transfer (Rehman et al., 2013). The negative effect of large biomass loading on pretreatment of lignocellulosic biomass is in agreement with previous study reported by Noureddini and Byun (2010) for dilute acid pretreatment of distiller’s grain and corn fibre without US.

Effect of acid concentration on sugar yield is also presented in Figure A.1.2. At low level of biomass loading, the sugar yield slightly increased with the acid concentration: pretreatment occurring at low biomass loading allows much effective dispersion of the chemical species in the solution so that their interaction with the lignocelluloses provides much effective biomass modification.

Increase in acid concentration allows larger lignin conversion. At fixed level of lignin conversion, increase in biomass loading leads to increase of the concentration of products from lignin depolymerisation. These products can locally reach values large enough to promote lignin recondensation in polymeric species (Bussemaker and Zhang, 2013). This phenomenon can hinder effective dissolution of lignin at both large biomass and acid concentrations so that the composition and structure of pretreated biomass provides decrease in sugar yield.

Analysis of inhibitors

The analysis of the liquid phases of hydrolysis pointed out that the fermentation inhibitors - furfural, HMF, ferulic acid and p-coumaric acid - were present at concentrations lower than 1 mg/L. The concentration was lower than the toxic levels known for various *Clostridium* sp. (Ezeji and Blaschek, 2008).

Conclusions

In this work the effect of biomass loading and acid concentration on ultrasound-assisted pretreatment of CSS were assessed. The decrease of biomass loading and the increase of acid concentration increased the sugar yield. The main inhibitors for acetone-butanol-ethanol fermentation (furfural, HMF, ferulic acid and p-coumaric acid) were present at concentration lower than the toxic limit for various *Clostridium* sp.

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Reference

Ballesteros, L. F., Teixeira, J. A., Mussatto, S. I., 2014, Chemical, functional, and structural properties of spent coffee grounds and coffee silverskin, Food Bioprocess Technology ,7, 3493–3503

Bussemaker, M., J., Zhang, D., 2013, Effect of ultrasound on lignocellulosic biomass as a pretreatment for biorefinery and biofuel applications, Ind. Eng. Chem. Res., 52, 3563−3580

Esquivel, P.; Jiménez, V. M., 2012, Functional properties of coffee and coffee by-products, Food Res. Int., 46, 488−495

Ezeji, T., Blaschek, H. P., 2008, Fermentation of dried distillers’ grains and soluble (DDGS) hydrolysates to solvents and value-added products by solventogenic clostridia, Bioresource Technology, 99, 5232–5242

Ezejiofor, T. I. N., Enebaku, U. E.; Ogueke, C., 2014, Waste to Wealth- Value Recovery from Agrofood Processing Wastes Using Biotechnology: A Review, British Biotechnology Journal, 4(4), 418-481

Ferone, M., Raganati, F., Olivieri, G., Salatino, P., Marzocchella, A., 2016, Succinic Acid Production from Hexoses and Pentoses by Fermentation of *Actinobacillus succinogenes*, Chemical Engineering Transactions, 49, 211-216 DOI: 10.3303/CET1649036

FitzPatrick, M., Champagne, P., Cunningham, M., F., Whitney, R., A., 2010, A biorefinery processing perspective: Treatment of lignocellulosic materials for the production of value-added products, Bioresource Technology, 101, 8915–8922

ICO (International Coffee Organization), Global Coffee Production - www.ico.org - accessed 22.11.2016

Kumar, P., Barrett, D. M., Delwiche, M., J., Stroeve, P., 2009, Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production" Ind. Eng. Chem. Res., 48, 3713–3729

Luo, J., Fang, Z., Smith Jr., R., L., 2013, Ultrasound-enhanced conversion of biomass to biofuels, Progress in Energy and Combustion Science, http://dx.doi.org/10.1016/j.pecs.2013.11.001

Noureddini, H., Byun, J., 2010, Dilute-acid pretreatment of distillers' grains and corn fiber, Bioresour. Technol., 101, 1060–1067

