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Effect of pretreatments on cellulase and biosurfactants production by *Bacillus* strains using lignocellulosic wastes

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I dedicate this work to:

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Table of contents

List of Figures

List of tables

Abbreviation list

AOP: Olive pomace from the previous year

CE: Catechin Equivalent

GAE: Gallic acid Equivalent

HMF: Hydroxymethylofurfural

H_2O_2: Hydrogen peroxidase

H_2SO_4: Sulfuric acid solution

NaOH: Sodium hydroxide

NOP: Olive pomace of the year

QE: Quercitin Equivalent

SCG: Spent coffee grounds

SFH: Sunflower hulls

Effect of pretreatments on cellulase and biosurfactants production by *Bacillus* strains using lignocellulosic wastes

Abstract

Over the last decades, research on the use of lignocellulosic biomass as an alternative energy feedstock to fossil fuels has gained considerable attention because of their high fermentable carbohydrates and bioactive products contents. One of the major limitations to lignocellulosic biomass valorization is its recalcitrance to enzymatic hydrolysis caused by the close inter-component association between main constituents of the plant cell wall. Pretreatments overcame barriers that make native biomass recalcitrant and make cellulose amenable to enzymatic hydrolysis. However, after pretreatments, large amounts of by-products such as polyphenols are released, which can inhibit the fermentation and decrease productivity.

In this study *Bacillus cereus* ATCC® 11778TM strain was used for the fermentation process and production of cellulase and biosurfactants. The main objectives of this work were to understand the effect of pretreatments and the nature and composition of substrates on polyphenols content, cellulase induction, and biosurfactants production by *Bacillus cereus* 11778. Also, to discover the effect of phenolic compounds on cellulase activity and biosurfactants production. Moreover, to understand the nature of the relationship between these three values added products.

For that, four substrates were collected: spent ground coffee (SGC), olive pomace of the year (NOP), olive pomace from the previous year (AOP), and sunflower hulls (SFH). These substrates were pretreated with sulfuric acid, sodium hydroxide, hydrogen peroxide, and chloroform/acetone separately. Different assays were done for polyphenols, flavonoids, cellulase, and biosurfactants.

After analyzing the pretreatment's effect on the different value-added products, the obtained results indicated that polyphenols and flavonoids content decreased after treatments in all substrates except in SFH. Cellulase inductivity also decreased, and reached its minimal activity with SCG after alkaline pretreatment at 48h (0.99 ± 0.02 U/ml), while olive pomaces induced a higher production after treatments, where NOP reached up to 2.02 ± 0.1 U/ml after oxidative treatment. High yields of biosurfactants were also recorded with untreated substrates after 72h, the highest value was obtained with SCG (22.18 ± 0.73 mg/ml), while AOP gave high productivity after treatments compared to untreated ones

However, pretreatments may cause a decrease in lipid compounds necessary for bacteria to produce biosurfactants, therefore, a decrease in cellulase activity. Correlation studies revealed that cellulase activity, polyphenols content, and biosurfactants production were strongly correlated and can influence each other positively, which indicates that biosurfactants promote the production of cellulase. However, polyphenols can also have a positive effect on yields and enhance cellulase activity by playing the role of nature surfactants.

Keywords: lignocellulosic biomass; pretreatment; cellulase; polyphenols; biosurfactants

Effet des prétraitements sur la production de cellulase et des biosurfactants par des souches de *Bacillus* en utilisant des déchets lignocellulosiques

Résumé

Au cours de la dernière décennie, la recherche sur l'utilisation de la biomasse lignocellulosique comme matière première d'énergie alternative aux combustibles fossiles a attiré une attention considérable en raison de ca teneur élevée en glucides fermentescibles et en produits bioactifs. L'une des principales limites de la valorisation de la biomasse lignocellulosique est sa récalcitrance à l'hydrolyse enzymatique causée par l'association étroite entre les principaux constituants de la paroi végétale. Les prétraitements surmontent ces barrières et rendent la cellulose accessible à l'hydrolyse enzymatique. Cependant, après les prétraitements, de grandes quantités de sous-produits tels que les polyphénols sont libérés, ce qui peut inhiber les fermentations et diminuer la productivité.

Dans cette étude, la souche *Bacillus cereus* ATCC® 11778TM a été utilisée pour le processus de fermentation et la production de cellulase et de biosurfactants. Les principaux objectifs de ce travail étaient de comprendre l'effet des prétraitements ainsi que la nature et la composition des substrats sur le taux des polyphénols, l'induction de la cellulase et la production des biosurfactants par *Bacillus cereus*11778. Aussi, pour découvrir l'effet des composés phénoliques sur l'activité de la cellulase et la production de biosurfactants. De plus pour comprendre la nature de la relation entre ces trois produits à valeur ajoutée.

Pour cela, quatre substrats ont été collectés : marc de café (SGC), grignons d'olive de l'année (NOP), grignons d'olive de l'année précédente (AOP), et les coques de tournesol (SFH). Ces substrats ont été traités avec de l'acide sulfurique, de l'hydroxyde de sodium, du peroxyde d'hydrogène et du chloroforme/acétone séparément. Différents dosages ont été effectués pour le taux de polyphénols, les flavonoïdes, la cellulase et les biosurfactants.

Après avoir analysé l'effet des prétraitements sur les différents produits à valeur ajoutée, les résultats obtenus ont indiqué que le taux de polyphénols et de flavonoïdes a diminué après les traitements dans tous les substrats sauf dans le SFH. L'inductibilité de la cellulase a également diminué et atteint son activité minimale avec le SCG après le prétraitement alcalin à 48h (0,99 ± 0,02 U/ml), tandis que les grignons d'olive ont induit une production plus élevée après les traitements, où NOP a atteint jusqu'à 2,02 ± 0,1 U/ml après le traitement oxydant. Des rendements élevés de biosurfactants ont également été enregistrés avec des substrats non traités après 72h, la valeur la plus élevée a été obtenu avec le SCG (22,18 ± 0,73 mg/ml) tandis que l'AOP a donné une productivité élevée après les traitements par rapport à un non traité.

Cependant, les prétraitements peuvent provoquer une diminution des composés lipidiques nécessaires à la production de biosurfactants par les bactéries, diminuant ainsi l'activité de la cellulase. Les études de corrélation ont révélé que l'activité de cellulase, le taux de polyphénols et la production de biosurfactants étaient fortement corrélées et peuvent s'influencer positivement, ce qui indique que les biosurfactants favorisent la production de cellulase, et que les polyphénols peuvent également avoir un effet positif sur les rendements et améliorer l'activité de la cellulase en jouant le rôle de surfactants naturels.

Mots-clés : biomasse lignocellulosique; prétraitement; cellulase; polyphénols; biosurfactants

تأثير المعالجة الأولية على إنتاج السيلولاز والمؤثرات السطحية الحيوية بواسطة سلالات عصوية باستخدام نفايات ليغنوسيلولوزية

ملخص

على مدى العقود القليلة الماضية، اكتسبت البحوث حول استخدام الكتلة الحيوية الليغنوسيلولوزية كطاقة بديلة للوقود الأحفوري اهتمامًا كبيرًا بسبب احتوائها على مستوى عال من الكربوهيدرات والمنتجات النشطة بيولوجيًا. احد العوائق التي تحد من استخدام الكتلة الحيوية الليغنوسيلولوزية هي بنيتها المعقدة الناجمة عن الترابط الوثيق بين المكونات الرئيسية لجدار الخلية النباتية والتي تجعلها غير قابلة للتحلل الإنزيمي. استطاعت المعالجة الأولية إزالة الحواجز التي تجعل الكتلة الحيوية معقدة وبذلك تجعل السيليلوز قابل للتحلل الإنزيمي، لكن مع ذلك فانه بعد المعالجة الأولية، يتم تحرير كميات كبيرة من المنتجات الثانوية مثل متعددات الفينول، والتي يمكن أن تمنع التخمير وتقلل الإنتاجية.

في هذه الدراسة تم استخدام سلالة *Bacillus cereus* ATCC® 11778TM لعملية التخمير وإنتاج السيلولاز والمؤثرات السطحية الحيوية. تتمثل الأهداف الرئيسية لهذا العمل في فهم تأثير المعالجات الأولية وطبيعة ومكونات الركائز على محتوى متعددات الفينول وإنتاج السيلولاز و والمؤثرات السطحية الحيوية بواسطة *Bacillus cereus* 11778TM. أيضا، لاكتشاف تأثير المركبات الفينولية على نشاط السيلولاز وإنتاج المؤثرات السطحية الحيوية. زيادة على ذلك يهدف هذا العمل أيضا لفهم طبيعة العلاقة بين هذه المنتجات الثلاثة ذات القيم المضافة.

من اجل ذلك، تم جمع أربعة ركائز تتمثل في : تفل القهوة (SGC)، ثفل الزيتون لهذا العام (NOP)، ثفل الزيتون من العام السابق (AOP)، وقشور بذورعباد الشمس (SFH). تمت معالجة هذه الركائز مسبقًا بحمض الكبريتيك وهيدروكسيد الصوديوم وبيروكسيد الهيدروجين وخليط الكلوروفورم/الأسيتون كل على حدة. تم إجراء فحوصات مختلفة لمتعددات الفينول والفلافونويد والسيلولاز والمؤثرات السطحية الحيوية.

بعد تحليل تأثير المعالجة الأولية على المنتجات ذات القيمة المضافة المختلفة، أشارت النتائج التي تم الحصول عليها إلى انخفاض محتوى متعددات الفينول ومحتوى الفلافونويد بعد العلاج في جميع الركائز باستثناء SFH. كما انخفض تحريض السليلوز، ووصل إلى الحد الأدنى من نشاطه مع SCG بعد المعالجة القاعدية في 48 ساعة (0.99 ± 0.02 وحدة / مل)، في حين تسبب ثفل الزيتون في إنتاج أعلى بعد العلاج، حيث وصل NOP إلى 2.02 ± 0.1 وحدة / مل بعد المعالجة التأكسدية. كما تم تسجيل إنتاج عالي للمؤثرات السطحية الحيوية مع الركائز الغير معالجة بعد 72 ساعة، وهي أعلى نسبة مع (22.18 ± 0.73 SCG مغ / مل) في حين أعطت AOP إنتاجية عالية بعد العلاجات مقارنة بالركيزة الغير المعالجة.

ومع ذلك، يمكن للمعالجة الأولية أن تسبب انخفاضًا في المركبات الدهنية اللازمة للبكتيريا لإنتاج المؤثرات السطحية الحيوية، وبالتالي زيادة نشاط السيلولاز. كشفت دراسات معاملات الارتباط أن نشاط السيلولاز ومحتوى متعددات الفينول وإنتاج المؤثرات السطحية الحيوية يرتبطون ارتباطًا قويًا ويمكنهم التأثير بشكل إيجابي على بعضهم البعض مما يشير إلى أن المؤثرات السطحية الحيوية تعزز إنتاج السيلولاز، كما أن متعددات الفينول يمكن أن يكون لها أيضًا تأثير إيجابي على الإنتاج وتعزز نشاط السيلولاز من خلال لعب دور المؤثر السطحي الطبيعي.

كلمات مفتاحية : الكتلة الحيوية الليغنوسيلولوزية; معالجة أولية; إنزيم السيلولاز ; متعددات الفينول; مؤثرات سطحية حيوية

Introduction

The world's scientific community is presently confronted with big crisis related to energy; the depletion of petroleum resources, deforestation, burning of fossil fuels and vegetation, environmental degradation due to emission of greenhouse gases, and global warming (Radhakrishnan, Prasad, Kumar, & Subramanian, 2020). Therefore, there is an immediate necessity to research alternative renewable, safer, and sustainable carbon sources. The intensive industrial activities had led to the generation of huge amounts of lignocellulosic materials; considered as wastes and are often burned. Since about half of the organic carbon in the biosphere is present in the form of cellulose; lignocellulosic wastes are a promising eco-friendly alternative and economical solution, its conversion into fuels and valuable chemicals has paramount importance (Abdeshahian, Kadier, Rai, & Silva, 2020; Isikgor & Becer, 2015). The major component of lignocelluloses is cellulose protected by other polymers of hemicelluloses and lignin which confers resistance to microorganisms, enzymes, and chemical attacks. This recalcitrance and crystallinity nature of lignocelluloses limit the utilization of this valuable source.