Procentese, A., Guida, T., Raganati, F., Olivieri, G., Salatino, P., Marzocchella, A., 2014, Process Simulation of Biobutanol Production from Lignocellulosic Feedstocks, Chemical Engineering Transactions, 38, 343-348 DOI: 10.3303/CET1438058

Procentese, A., Johnson, E., Orr, V., Garruto Campanile, A., Wood, J., A., Marzocchella, A., Rehmann, L., 2015, Deep eutectic solvent pretreatment and subsequent saccharification of corncob, Bioresource Technology, 192, 31–36

Raganati, F., Procentese, A., Olivieri, G., Salatino, P., Marzocchella, A., 2014, Biobutanol Production from Hexose and Pentose Sugars, Chemical Engineering Transactions, 38, 193-198 DOI: 10.3303/CET1438033

Ragauskas, A. J., Williams, C. K., Davison, B. H., Britovsek, G., Cairney, J., Eckert, C. A., et al., 2006, The path forward for biofuels and biomaterials. Science, 311, 484–489

Rehman, M., S., U., Kim, I., Chisti, Y., Han, J.-I., 2013, Use of ultrasound in the production of bioethanol from lignocellulosic biomass, Energy Education Science and Technology Part A: Energy Science and Research Volume (issues) 30(2): 1391-1410

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D., 2012, NREL Technical Report, NREL/TP-510-42618

Subhedar, B. P., Gogate, P. R., 2013, Intensification of Enzymatic Hydrolysis of Lignocellulose Using Ultrasound for Efficient Bioethanol Production: A Review, Ind. Eng. Chem. Res., 52, 11816−11828

Sud, D., Mahajan, G., Kaur, M. P., 2008, Agricultural waste material as potential adsorbent for sequestering heavy metal ions from aqueous solutions – A review, Bioresource Technology, 99, 6017–6027

Velmurugan, R., Muthukumar, K., 2011, Utilization of sugarcane bagasse for bioethanol production: Sono-assisted acid hydrolysis approach, Bioresour. Technol., 102, 7119–7123

Wood, I. P., Elliston, A., Ryden, P., Bancroft, I., Roberts, I. N., Waldron, K. T., 2012, Rapid quantification of reducing sugars in biomass hydrolysates: Improving the speed and precision of the dinitrosalicylic acid assay, Biomass and Bioenergy, 44, 117-121

A.2 Clostridial conversion of corn syrup to Acetone-Butanol-Ethanol (ABE) via batch and fed-batch fermentation

Saverio Niglio[a], Antonio Marzocchella[a], Lars Rehmann[b]

[a] Dipartimento di Ingegneria Chimica, Dei Materiali e della Produzione Industriale, Università degli Studi di Napoli Federico II, P.le V. Tecchio 80, 80125 Napoli, Italy

[b] Department of Chemical and Biochemical Engineering, University of Western Ontario, 1151 Richmond Street, London, ON, Canada

Abstract

Corn syrup - a commercial product derived from saccharification of corn starch - was used to produce acetone-butanol-ethanol (ABE) by *Clostridium* spp. Screening of commercial *Clostridium* spp., substrate inhibition tests and fed-batch experiments were carried out to improve ABE production using corn syrup as only carbon source. The screening tests carried out in batch mode using a production media containing 50 g/L corn syrup revealed that *C. saccharobutylicum* was the best performer in terms of total solvent concentration (12.46 g/L), yield (0.30 g/g) and productivity (0.19 g/L/h) and it was selected for successive experiments. Concentration of corn syrup higher than 50 g/L resulted in no solvents production. Fed-batch fermentation improved ABE production with respect to batch fermentation: the butanol and solvent concentration increased up to 8.70 and 16.68 g/L, respectively. The study demonstrated the feasibility of producing solvents via ABE fermentation using a concentrated sugar solution (corn syrup) as substrate.

Keywords: Corn syrup, ABE fermentation, *Clostridium spp.*, Butanol.