Therefore, to valorize these wastes to value-added products; pretreatment technologies are required before that conversion. For that, many physical, chemical, and biological pretreatment methods have been developed to open up that recalcitrance structure and allow the access to the desired fractions (Flórez Pardo, Salcedo Mendoza, & López Galán, 2019; Romaní, Rocha, Michelin, Domingues, & Teixeira, 2020). However, treatment processes release a range of molecules; depending on the starting materials and type of treatment. Functionalized molecules such as lipids and monosaccharides are released, as well as inhibitory co-products, such as 5-hydroxymethylfurfural, furfural, acetic acid, phenolic compounds, which can act as inhibitors of microbial metabolism in the fermentation process, decreasing cellulosic conversion or preventing it (Ferrand, Vasco, & Gamboa-Santos, 2020; Vázquez, Leos, Rodriguez-Duran, & Torres, 2020).

Many solutions have been developed to treat this problem. Surfactants like Tween 80 and Triton-X are often used to remove that inhibitory effect and increase cellulolytic activity. Furthermore, recent studies used microbial strains like *Bacillus* to produce eco-friendly and biodegradable alternatives biosurfactants like rhamnolipids, which could be a sustainable solution (J. Liu *et al*., 2017; Q. Zhang, Cai, & Wang, 2008).

This study aims to reveal the effects of the nature and composition of substrates and their impact on cellulase production by *Bacillus cereus* ATCC® 11778TM. The effect of four different chemicals pretreatments on cellulase induction, polyphenols yields, and biosurfactants production has been studied. Also, the relationship between cellulase production, phenolic compounds of treated and untreated substrates and the production of biosurfactants have been investigated.

Literature review

1. Lignocellulosic biomass and lignocellulosic wastes

Ecologically talking, biomass is the total mass of all living things occupying at a given time, a well-defined biotope, but in terms of energy, it represents all organic matter that can be transformed into energy. Lignocellulosic biomass is considered to be any plant-derived renewable organic matter (Hughes & Qureshi, 2014). It contains about 50 to 90% of total organic matter (Bernal *et al*., 2017). Lignocellulosic biomass's global annual primary production is about 220 billion tones on dry weight matter (Kumari & Singh, 2018) which makes lignocellulosic based wastes one of the most abundant renewable resources on the planet (Kricka, Fitzpatrick, & Bond, 2015).

1.1 Lignocellulosic wastes biomass sources

Lignocellulosic wastes have different sources, agricultural crops residues (corn cob, vine pruning residues, corn husk, brewers spent grain, oat straw, sugarcane bagasse, hazelnut pruning residues, rice straw….) (Romaní, Rocha, Michelin, Domingues, & Teixeira, 2020). Forestry residues include hardwood and softwood (wastes from wood, pulp, and paper industries...), material resulting from forest management operation, dead and dying trees (Hughes & Qureshi, 2014). Dedicated crops, and short rotation crops including grasses as switchgrass, bamboo, sweet sorghum, wheatgrass, and others (Hughes & Qureshi, 2014). Wastes from agro-food industries (Pomaces, bagasse, barks, cereal husks, straws, stalks, leaves, peels, shells…) (Romaní *et al*., 2020).

1.2 Applications and valorization of Lignocellulosic wastes biomass

Availability and wide variety of lignocelluloses sources make it the most abundant biopolymer available on earth as waste biomass (Chandra & Madakka, 2019), which could be successfully valorized by its conversion into bioproducts. Biofuels and energy were the main drivers for lignocellulosic biomass exploitation, specially for bioehanol production, but now the main challenge is a zero-waste producing society with maximum biomass valorization (Romaní *et al*., 2020). Biorefinery with the concept of biomass fractionation into its main components offers myriad benefits to the bioprocessing industries harnessing the various feedstocks into a wide range of value-added products (Ingle, Chandel, & da Silva, 2020) such as biofuels for energy (bio-hydrogen, bio-methane, bioethanol, bio-methanol, bio-butanol...) (Kumari & Singh, 2018). Biomaterials like biodegradable plastics and paper; valuable chemicals such as organic acids, biopigments, biosurfactants, biofertilizers, and enzymes production… (Ingle *et al*., 2020). Also, residues derived from food industry provide a high percentage of nonstructural components (phenolic compounds, essential oils) with interesting bioactive activities (Romaní *et al*., 2020).

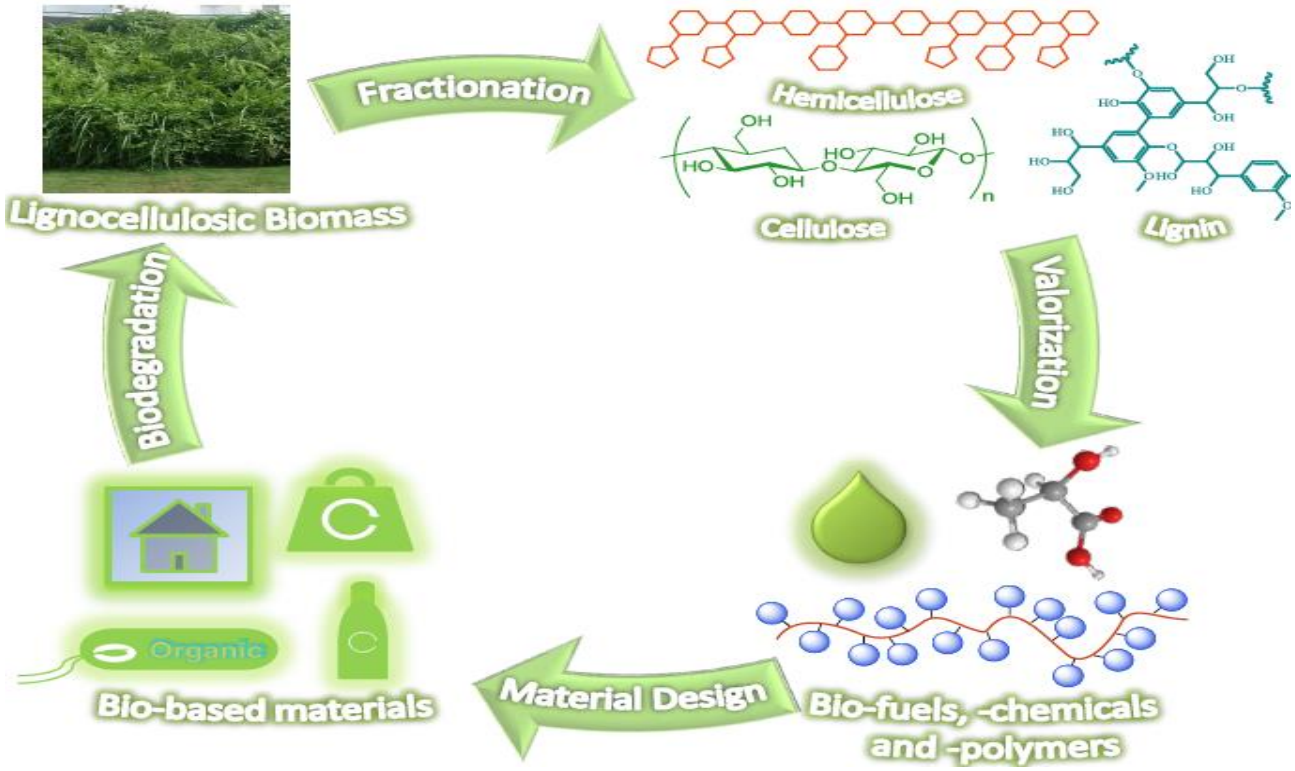

Figure 01: Main lignocellulosic fractions and some products derived from its biorefineries (Isikgor & Becer, 2015)

2. Lignocellulosic biomass structure and composition

Lignocelluloses is a complex of structural and nonstructural biopolymers, which are mainly composed of two carbohydrates polymers, cellulose [30%-50%], hemicelluloses [15%-35%] and non-carbohydrate phenolic polymer, lignin [10%-20%] and pectin, with trace amounts of other molecules, such as proteins, lipids, and ashes (Abdel-Hamid, Solbiati, & Cann, 2013; Dharmaraja *et al*., 2020). These components are strongly interlinked by noncovalent forces and covalent cross-linkages, forming an intricately linked network that provides strength to the plant cell wall (Naraian & Gautam, 2018). However, their compositions vary greatly, depending on plants type, cultivation conditions, and plants or residues age (Yang, 2007).

2.1 Cellulose

Cellulose is the most common skeletal polysaccharide, responsible for mechanical strength. It is a linear polymer of D-anhydro-glucopyranose molecules joined by β-1,4-glycosidic bonds (Ferrand, Vasco, & Gamboa-Santos, 2020), with disaccharide cellobiose as a fundamental repeating unit (Isikgor & Becer, 2015). Cellulose chains are made up with linearity and regular structure of 500–1400 D-glucose units (Zoghlami & Paës, 2019), linked together by hydrogen bonds and Van Der Walls forces (Kumari & Singh, 2018), their arrangement together forms microfibrils, which are

packed to form cellulose fibrils (Zoghlami & Paës, 2019). Each fibril has cellulose molecules that are laterally bound, while the adjacent molecules are parallel, placed in opposite directions with different degrees of orientation (Abdeshahian, Kadier, Rai, & da Silva, 2020), which makes a robust crystalline structure. Polymerization's degree defines solubility in water and most typical solvents; to solubilize it, the majority of hydroxyl bonds should break simultaneously (Ingle *et al*., 2020).

2.2 Non cellulosic Polysaccharides

Non cellulosic polysaccharides fraction is mainly composed of hemicelluloses (d-xylans, d-glucans, d-mannans, etc.) and pectins (d-galacturonans) (Ingle *et al*., 2020). Unlike cellulose, hemicelluloses have a random and amorphous structure, composed of several heteropolymers with different monosaccharide building units, pentoses (xylose and arabinose) and hexoses (mannose, glucose, and galactose) linked by more weak -glycosidic bonds (Isikgor & Becer, 2015; Zhu, Abdelaziz, Hulteberg, & Riisager, 2020), forming a complex network of bonds that provide structural strength by linking cellulose fibers into microfibrils and cross-linking with lignin (Isikgor & Becer, 2015). Polymerization's degree is much lower than that of cellulose, in a range of 100–200 units. Hemicelluloses can be extensively acetylated with acetyl groups, forming a limiting physical barrier for cellulose accessibility, by preventing cellulose binding to the catalytic domain of cellulase through increasing cellulose chain diameter or changing its hydrophobicity (Pan, Gilkes, & Saddler, 2006).

Pectin is a complex and heterogeneous polysaccharide molecule found in the middle lamella and primary cell wall of higher plants and considered as a common ingredient of all higher plants. Formed of two structural regions: smooth regions, consist of α1, 4-linked D-galacturonic acid residues, whereas the hairy regions, consists of α1, 4-linked D-galacturonic acid and L-rhamnose (Naraian & Gautam, 2018).

2.3 Lignin

Lignin is kind of three-dimensional amorphous and highly branched polyphenolic polymer (Romaní *et al*., 2020) composed of three monomer units (coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol) through C-C or C-O linkages and with polysaccharides by ether and ester bonds, leading to cross-links between them (L. Dai *et al*., 2020). It gives rigidity and cohesion to the material cell wall, confers water impermeability to xylem vessels, and forms a physicochemical barrier against microbial attack (Kumari & Singh, 2018). Lignin can be isolated with different extraction processes that influence its structure through the cleavage of bonds between different lignin monomers, or the covalent bonds to the polysaccharides, these changes create smaller fragments of lignin with different physicochemical properties, and then with suitability for different applications (Romaní *et al*., 2020).

3. Lignocellulosic enzymes

Enzymatic hydrolysis of lignocellulosic wastes needs macerating enzymes complex working synergistically including cellulase, xylanase, pectinase, and lignin-degrading enzymes, with different frequencies depending on lignocellulosic waste nature. All lignocellulosic enzymes are important in conversion bioprocesses of lignocellulosic wastes into value-added bioproducts.

3.1 Cellulase

Cellulase refers to a multi-enzyme system required for full hydrolysis of cellulose, including β-1.4-endoglucanase (EC 3.2.1.4), which catalyzes the endohydrolysis of β-1.4 D-glycosidic bonds in cellulose and β-D-glucans; exoglucanase (EC. 3.2.1.91), which also catalyzes the hydrolysis of β-1,4 D-glycosidic bonds but from the non-reducing end, releasing cellobiose units; and β-glucosidase or cellobiase (EC. 3.2.1.21), which hydrolyzes cellobiose into glucose. In synergy with other enzymes (pectinases, xylanase, and hemicellulases…), disrupting the structural integrity of lignocelluloses and enhancing the extraction of targeted molecules (J. Singh, Kundu, Das, & Banerjee, 2019). These enzymes can be isolated from different microorganisms such as bacteria and fungi (Naraian & Gautam, 2018). They are used in various industrial applications, including starch processing, animal feed production, fermentation of grain alcohol, malting and brewing, fruit and vegetable juice extraction, pulp and paper, and textile industry (Abdeshahian *et al*., 2020).

3.2 Other enzymes

The enzymatic hydrolysis of polysaccharides to soluble sugars occurs under the action of different enzymes acting in concert such as xylanases (EC 3.2.1.8), which are glycosidases catalyzes the endohydrolysis of 1,4-β-D-xylosidic linkages in xylan, involved in the production of xylose. These enzymes can be produced by many microorganisms and have potential applications in a wide range of industrial processes such as paper and food (Collins, Gerday, & Feller, 2005).

Pectinases constitute a heterogeneous group of enzymes that hydrolyze pectic substances, by depolymerization or de-esterification (esterases) reactions. Pectinolytic enzymes are naturally produced by many organisms like bacteria, fungi, and yeasts; they have been used in several industrial processes such as tea and coffee fermentation and treatment of industrial wastewater (R. S. Singh, Singh, & Pandey, 2019). However, lignin is thought to restrict the access of cellulase to their substrate because of its recalcitrance to degradation and its molecular architecture. Most of the studies established peroxidase (EC 1.11.1.X) as the most effective “delignifyer” include lignin peroxidase (EC 1.11.1.14) and manganese peroxidase (EC 1.11.1.13), which are distributed widely in plants, animals, and microbes (Falade *et al*., 2017).

3.3 Lignocellulosic wastes as substrates for enzymes production by microorganisms

Pretreated lignocellulosic wastes provide a low-cost substrate for economically viable production of enzymes in large scale bioprocesses. To grow, microorganisms need carbon sources, carbohydrate polymers of lignocellulosic wastes can be utilized as a low-cost carbon source for their growth and metabolic activities. Many microorganisms, including bacteria, yeast, actinomycetes, and fungi, can produce and secrete many enzymes (cellulases, xylanases, mannan, degrading enzymes, laccase, lignin peroxidase, manganese peroxidase, lipase, protease, amylase, pectinase, glutaminase…) to hydrolyze polysaccharides of various lignocellulosic materials by various fermentation process (Abdeshahian *et al*., 2020).

4. Pretreatment

Structural and chemical properties of native lignocellulosic biomass make it resistant to enzymatic and microbial biodegradation; to remove these obstacles, pretreatment is an initial step in exposing cellulose and hemicelluloses content for hydrolysis and promote an effective high yield conversion to fermentable sugars. Different used pretreatment technologies must be energetically and chemically economical (McMillan, 1994).

4.1 Pretreatments goals

Natural enzymatic hydrolysis of lignocellulose gives less than 20% yield because of accessibility surface area (porosity), crystallinity, lignin, and hemicelluloses content; that contribute in its recalcitrance. Pretreatment process objectives are altering such characteristics. An effective pretreatment decrease crystallinity of cellulose, increase surface area and provide easily accessible binding sites for enzymes, remove lignin and hemicelluloses, limit inhibitory products formation that prevents hydrolysis and fermentation process (Mosier *et al*., 2005).

4.2 Categories of pretreatment

Pretreatment methods have been classified into biological (fungi, bacteria) and non-biological strategies which include chemical, physical or physicochemical approaches (Hassan, Williams, & Jaiswal, 2018). Pretreatment's choice depends on the lignocellulosic waste type and efficiency in the bioconversion process (Zheng, Zhao, Xu, & Li, 2014).

4.2.1 Physical pretreatment

Physical pretreatment includes mechanical, irradiation (microwaves and ultrasounds), and all methods applied to alter biomass polymerization's degree and to modify particles size (Jędrzejczyk, Soszka, Czapnik, Ruppert, & Grams, 2019). Physical techniques avoid the use of

chemicals and microorganisms in the pretreatment process, these methods are less environmentally harmful but require high energy consumption and high production cost (Sankaran *et al*., 2020).

4.2.1.1 Mechanical pretreatments

Size reduction is a conventional method necessary to modify the crystalline structure of cellulose and increases the surface area (Dell'Omo & Spena, 2020). Principal mechanical methods are: milling, grinding, and chipping; their main disadvantage is high production costs due to high energy consumption (Jędrzejczyk *et al*., 2019).

4.2.1.2 Irradiation

Irradiation can easily penetrate lignocellulosic biomass structure, causing modification in physical and chemical proprieties, including alteration of lignin and breakdown cellulose's crystal and amorphous region, increasing surface area and decreases thermal stability. Irradiation can be done with gamma rays, microwaves, ultrasounds, and electron beam (Hassan *et al*., 2018).

4.2.2 Chemical pretreatment

Chemical processes of different natures are used, most are done with alkaline, acid, oxidizing agents, and organic solvents that promote hydrolysis and improve glucose's yield by removing hemicelluloses and lignin (Bhatia *et al*., 2020; Jędrzejczyk *et al*., 2019). But they have many disadvantages including the formation of by-products, low glucose yields, low efficiency, and high costs (Bhatia *et al*., 2020). Despite all this, they are considered very promising since they can degrade more complex structured (Pellera & Gidarakos, 2018).

4.2.2.1 Acid pretreatment

Acid pretreatment is one of the most effective chemical pretreatment. It has been proved that acid pretreatments are optimal for a wide range of substrates (hardwoods, herbaceous plants, co-products agricultural) (Ogier, Leygue, Ballerini, Pourquie, & Rigal, 1999).

The main reactions during acid treatment are the breakdown of glycosidic bonds, solubilization of hemicelluloses, precipitation of lignin, making cellulose more accessible for enzymes and microorganisms. Many acid agents are used such as sulfuric acid, hydrochloric acid, and phosphoric acid (Jędrzejczyk *et al*., 2019). But these methods have some limitations like corrosively and high toxic reaction medium, the formation of inhibitors (hydroxymethylfurfural (HMF), furfural, and acetic acid).

4.2.2.2 Alkaline pretreatment

Bases like NaOH, KOH, $Ca(OH)_2$ cause intermolecular saponification of ester bonds in lignocellulosic which leads to increase porosity and internal surface area, decrease polymerization

degree and crystallinity (Zheng *et al*., 2014). These Pretreatments are more efficient for lignocellulosic materials characterized by low lignin content. The main limitations of alkaline treatment are long duration and neutralization difficulties of post-treatment mixture. On the other hand, these methods use cheap chemicals, mild reaction conditions and give effective removal of lignin (Jędrzejczyk *et al*., 2019), many researchers have reported a positive effect of NaOH pretreatment for rice straw, spruce wood wastes, sugarcane, cassava and peanuts wastes, corn cob and organic fraction of municipal solid wastes (Mtui, 2009).

4.2.2.3 Oxidative pretreatment

Oxidizing agents like hydrogen peroxide and ozone causes many chemical reactions, peroxides are transformed into hydroxyl radicals which are responsible for delignification by converting lignin to acids, which may act as inhibitor products, but it also degrades lignin and hemicelluloses which exposes cellulose to enzymatic hydrolysis (Zheng *et al*., 2014).

4.2.2.4 Organosolv methods

Several organic solvents like methanol, ethanol, acetone, and ethylene glycol are used to extract lignin and solubilize hemicelluloses. This pretreatment has high efficiency, mild conditions (temperature and pressure), and does not produce toxic and inhibitory products for fermentation and enzymatic hydrolysis processes. The main disadvantage is the high costs of solvents and processes (A. K. Kumar & Sharma, 2017).

4.2.3 Physicochemical pretreatment

A combination of chemical and physical pretreatments can be beneficial for more complex biomass by providing improved accessibility of the cellulose for hydrolytic enzymes, which gave higher glucose yields. The most successful physicochemical pretreatments include liquid hot water, ammonia fiber explosion, steam explosion which is typically a combination of mechanical forces (pressure drop) and chemical effects (autohydrolysis of acetyl groups of hemicelluloses) in this process an explosive action (high-pressure) on the biomass is required to prepare them for the hydrolysis (A. K. Kumar & Sharma, 2017).

4.2.4 Biological pretreatment

Biological pretreatments are safe, environmentally friendly, and low energy methods, by using microorganisms (bacteria, fungi) or enzymes. Fungal treatment can be carried out by brown rots fungi which mainly attack cellulose, white-rot fungi (presence of peroxidases and laccases), and soft-rot fungi which mainly degrade lignin and hemicelluloses and little amount of cellulose (P. Kumar, Barrett, Delwiche, & Stroeve, 2009). Bacterial pretreatment is done with several species, they are extra promising because they have a faster growth rate and high

metabolic activity compared to fungus (Sankaran *et al*., 2020). Enzymatic pretreatment use microorganisms hydrolytic and oxidative enzymes, such as cellulase for cellulose, hemicellulase for hemicelluloses, and lignin peroxidase for lignin (Mtui, 2009).

5. **Unwanted effect of released co-products**

5.1 Inhibitory co-products

Several pretreatment methods have been developed for lignocellulosic biomass conversion, but each release a wide range of microorganism's and enzyme's inhibitors, divided to three types: weak acids, furan derivatives, and phenolic compounds (Palmqvist & Hahn-Hägerdal, 2000), their formation depends on feedstock's type and pretreatment method (Martín, Wu, Wang, Stagge, & Jönsson, 2018). Furan derivatives (HMF, furfural…) are co-products of cellulose and hemicellulose solubilization into hexoses, pentose, and sugar acid. They affect microbial amino acid biosynthesis and damage cell organelles. (Bhatia *et al*., 2020). Weak acids (acetic, formic, and levulinic acids…) are also co-products of hemicellulose degradation, furfural, and HMF co-products, they are liposoluble weak acids so they can cross plasma membrane which decrease intracellular PH and inhibit cells growth (Palmqvist & Hahn-Hägerdal, 2000). Phenolic compounds (vanillin, 4-hydroxybenzoic acid, tannin) are aromatic byproducts drives from lignin's breakdown, they are the most toxic, due to their low molecular weight they easily penetrate the cell membrane and they are known by their high banding ability to enzymes which inhibits their activity (V. Kumar, Yadav, Kumar, & Ahluwalia, 2020).

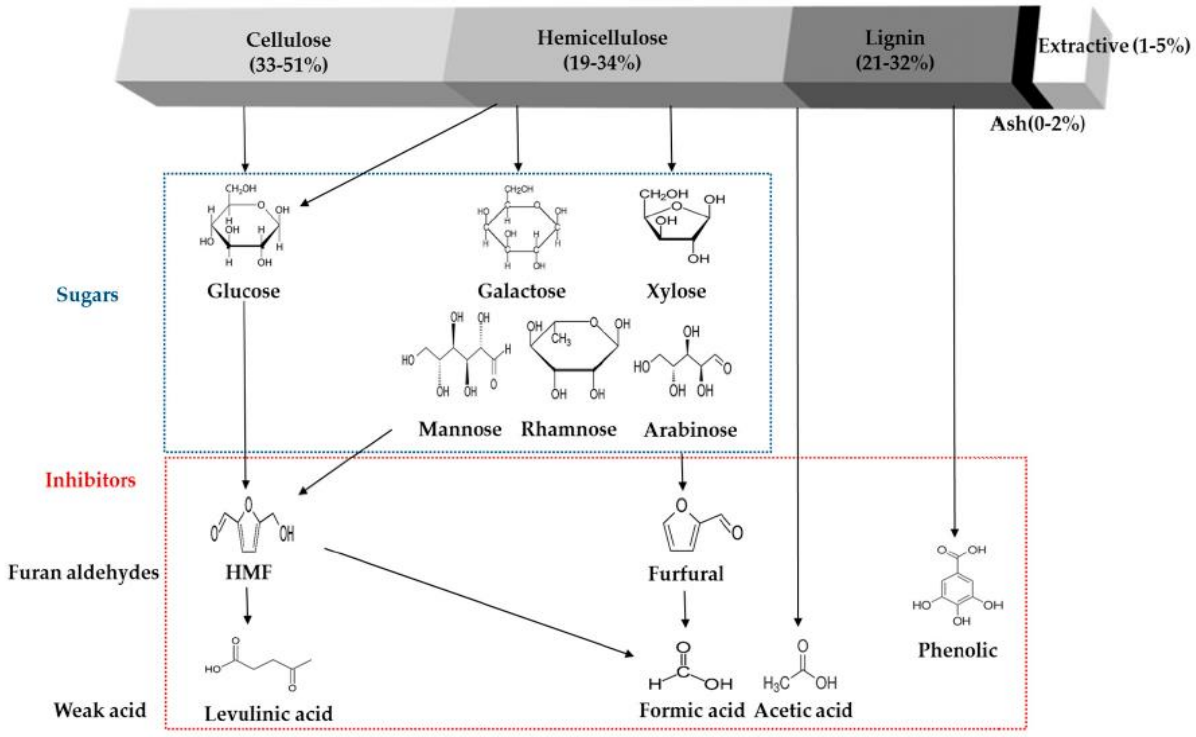

Figure 02: The main inhibitory compounds released after lignocelluloses breakdown (Kim, 2018)

5.2 Inhibitors effect on microorganisms and enzymes during hydrolysis

After pretreatment, different inhibitors are released, which affect negatively cell growth and microbial fermentation by different mechanisms such as loss in membrane integrity, inhibiting metabolism, thus, decreasing the production of the target product. Bhatia *et al*. (2020) showed that inhibitors have different effect levels on *Saccharomyces cerevisiae* glucose fermentation, as follows: vanillin > phenol > 5-HMF > furfural > levulinic acid > acetic acid > formic acid. All these toxic co-products have also an inhibitory effect on enzyme hydrolysis efficiency by reducing enzyme availability, adsorbing cellulose, and inhibiting its contact with enzymes, hiding their catalytic domain, and inhibiting their activity (Bhatia *et al*., 2020).

5.2.1 Some solutions for unwanted effects

Inhibitors removal from hydrolysate is an essential step before microbial fermentation, different detoxification strategies have been developed to reduce toxic effect: physical method such as membrane separation (filtration process) including microfiltration, ultrafiltration, and nanofiltration, which allow inhibitors with small molecular weight pass through the membrane (V. Kumar *et al*., 2020). Chemical neutralization by using alkaline solution (calcium hydroxide, sodium hydroxide) to maintain pH and precipitating inhibitors. Chemical adsorption using ion exchange resin that involves inhibitors adsorption from the liquid phase to solid phase with weak chemical bonds. Enzymatic detoxification by laccase is responsible for high phenol reduction but the main disadvantage is their high costs (Bhatia *et al*., 2020).

5.2.2 Surfactants

Surfactants are surface-active amphiphilic molecules with long hydrophobic chain and ionic hydrophilic head which gives them properties of reducing surface tension, stabilizing emulsions by increasing their kinetic stability and promoting foaming by micelles formation. Several studies reported that their specific structure makes them capable of establishing interactions with polyphenols especially nonionic surfactants like Tween 80, but it has been also proved that these chemicals are harmful and toxic for human health and the environment (Skrypnik & Novikova, 2020).

Biosurfactants are low-cost, eco-friendly and biodegradable competitive, produced by many microorganisms such as *Pseudomonas, Candida, and Bacillus* using lignocellulosic wastes as substrates, it has also been demonstrated that the addition of lipids in fermentation medium increase biosurfactants production (Moldes, 2014) because of their lipidic nature either glycolipids, fatty acids or lipopeptides in most cases rampholipides (Morita, Fukuoka, Imura, & Kitamoto, 2016).

Materials and Methods

1. Materials

1.1 Chemical products

All chemical products were purchased from **Sigma-Aldrich**

- 2% sulfuric acid solution (H_2SO_4) prepared from 99.9% stock solution
- 2% Sodium hydroxide (NaOH) prepared from 98% stock solution
- 3% Hydrogen peroxide (H_2O_2)
- 25% Ethanol (C_2H_5OH) prepared from 99.8% stock solution
- 25% methanol (CH3OH) prepared from 99.8%stock solution

1.2 Biological materials

- Lignocellulosic waste (banana peel, olive pomace, coffee grounds, potato peel, orange peel, tea waste, soybean waste).
- Microorganisms: bacterial strain used in this study is *Bacillus subtilis* TLO3.

2. Method

2.1 Microorganisms and culture conditions

The strain used in this study is *Bacillus subtilis* TLO3 isolated in 2013 from samples of rhizospheric soil of an olive tree in Tlemcen, Algeria by **Slimane Choubane** (Choubane, Gabed, Khelil, & Cheba, 2018). One milliliter of the received culture was taken using a sterile micropipette and introduced into a sterile cryotube containing 60 ml of nutritive broth; the inoculum was incubated at 45°C for 24 hours. Thereafter 500 µl of the culture was taken and introduced into a sterile eppendorf containing 500 µl of 80% glycerol (V / V), then conserved in the freezer at -20°C until use.

2.2 Lignocellulosic substrates

Seven lignocellulosic substrates were collected (table 01), 400 g of each was oven-dried at 75°C until a constant weight was obtained, in order to eliminate moisture content, then ground and sieved into powder (250 µm to 500 µm). Finally, they were kept away from humidity using plastic film until use.

Figure 03: Lignocellulosic substrates

a: Banana peel; b: Olive pomace; c: Coffee grounds; d: Potato peel; e: Orange peel; f: Tea waste

Table 01: Lignocellulosic substrates sources

lignocellulosic substrates	Source	sampling date
Coffee grounds	Local cafeteria (Oran)	15/02/2020
Tea waste	Local cafeteria (Oran)	15/02/2020
Banana peel	Local market (Oran)	20/02/2020
Olive pomace	Oil mill plant of Chetouan (Tlemcen)	15/20/2020
Potato peel	University residence restaurant C4 (Oran)	20/02/2020
Orange peel	University residence restaurant C4 (Oran)	20/02/2020
Soybean waste	El-Bahia oil plant (Oran)	24/02/2020

2.3 Pretreatments of substrates

All substrates were pretreated with 4 different treatments under the same conditions. Treatment solutions were prepared in volumetric flasks then poured into bottles containing 10 g of the substrate (each substrate separately), the treatments were done using a hot plate at 120°C for 60 minutes with stirring, then each treated sample was rinsed and filtered several times (four times) with distilled water and fabric filter, pH was measured after each rinse until a neutral value was obtained.

Table 02: Treatments used

Type of treatment	Treatment
Alkaline	NaOH (2%)
Acid	H_2SO_4 (2%)
Oxidative	H_2O_2 (3%)
Organic solvent	Ethanol (25%) + Methanol (25%)

***NB**. Fermentation process using treated substrates and different assays for enzymatic activity, polyphenols, flavonoids, and biosurfactants, could not be done because of Coronavirus disease COVID-19 pandemic

2.4 Data analysis

Data used in this section were provided by Omar Khelil, which is a part of his thesis entitled ‘‘ Production de cellulase et d’enzymes associées par des souches de *Bacillus sp*: Le rôle des prétraitements et l’effet des polyphénols, des flavonoïdes et des biosurfactants’’, defended at the University Of Sciences And Technology Mohamed-Boudiaf Oran on September 2017 (Khelil, 2017). During his work, four substrates were collected (spent ground coffee (SGC), olive pomace of the year (NOP), olive pomace from the previous year (AOP), sunflower hulls (SFH)). The substrates were pretreated with four pretreatments which are: 2% sulfuric acid (H_2SO_4), 2% sodium hydroxide (NaOH), 2% hydrogen peroxide (H_2O_2) under the same conditions (60min /Autoclave 121°C) and another pretreatment which consisted of an organosolv treatment (a mixture of ethanol and methanol (25/25 v/v)) for 5h at room temperature.

For fermentation processes, *Bacillus* cereus ATCC® 11778TM was used in this study. The strain was cultured on nutrient broth media for 18 hours at 30°C under continuous shaking at 150 rpm. Enzymes production was carried out in Erlenmeyer flask containing 1% substrate, 0.1% peptone, 0.1% beef extract, and the flasks were inoculated with a 5% (v/v) of *Bacillus cereus* 11778. The cultures were maintained at 37°C under continuous shaking at 150 rpm. At an interval of 24, 48, and 72h of incubation; cultures were then centrifuged at 10000 rpm for 10 min at 4°C and supernatants were conserved for further assays.

2.4.1 Assays of total cellulase activity, biosurfactants, flavonoids, and polyphenols content

Total cellulase activity (FPA) was determined by the filter paper assay using Whatman No.1 filter paper as a substrate. Total reducing sugars were estimated in the supernatant by the dinitrosalicylic acid (DNSA) method (Miller, 1959). The color formed is measured by spectrophotometer at 540 nm.

Biosurfactants content was determined by the orcinol method, and the absorbance was measured at 421nm, the results are expressed in rhamnose equivalents (ER) (mg/ml) (Wang *et al*., 2007; Bharali *et al*., 2014).

After extraction by autoclaving of the treated and untreated substrates, the suspensions were filtered and the filtrates were used to determine polyphenols and flavonoids contents. Polyphenol content was determined according to the Folin-Ciocalteu method (Aguilar-Garcia, Gavino, Baragaño-Mosqueda, Hevia, & Gavino, 2007), and the absorbance was measured at 760 nm. The results are expressed in mg of gallic acid equivalent per gram of the sample (mg GAE /g).

Total flavonoids content was determined according to the Dowd method (Arvouet-Grand, Vennat, Pourrat, & Legret, 1994), and absorbance was measured at 415 nm. Values were expressed as mg catechin equivalent per gram of dry weight material (mg CE/g samples).

2.4.2 Correlation studies

The provided data has been reorganized in a way to highlight the relationships between the different parameters, for that, statistical tools have been used. The graphs focused on the effect of the different pretreatments on the rate of polyphenols, the inducibility of the enzymatic activity, and the production of biosurfactants, by different substrates, and the correlation tests to understand the relationship between the different parameters per substrate.

Correlation studies were done by Microsoft Excel 2007 software and SPSS IBM (version 26.0). These studies allow assessing the possible existence of a relationship between the different studied parameters (cellulase/polyphenols; cellulase/biosurfactants; polyphenols/biosurfactants). Whereas the p-value associated with the correlation coefficient brings the information, if results are statistically significant and the correlations obtained are not random.

NB. All the experiments were carried out in three parallel replicates and the results presented were the average values of three determinations.

Results and Discussion

This study aims to provide information that helps to valorize lignocellulosic wastes; by revealing the effect of pretreatments and the nature and composition of substrates on polyphenols content, cellulase induction, and biosurfactants production by *Bacillus cereus* 11778; to discover the effect of phenolic compounds on cellulase activity and biosurfactants production. Moreover, it aims to understand the nature of the relationship between these three value added products.

Before COVID-19, the first steps of substrates pretreatment were done; moister content, pH, and weight loss after pretreatment were measured, and results are represented in annexes. While the results of the provided data by Omar Khelil are represented and discussed in this section.

1. Treatments effect on polyphenols content

Total polyphenols content in the untreated substrates is represented in figure 04 a; where the highest content was obtained with SCG (17.21 ± 0.65 mg GAE/g), and the lowest with SFH (4.11 ± 0.07 mg GAE/g). While a small difference was observed between NOP and AOP with 7.18 ± 0.12 and 5.31 ± 0.17 mg GEA/g respectively.

A considerable decreasing of phenolic content in SCG, NOP and AOP (12.26 ± 0.8, 4.60 ± 0.22 and 4.95 ± 0.4 mg GAE/g respectively) was observed after sulfuric acid treatment (fig. 04 b), otherwise SFH phenolic content slightly raised to 4.60 mg GAE/g with the same treatment.

For alkaline treatment polyphenols content in SCG reached its lowest recorded value with 5.01 ± 0.66 mg GAE/g, in the same trend NOP also decreased to 4.80 ± 0.21 mg GAE/g. However, SFH and AOP raised a little bit compared to untreated substrates (5.68 ± 0.32 mg GAE/g 7.77 ± 0.08 mg GAE/g respectively) (fig. 04 c).

Oxidative treatment (fig. 04 d) had the same effect on the substrates, where SCG and NOP decreased to 7.45 ± 0.79 and 5.60 ± 0.39 mg GAE/g respectively, and AOP raised to 6.64 ± 0.21 mg GAE/g, while SFH remained the same as untreated one (4.85 ± 0.35 mg GAE/g).

Finally, the last treatments with organosolv (fig. 04 e) gave higher yields for SFH, NOP and AOP (6.44 ± 0.71, 5.67 ± 0.08 and 6.96 ± 0.31 mg GAE/g respectively) comparing to untreated ones, while SCG decreased to 10.78 ± 0.33 mg GAE/g.

The obtained results indicate that polyphenols content differs from substrates to another and from untreated to treated ones. The yield obtained from untreated SCG corresponds to what has been found by several studies, ranged from 17 to 35 mg GAE/g (Choi & Koh, 2017; Panusa, Zuorro, Lavecchia, Marrosu, & Petrucci, 2013). Also for SFH polyphenols content matches to what Menzel *et al*. (2019) found (4.98 mg GAE/g) in their study (Menzel, González-Martínez, Chiralt, & Vilaplana, 2019). Otherwise, olive pomace's content is variable and depends on many factors such as olive cultivars, the extraction process, and the type of pomace generated as a by-product (Cioffi

et al., 2010). Also, the difference observed between olive pomaces in this study is probably due to storage conditions (Kulak & Çetinkaya, 2018).

Diluted sulfuric acid treatment can destroy hemicelluloses polymeric bonds and hydrolyze it to its monomers. It can also disrupt lignin's structure but it's not so effective in dissolving it (Keskin, Nalakath Abubackar, Arslan, & Azbar, 2019), depending on processing time, temperature and acid concentration (Martínez-Cartas, Olivares, & Sánchez, 2019). This explains the little loss of phenolic compounds in SCG, NOP, and AOP which is probably due to lignin partial hydrolysis.

The increasing of SFH phenolic compounds might be due to their bound forms. Knowing that in some cases acid treatment gives a higher yield of polyphenols (Martínez-Cartas *et al*., 2019).

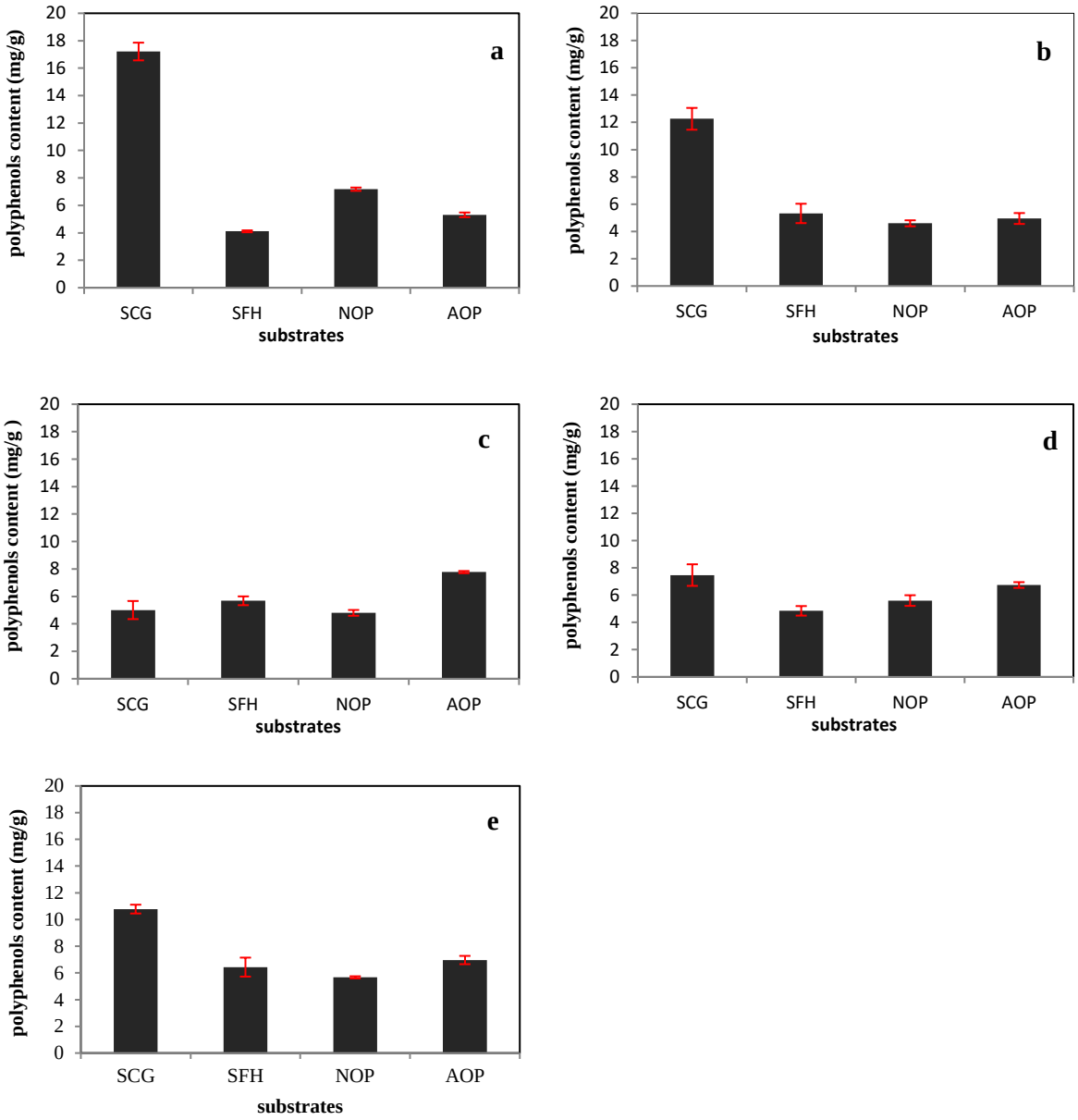

Figure 04: Treatments effect on polyphenols content (SCG: spent coffee grounds; SFH: sunflowers hulls; NOP: olive pomace of the year; AOP: olive pomace of the previous year)

(a): untreated; (b): H_2SO_4 2%; (c): NaOH 2%; (d): H_2O_2 2%; (e): chloroform/acetone

Unlike acid treatment, diluted alkaline solutions of NaOH mainly targets lignin causing its degradation and solubilization by changing the structural linkage between lignin and carbohydrates network at elevated temperatures (Dhanya, Mishra, Chandel, & Verma, 2020); as a result, phenolic compounds are released and lost which explains the huge losses recorded in SCG and NOP in this study. However, the rise in SFH and AOP phenolic content is probably due to their phenolic acids and flavonoids profiles, Sindhu *et al*. (2015) mentioned that alkaline pretreatment yield depends on the type as well as the composition of biomass (Sindhu, Pandey, & Binod, 2015).

For oxidative treatment, Mussatto *et al*. (2011) found the same yield with SCG (7.4 mg GAE/g) (Mussatto, Ballesteros, Martins, & Teixeira, 2011). Since treatment by H_2O_2 is considered as an alkaline treatment too, it also causes a loss in polyphenols because oxidizing agents provide delignification by several reactions like displacement of side chains, electrophilic substitutions, and oxidative cleavage of aromatic cycles as a result, phenolic compounds forms soluble fragments with oxidative agents causing its detachment (Kumari & Singh, 2018).

It was reported that solid-liquid extraction with mixtures of organic solvents is the most suitable for extraction of phenolic compounds from plant sources but its efficiency is affected by several factors such as the type of solvent and its concentration, the solvent/solid ratio, the number of extraction steps, pH, time of contact, temperature and particle size (Mussatto *et al*., 2011). In this study, all substrates were subjected to the same conditions while there were differences in polyphenols content between substrates. This may be explained by the variation of wide-ranging polarities and chemical characteristics of phenolic compounds depending on substrates, that affect their solubility even when using the same solvent (Vural, Cavuldak, Akay, & Anlı, 2020). It has been also proven that when acetone is mixed with other solvents, its efficacy for polyphenols extraction decreases, while methanol/water mixture gives the best results (Venkatesan, Choi, & Kim, 2019). Although, Mussatto *et al*. (2011) used 50% methanol mixture for SCG and got approximately the same yield as obtained in this study (11 mg GAE/g) (Mussatto *et al*., 2011).

2. Treatments effect on flavonoids content

Flavonoids content of untreated substrates are represented in figure 05 a. SCG showed a high flavonoids content (9.24 ± 0.08 mg CE/g), while NOP and AOP presented only 2.58 ± 0.32 mg CE/g and 2.11 ± 0.04 mg CE/g respectively. On the other side, SFH had the lowest flavonoids content (1.58 ± 0.006 mg CE/g).

After acid pretreatment with H_2SO_4 (fig. 05 b), a reduction of flavonoids content was recorded in SCG (3.85 ± 0.04 mg CE/g), while NOP increased to 1.87 ± 0.21 mg CE/g, and SFH and AOP remained the same as untreated ones (1.71 ± 0.07 and 2.14 ± 0.06 mg CE/g respectively).

A huge lost was observed with alkaline treatment (fig. 05 c), where SCG, NOP, and AOP reached the lowest flavonoids content (2.19 ± 0.14, 2.33 ± 0.16 and 1.86 ± 0.1 mg CE/g respectively) except for SFH which slightly increased to 1.92 ± 0.05 mg CE/g. The same effect was recorded after oxidative treatment with H_2O_2 where SFH highly increased to 3.11 ± 0.14 mg CE/g (fig. 05 d).

In the other hand, after organosolv treatment, AOP and NOP gave almost the same yield as untreated one ranging from 2.10 to 2.65 mg CE/g, while SFH increased to 2.26 ± 0.07 mg CE/g and SCG decreased to 5.24 ± 0.08 mg CE/g (fig. 05 e).

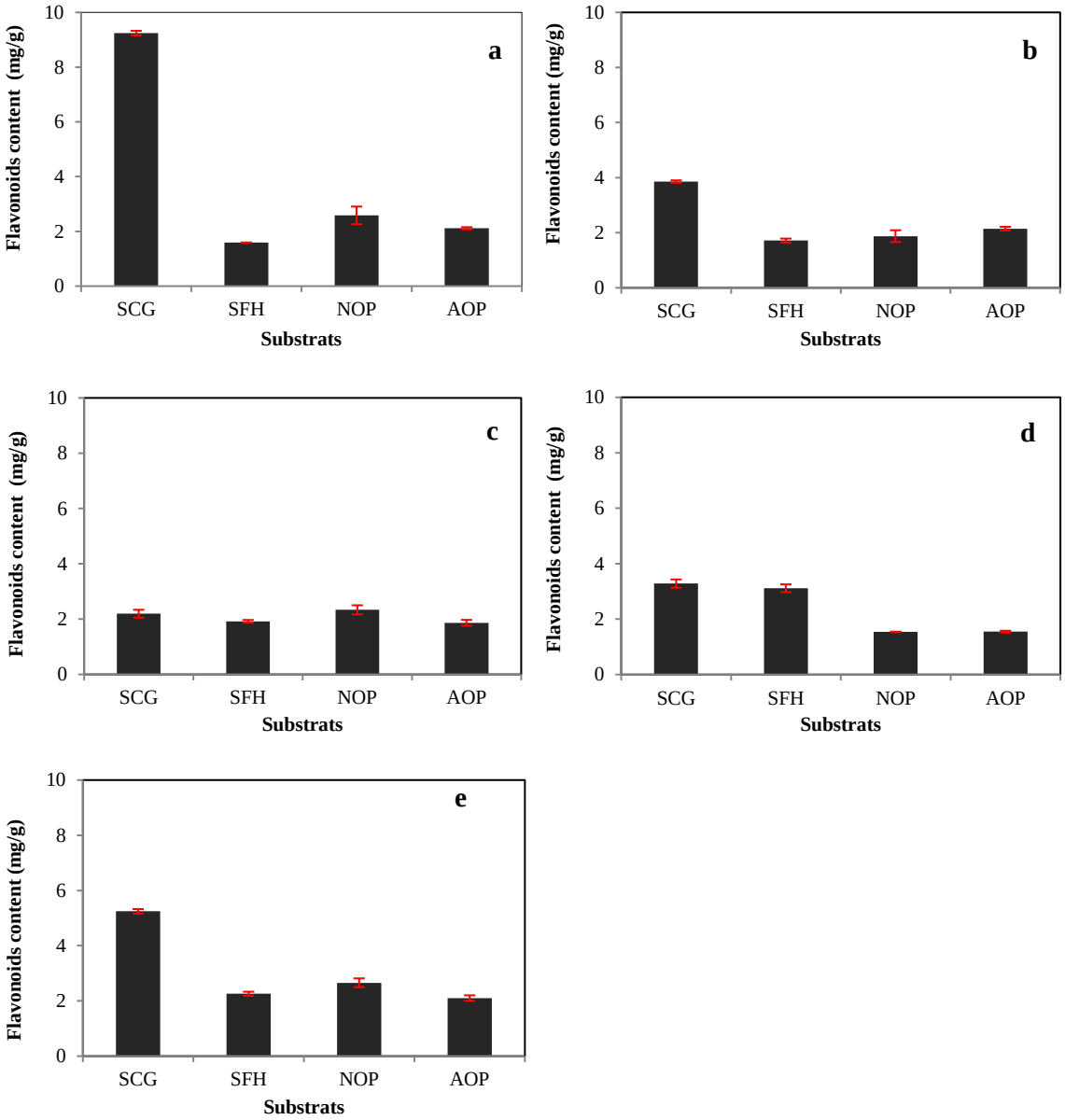

Figure 05: Treatments effect on flavonoids content (SCG: spent coffee grounds; SFH: sunflowers hulls; NOP: olive pomace of the year; AOP: olive pomace of the previous year)

(a): untreated ; (b): H_2SO_4 2%; (c): NaOH 2%; (d): H_2O_2 2%; (e): chloroform/acetone

Many researchers proved that flavonoids content differs according to the substrates and the pretreatment method used. Comparing the results of the untreated SCG obtained in this study (9.24 ± 0.08 mg CE/g) with different studies; total flavonoids content in SCG fits to what was found in the literature which ranges between 1.81 mg QE/g and 29 mg QE/g, depending on the extraction method used (Wu *et al*., 2019). For olive pomace, different amounts of flavonoids were quantified and identified in the extracts with concentration ranging from 3.1 to 199.9 mg/kg dry weight (Skaltsounis, Argyropoulou, Aligiannis, & Xynos, 2015). In sunflower seeds, flavonoids content varies generally between 25 and 45 mg QE/g. Flavonoids are mainly located in husks more than seeds (Guo, Ge, & Jom, 2017).

After treatment of substrates with H_2SO_4, a reduction of flavonoids content in SCG and AOP was noticed, which may be explained by several parameters such as the use of strong acid, extraction time, solid-liquid extraction, temperature (Putnik, Bursać Kovačević, Radojčin, & Dragović-Uzelac, 2016). Some studies have reported that acid solutions may cause pigment's degradation (Deng *et al*., 2019).

Flavonoids content has significantly decreased in SCG, AOP, and NOP under NaOH treatment, this phenomenal may be due to more repolymerization reactions between lignin and sugars (Wang, Jiang, Xu, & Sun, 2009); oxidation of flavonoids and phenolic compounds in an alkaline medium (Laguna, 2019), or partial removal of pigments with an alkaline solution and temperature extraction (Petrakis, 2006). Otherwise, recent studies have shown that flavonoids content may increase due to the liberation of polyphenols by-products after applying alkaline treatment and steam explosion (V. Kumar *et al*., 2020).

For H_2O_2 treatment and to the best of our knowledge, there is a lack of data concerning the oxidative extraction of flavonoids content. The decrease of flavonoids content is probably due to the oxidation of flavonoids and their conversion to other compounds at high temperature (Papoutsis *et al*., 2018).

A comparison between flavonoids content with chloroform/acetone and other pretreatments (acid, alkaline), shows that maceration with various organic solvents such as methanol, ethanol, acetone, ethyl acetate, and their combinations is the most common technique for flavonoids extraction, which gives a high yield. According to previous studies, flavanols are better extracted with aqueous acetone. This explains the high yields obtained with SFH, AOP, and NOP comparing to the other treatments. Also, the recovery of flavonoids from materials is also influenced by the extraction time, temperature, the level of agitation, and type of solvents (J. Dai & Mumper, 2010).

Figures 04 and 05 clearly shows that polyphenols content including flavonoids after treatments decreases in all substrates (except SFH) this large different might be caused by drying

processes and treatment's timing at high temperatures above 70°C and repeated washes after treatment, that all substrates were subjected to, those undesirable conditions may cause polyphenol's degradation, oxidation, and conversion to other compounds leading to lower extraction yields (Tzortzakis & Chrysargyris, 2017).

3. Treatments effect on cellulase production by *Bacillus cereus* 11778

Total cellulase activity was recorded after 24h, 48h and 72h of fermentation by *Bacillus cereus* 11778 using untreated (T0) and treated substrates (T1, T2, T3, T4) as carbon source (fig. 06).

The highest level of cellulase production was obtained at 72h for all untreated substrates (SCG, SFH, NOP and AOP with 1.43 ± 0.03, 1.99 ± 0.07, 1.7 ± 0.1 and 1.68 ± 0.01 U/ml respectively). While the lowest production was recorded at 48h with SCG and NOP (1.16 ± 0.04 and 1.19 ± 0.12 U/ml respectively) (fig. 06 a).

After substrates treatment with H_2SO_4 (fig. 06 b), cellulase activity decreased for all substrates except for SFH that slightly induced a higher production compared to untreated ones. After 48h, SCG cellulase induction dropped to its lowest value of 1.1 ± 0.02 U/ml. Otherwise, for all substrates, cellulase productions increased over time reaching their highest level after 72h.

For SFH, NOP and AOP, after NaOH treatment (fig. 06 c), gave nearly the same yields in a range of 1.71 to 1.81 U/ml at 72h, while the activity was reduced by 25% for SCG with the lowest value of 0.99 ± 0.02 U/ml at 48h.

H_2O_2 treatment gives almost the same results as the alkaline treatment. The highest yield reached was 2.02 ± 0.1 U/ml with NOP at 72h. Also, all the other substrates attain their uppermost inducibility at 72h (fig. 06 d).

Treated substrates with chloroform/acetone induced the highest cellulase activity at 72h (fig. 06 e) in a range of (1.80-1.90 U/ml) for SFH, AOP and NOP while SCG gave the minimal cellulase activity (1,22 ± 0.01 U/ml).

Enzymatic hydrolysis of lignocellulose without pretreatment is usually not so effective because of the high resistance of materials to enzymatic or bacterial attacks (Taherzadeh & Karimi, 2008). Therefore, for efficient and rapid hydrolysis of carbohydrates, the pretreatment step is needed to disrupt the recalcitrant structures of the lignocellulosic materials to increase the digestibility of the materials prior to its enzymatic hydrolysis (da Costa Correia, Júnior, Gonçalves, & Rocha, 2013).

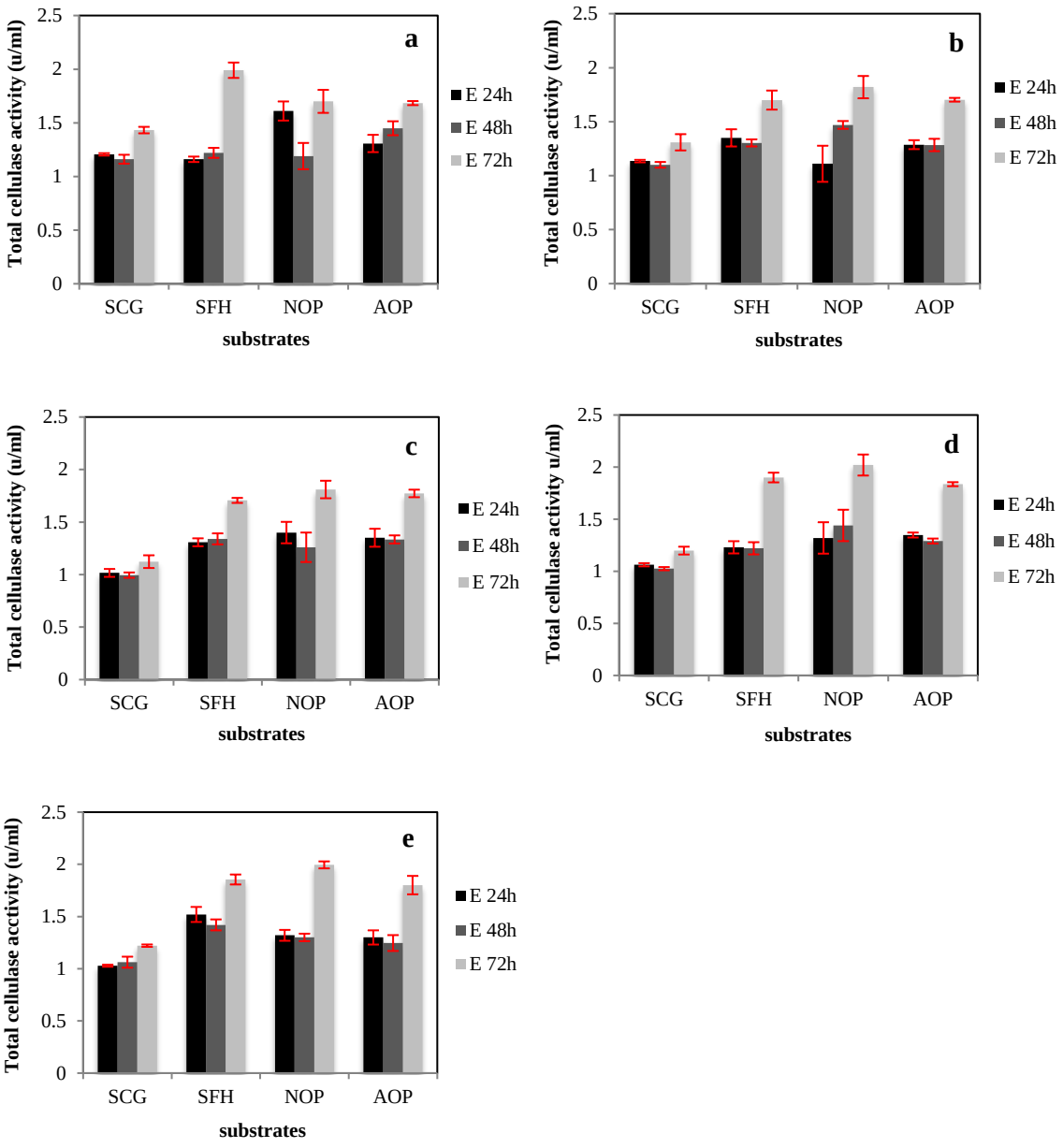

Figure 06: total cellulase activity after 24h, 48h and 72h of substrates fermentation by *Bacillus cereus* 11778 (SCG: spent coffee grounds; SFH: sunflowers hulls; NOP: olive pomace of the year; AOP: olive pomace of the previous year)

(a): untreated; (b): H_2SO_4 2%; (c): NaOH 2%; (d): H_2O_2 2%; (e): chloroform/acetone

The decrease in cellulase activity in untreated substrates after 48h in the present study, can be explained by the peptone's concentration in the culture medium, it has been reported that cellulase production decrease when excess nitrogen sources such as peptone were presented, and only when nitrogen runs out (usually after exponential phase), cellulolytic enzymes production is induced (Dias, Melo, Schwan, & Silva, 2015), which explains why cellulase reached its maximal activity after 72h. It was also shown by Chia-wen C. Hsieh *et al*. (2014) that cellulase components can be inhibited by high monosaccharides concentration such as glucose, cellobiose, xylose, and mannose (Hsieh, Cannella, Jørgensen, Felby, & Thygesen, 2014).

The increase of cellulase activity with the prolongation of culture time within 72 h might be due to the reduction of monosaccharide concentration in the medium (Gautam *et al*., 2011).

Recent studies have reported that acid treatment with H_2SO_4 degrades hemicelluloses by cutting off Van Der Waals forces and hydrogen bonds, this allows enzyme accessibility to cellulose, but in the other hand, it causes the accumulation of toxic byproducts in the form of furfural and hydroxymethylfurfural (HMF), which decrease cellulase activity (Sharma, Kalra, & Kocher, 2004). This might explain the low cellulase induction by SCG and SFH compared to untreated ones in the present study, because of their high hemicelluloses fraction (tab. 03), thereby high amounts of possible toxic co-product.

NaOH treatment of lignocellulosic materials promotes swelling, leading to the rise of internal surface area, reduction in the degree of polymerization, and disruption of lignin structure, as well as the breakdown of the linkages between lignin and carbohydrates to enhance the accessibility to substrates for efficient hydrolysis. However, phenolic compounds derived from lignin breakdown, are among the most influential inhibitors in enzymatic reaction and microbial fermentation (Kim, 2018). Other works suggest that pretreatment conditions were not enough for the dissolution of lignin and hemicelluloses, which impeded the enzymatic efficiency (H. Zhang, Huang, Wei, Zhang, & Xie, 2019). This might be the cause of yield's decreasing with SFH and SCG compared to untreated ones, where phenolic compounds and/or treatment conditions affected negatively cellulase activity.

In this work, cellulase activity yields after H_2O_2 treatment was much higher than that of NaOH treatment, this result suggested that H_2O_2 treatment gives a better improvement of enzymatic hydrolysis efficiency (Luo, Zhao, Yang, Shen, & Rao, 2009). During the pretreatment, hydroxyl radicals (HO^-) and superoxide anion radicals (O_2^-), produced from the decomposition of hydrogen peroxide in alkaline solution, can oxidize and degrade hemicelluloses and lignin, which increase surface area making cellulose accessible to cellulase. The main advantage that H_2O_2 pretreatment can be degraded to oxygen and water without any residues or toxic products left in the process (H. Zhang *et al*., 2019).

Like what was found in literature, the obtained results in this study revealed that organosolv pretreatment followed by bacterial hydrolysis and fermentation gives higher conversion efficiency. Organosolv pretreatment is an attractive method for delignification (it extracts almost pure lignin) and enzymatic glucose conversion in the same process. During the reaction, pure cellulose is obtained with only a slight degradation in the solid phase, while hemicelluloses; lignin and degradation products (acetic acid, HMF, and furfural) are obtained in the liquid phase. The removal of inhibitory products induces a high rate of cellulose conversion and increases cellulase activity.

In a comparative study between solvents, maximum cellulose concentration was found 72%, and delignification yield 79% for acetone with low furfural formation, which gives higher accessibility for enzymes (Borand & Karaosmanoğlu, 2018). Polyphenols contribute to high toxicity and antimicrobial activity of olive pomace (Massadeh & Fandi, 2014), and organosolv pretreatment can remove that toxicity by removing polyphenols.

From the obtained results in this study and literature, production yields depend strongly on feedstock composition. In particular, cellulose and lipids content, as example SFH contains 42.60% cellulose and 4.80% lipids (Ph Evon, Virginie Vandenbossche, Pierre-Yves Pontalier, & Luc Rigal, 2007), while SCG contains 12.40 % cellulose and 16% lipids (Lavecchia, Medici, Patterer, & Zuorro, 2016; Loyao Jr, Villasica, Peña, & Go, 2018). In this study, treated substrates show a high decrease in cellulolytic activity compared to untreated ones, especially with SCG, and it's known that treatments remove lipids, leading to low enzymatic activity, Khelil *et al*. (2016) reported that untreated SCG induced high activity, while after the removal of cell components (including lipids), cellulase activity decreased (Khelil, Choubane, & Cheba, 2016). A relation might exist between cellulase activity and lipid content.

4. **Effect of pretreatment on biosurfactants production by *Bacillus cereus* 11778**

Figure 07 represent results of biosurfactants production by *Bacillus cereus* 11778 after 24h, 48h, and 72h of fermentation using untreated (T0) and treated (T1, T2, T3, T4) substrates separately as carbon source.

Untreated substrates are represented in figure 07 a, for all substrates biosurfactants yield increased over time, with SCG as the best inducer during all fermentation days, reaching a peak of 22.18 ± 0.73 mg/ml after 72h, while other substrates gave converging values between 15 and 20 mg/ml.

However biosurfactants production with all substrates after sulfuric acid treatment decreased slightly and no large difference between substrates was recorded (fig. 07 b), except for SCG that also reached the highest yields after 48h and 72h with 18.50 ± 0.46 and 17.54 ± 0.15 mg/ml respectively.

Otherwise, after alkaline treatment, SCG hit the lowest value of 9.95 ± 0.6 mg/ml after 24h then enhanced considerably until its highest yield after 72h (19.95 ± 0.39 mg/ml). The other substrates also reached their highest value after 72h (fig. 07 c) with values of 16.4 ± 0.2, 19.23 ± 0.59, 15.42 ± 1.77 mg/ml for SFH, AOP, NOP respectively.

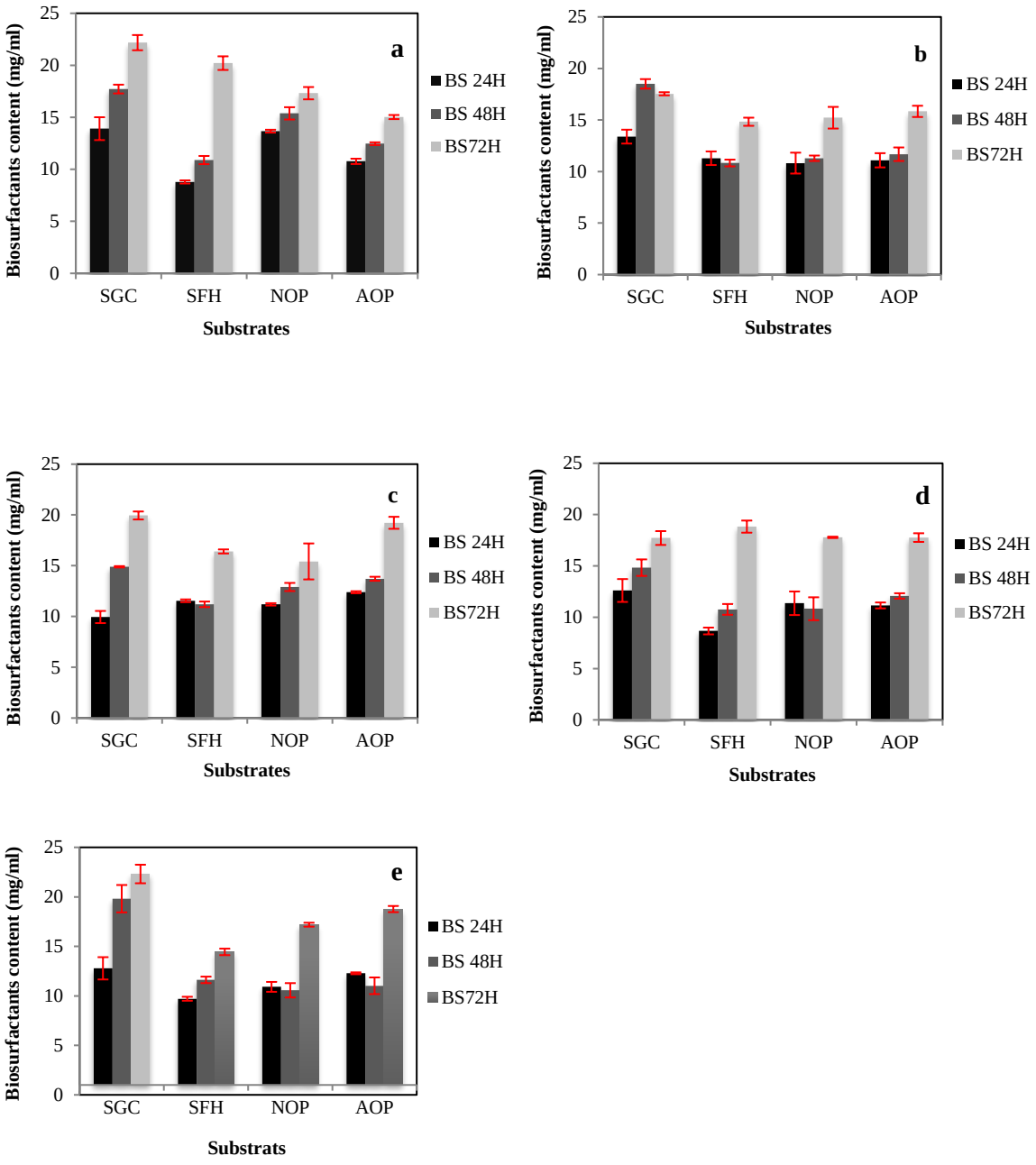

Figure 07:biosurfactants production by *Bacillus cereus* 11778 after 24h, 48h and 72h of substrates fermentation (SCG: spent coffee grounds; SFH: sunflowers hulls; NOP: olive pomace of the year; AOP: olive pomace of the previous year)

(a): untreated ; (b): H_2SO_4 2% ; (c): NaOH 2% ; (d): H_2O_2 2% ; (e): chloroform/acetone

Figure 07 d shows that H_2O_2 treatment made biosurfactants production rise significantly over time for all substrates with optimum value in the third day in the range of 17-18 mg/ml. It was noticed that SFH yield for all fermentation days remained almost the same as untreated one from 10 to 20 mg/ml.

Though organosolv treatment provoked the uppermost production comparing to other treatments, figure 07 e shows that SCG gradually reaches its highest productivity with 22.31 ± 0.94 mg/ml after 72h, while SFH production was at its lowest value after 24h (9.71 ± 0.2 mg/ml).

The obtained results show that biosurfactants production with almost all substrates decreases after pretreatments. Otherwise, for treated and untreated substrates the highest yields are obtained after 72h of fermentation.

Several studies have been devoted to microbial biosurfactants due to various advantages, mainly their ability to decrease interfacial and surface tension, to increase the surface area of contact and the possibility of being produced from low-cost raw materials such as lignocellulosic waste. Many bacterial strains, like *Bacillus subtilis* and *Pseudomonas aeruginosa*, are being studied, biosurfactants production doesn't depend only on the producer strain and fermentation conditions but mainly on the nature of carbon source (Srivastava, Kumar, & Srivastava), which explains the differences in biosurfactants yields in the present study, despite they were under the same conditions in each state separately, each substrate differs in its source, chemical composition, and properties.

Substrates in this study are considered oleaginous due to their lipidic fraction (tab. 03), and it was proven that there are numerous secondary metabolism biosynthetic pathways for biosurfactants production and it is possible for bacterial metabolism to shift toward different sources of carbon (Martinez-Toledo & Rodriguez-Vazquez, 2018) to increase biosurfactants yield, Zhang *et al*. (2014) demonstrate that a mixture of fermentable sugars with fatty acids produced an increasing of rhamnolipid's yield from 2.1 g/l to 14.3 g/l with increasing fatty acids concentration with different chain length (L. Zhang, Pemberton, & Maier, 2014). This explains the obtained biosurfactants high amounts, up to 22.18 ± 0.73 g/l for untreated SCG.

Unlike other studies, substrate's pretreatment didn't make a large difference in biosurfactants yield with untreated ones, while it is known that pretreatment is a key step to use lignocellulosic waste for biosurfactants production to breakdown cellulose and converting it to fermentable sugars, which serves latter as carbon sources for biosurfactants production (Tan & Li, 2018). The obtained results with untreated substrate might be due to hemicellulosic and cellulosic fraction in each substrate (tab. 03) because during lignocelluloses break down, hemicelluloses are the first to be degraded liberating fermentable monosaccharides, which increase biosurfactants production more than cellulose with the presence of fatty acids in the medium. As Prado *et al*. (2019) observed that by using pentose such as xylose and arabinose; biosurfactants production from *Bacillus subtilis* was improved (Prado *et al*., 2019). While after pretreatments; on one hand, lipids content decrease, and the removal of hemicelluloses and lignin is accelerated, this means more inhibitory co-products that

affect microbial metabolism. On the other hand, usually for biosurfactants production, after treatment of lignocellulosic waste, several washes take place and only resulting sugars and lipids are used as carbon source, also untreated substrates are having an enzymatic treatment in the same time with fermentation by bacterial cellulase enzymatic machinery, and it was demonstrated by Ramirez *et al* (2016), that after fermentation of treated olive mill wastes with different treatment (enzymatic, acidic and combined acid with enzymatic treatment), the enzymatic hydrolysis was the best pretreatment, yielding up to 29.5mg/L of biosurfactants (Ramírez *et al*., 2016), the same as the obtained results in this study with SCG and SFH, where their highest yield was produced by untreated one.

Many researchers reported that stimulating the biomass with H_2O_2 gives a better yield of biosurfactants (Rezaei, Moussavi, Naddafi, & Johnson, 2020). H_2O_2 molecule increases oxidative enzyme activity such as peroxidase and catalase in breaking down the aromatic structures, thereby increasing biodegradation of hydrophobic hydrocarbons (C. Liu *et al*., 2017). H_2O_2 degrades to H_2O and O_2 so it provides oxygen in aerobic fermentation, promoting better conditions for biosurfactants production (Kaushal, Mehandia, Singh, Raina, & Arya, 2018). Rezaei *et al*. (2020) demonstrated that biomass treatment with H_2O_2 gives 252 mg/l, while untreated biomass gives 172 mg/l (Rezaei *et al*., 2020), which is contrary to our results where biosurfactants yields decreased with all substrates comparing to untreated ones after oxidative treatment, this might be due to the removal of lipids by this treatment, thereby a low production of biosurfactants.

After alkaline treatment, AOP increased its productivity at 72h, which was also found by other research, where alkaline pretreatment of rice husk, raises the quality and amount of biosurfactants produced compared to untreated substrates from 5.3% to 11.4% (Oje *et al*., 2016).

Another recent research shows that acids can affect and inhibit β-oxidation pathway and favorite other pathways for biosurfactants (rhamnolipids) precursors, depending on the type and amount of fatty acids contained in carbon source *(*Abdel-Mawgoud, Lépine, & Déziel, 2014*)*, maybe this can explain why SCG in the present study gives higher yields of biosurfactants (24h and 48h) with acidic pretreatment then other substrates, knowing that it contains the highest yields of lipids (16%) (tab. 03).

For better recovery of biosurfactants, organosolv is the most used (Shah, Sivapragasam, Moniruzzaman, & Yusup, 2016), which matches with olive pomaces behavior after organosolv treatment. This might have a relation with the physicochemical interaction of its main fatty acid.

The fact that almost practically all substrates treated and untreated reach their upmost productivity after 72h, is probably due to a nitrogen source. Several studies reported that limiting concentrations of nitrogen favors the production of biosurfactants by various microorganisms. It

was observed that *Bacillus subtilis* increased its production only when nitrate decreased in the culture medium, also the synthesis of rhamnolipids by *Pseudomonas aeruginosa* takes place when the source of nitrogen run out and when its growth cycle is at its latency phase, and that's why, when high nitrogen concentrations are added, biosurfactants production is inhibited (Martinez-Toledo & Rodriguez-Vazquez, 2018).

It was also proven that potassium, iron, magnesium, sodium, and calcium can strongly affect biosurfactants production; a study reported that the addition of iron to culture medium increases rhamnolipids production by *Pseudomonas aeruginosa.* While *Bacillus subtilis* has an active transportation system for ions and manganese which may act as a cofactor to many enzymes involved in the metabolism of nitrogen, thereby increasing biosurfactants productivity (Martinez-Toledo & Rodriguez-Vazquez, 2018). In the present study, SCG has the highest inducibility with all treatment, while SFH has the lowest inducibility of biosurfactants, this might have a relation with their iron and ions content (Fe, Na, K, Mn), where SCG has higher amounts of this elements then SFH (Barišić, Netinger Grubeša, Dokšanović, & Marković, 2019; Lavecchia *et al*., 2016). Biosurfactants production isn't constant with a typical substrate or treatment; it depends on many factors, so for better production optimal conditions need to be studied.

Table 03: chemical composition of substrates

Component (g/100g dry matter)	cellulose	hemicelluloses	lipids	references
SCG	12.4	39.1	16	(Lavecchia, Medici, Patterer, & Zuorro, 2016)
SFH	42	16.6	4.8	(Evon, Vandenbossche, Pontalier, & Rigal, 2007)
Olive pomace	23.4	29.7	11.1	(Morillo-Pérez, Antizar-Ladislao, Monteoliva-Sánchez, Ramos-Cormenzana, & Russell, 2009)

5. Relation between cellulase activity, polyphenols and biosurfactants

Correlation coefficients were calculated to determine the strength and direction of the relationship between cellulase/polyphenols, cellulase/biosurfactants, andpolyphenols/biosurfactants. The results are presented in table 04.

5.1 Cellulase/polyphenols

There was a direct positive relationship between cellulase activity and polyphenols content in SCG, NOP, AOP, and SFH after 24h, with correlation coefficient 0.89; 0.83; 0.74 and 0.95 respectively, which indicates that there is a strong linear correlation between cellulase activity and polyphenols.

After 48h of fermentation, a perfect correlation for SCG (R= 1) and a very strong correlation for SFH (R= 0.96) were recorded, while an average negative correlation (R= -0.63) between the two parameters in NOP and a weak positive correlation in AOP (R= 0.42) were noticed (P values less than 0.01).

At 72h with SCG and AOP a linear positive correlation was recorded (R= 0.98 and 0.76 respectively), while with NOP the correlation became weak negative (R= -0.29) and moderately weak negative with SFH (R= -0.55) (P values less than 0.01).

5.2 Cellulase/biosurfactants

Different relationships have been recorded between biosurfactants and cellulase production. After 24h a strong linear association between the two parameters in SCG (R= 0.76) and NOP (R= 0.86) was recorded. However, a very weak uphill linear relationship was noticed in AOP (R= 0.28) and SFH (R= 0.37).

A moderate positive correlation has been recorded in SCG and AOP (R= 0.60 and R= 0.50 respectively) after 48h of fermentation. However, a strong negative correlation was obtained with NOP (R= -0.76), while with SFH a strong uphill straight line was observed (R= 0.91).

After 72h, a very weak correlation in SCG (R= 0.28) compared to SFH and AOP in which strong linear correlation was observed (R= 0.74 and 0.80 respectively). NOP recorded also a weak correlation (R= 0.42) with a P value of less than 0.01.

5.3 Polyphenols/biosurfactants

Correlation coefficients in SCG, NOP, and AOP after 24h were between 0.84 and 0.90 indicating that polyphenols content with biosurfactants production are highly correlated, while in SFH a weak correlation has been observed (R= 0.49).

Table 04: correlation between parameters after 24h, 48h and 72h of lignocellulosic substrates fermentation

Relation	24h	48h	72h
Spent coffee grounds			
Cellulase/polyphenols	0,89	1,00	0,98
Cellulase /biosurfactants	0,76	0,60	0,28
Polyphenols/biosurfactants	0,87	0,63	0,42
Olive pomace of the year			
Cellulase/polyphenols	0,83	-0,63	-0,29
Cellulase /biosurfactants	0,86	-0,76	0,42
Polyphenols/biosurfactants	0,90	0,65	0,73
Olive pomace from the previous year			
Cellulase/polyphenols	0,74	0,42	0,76
Cellulase /biosurfactants	0,28	0,50	0,80
Polyphenols/biosurfactants	0,84	0,38	0,95
Sunflower hulls			
Cellulase/polyphenols	0,95	0,96	-0,55
Cellulase /biosurfactants	0,37	0,91	0,74
Polyphenols/biosurfactants	0,49	0,82	-0,89
(P <0.01)			

However, after 48h of fermentation, results in table 04 shows a positive moderate correlation between the two parameters with SCG (R= 0.63) and NOP (R= 0.65), and a weak correlation with AOP (R= 0.38), whereas with SFH a strong uphill linear relationship was observed (R= 0.82).

After 72h, the degree of linear association became weak with SCG (R= 0.42), strong with NOP (R= 0.73), and very strong nearly perfect with AOP (R= 0.95), while with SFH a negative linear strong correlation was observed (R= -0.83) (P values less than 0.501).

Correlation results showed that there is a strong positive relation between cellulase activity, biosurfactants production, and polyphenols content after oleaginous substrate's fermentation by *Bacillus cereus*. Recently, several studies focused on improving cellulase activity during biomass fermentation by adding surfactants or biosurfactants. In a comparative study, Zhang *et al*. (2008) evaluated the ability of different surfactants (Triton X-100, SDS, Tween 20, Tween 80, and pure rhamnolipids) to enhance the hydrolysis of rice straw by *Pseudomonas aeruginosa*. The production of reducing sugars greatly increased by rhamnolipids (22.30% compared to control) better than any other chemical surfactant, followed by non-ionic surfactant Tween 80, while anionic charged SDS (sodium dodecyl sulfate) reduced the hydrolysis. The stimulatory effect of biosurfactants was interpreted by the surfactant's ability to improve cell membrane permeability leading to more enzymes being excreted; also it improves the cellulase stability and prevents the denaturation of enzymes during hydrolysis by desorbing it from the cellulose substrate. Surfactants can also modify the cellulose surface property and minimize irreversible binding, thus promoting

the production of cellulase, and enhancing the enzymatic hydrolysis of cellulose (Q. Zhang *et al*., 2008)

It was also found, that the enzymatic hydrolysis of lignocellulosic biomass was enhanced by biosurfactants from *Bacillus sp.* W112, which increase cellulase thermostability, reduce the non-productive binding of cellulase to lignin and increase the binding of cellulase to cellulose (J. Liu *et al*., 2017), which explain the strong positive association found between cellulase activity and biosurfactants production with all substrates used in this study.

Researchers showed that the relation between polyphenols and cellulase is strongly negative, it has been shown that phenolic compounds are strong inhibitors to cellulolytic reaction and microbial fermentation (D. Kim, 2018). However, the correlation results showed the opposite, polyphenols content and cellulase activity had a strong positive relationship and even perfect with SCG at 48h, this might be explained by the main phenolic compound in all substrate, several studies proved that chlorogenic acid and caffeic acid are the main phenolic compounds in SCG, ranging from 0.09 to 4.8 mg/g and 0.06 to 9.7 mg/g respectively (Burniol-Figols, Cenian, Skiadas, & Gavala, 2016; Choi & Koh, 2017; Ramón-Gonçalves *et al*., 2019). For sunflowers hulls it's the same thing, but with less content ranging from 0.03 to 0.08 mg/g for chlorogenic acid, and from 0.01 to 0.03 mg/g for caffeic acid (Pedrosa *et al*., 2000) representing 79.4% and 4.1% of the total phenolic content in sunflower hulls respectively. The interesting thing about these phenolic compounds, it's that, it has been proved that they can serve as a natural surfactant, Shweta *et al*. (2019) explored the surface-active potential of chlorogenic and caffeic acid in coffee oil and found that caffeic acid and their derivates help in the self-assembly (chlorogenic acid – hydrophilic head, hydrocarbon – hydrophobic tail), which is responsible for the stability of microbubbles at the air-water interface, forming micelle and facilitating its surfactant behavior (Deotale, Dutta, Moses, & Anandharamakrishnan, 2019).

For olive pomaces, hydroxytyrosol is the main phenolic compound in the range of 5.3 to 8 mg/g (Tamasi *et al*., 2019), followed by caffeic, gallic and chlorogenic acids (Alhamad *et al*., 2017), another research showed that a combination of hydroxytyrosol and gallic acid and their derivates have an antioxidant, emulsifiers and surfactant proprieties (Alonso *et al*., 2015). All this explain that strong positive relationship between the two parameters and the difference amount in this phenolic compound might be the reason why SCG reached a perfect correlation, followed by SFH, AOP, and NOP, and the difference between olive pomaces might be due to the storage effect because chlorogenic acid content is higher in stored one (Kulak & Çetinkaya, 2018).

Biosurfactants production and polyphenols content also showed a strong positive relation, it might be since nonionic surfactants can give higher extraction efficiency of polyphenols due to their physicochemical properties, surfactants forms micelles composed of a hydrophilic surface and hydrophobic core, this specific structure makes them capable to establish chemical and physical interactions with either hydrophilic or lipophilic substances and bind to phenolic compounds (Skrypnik & Novikova, 2020).

Other studies revealed that chlorogenic and caffeic acids can accelerate the beta-oxidation of free fatty acids pathway in human and rat's liver and intestinal microbiote by effecting transcriptional genes regulators (Skrypnik & Novikova, 2020; Xu *et al*., 2019). There is no detailed research about microbial regulation of beta-oxidation by chlorogenic acid, it might exist in bacteria, which provided a high level of biosurfactants production especially rhamnolipids by increasing polyphenols levels and vice versa, and this can explain the positive relationship obtained between the two parameters.

Conclusion and perspectives

Pretreatment of lignocellulosic biomass is required to overcome its recalcitrance, therefore making cellulose more accessible to enzymatic hydrolysis for sugar conversion. However, lignocellulose-derived byproducts, which can inhibit or deactivate enzymes and microbial biocatalysts, are formed. Results obtained in this study showed that polyphenols and flavonoids content differs from substrates to another and from untreated to treated ones. Thus, pretreatments caused a loss of polyphenols and flavonoids content in lignocellulosic substrates except for SFH, probably due to their phenolic compounds profiles and composition.

Results of treatment's effect on cellulase production indicated that pretreatments cause a decrease in cellulase activity and conversion efficiency. However, it is shown that pretreatments remove cell components including lipidic compounds, and the obtained results reveal that treated substrates induced a high decrease in cellulase activity especially in spent coffee grounds and olive pomace of the year, which indicate that a positive relationship might exist between lipids and cellulase production by *Bacillus cereus* 11778.

The effect of pretreatments on the production of biosurfactants by *Bacillus* cereus 11778 varies according to feedstock nature and composition and fermentation time. The present study showed that biosurfactants production decrease after treatments with all substrates, this might be due to the removal of lipids by treatments that plays a very important role in the synthesis of biosurfactants. These results indicate that a strong association exists between lipids, cellulase activity, and biosurfactants production.

Correlation studies show that cellulase activity, biosurfactants production, and polyphenols content have a strong relation, and can influence each other positively. Biosurfactants prevent the denaturation of enzymes during hydrolysis, thus promoting the production of cellulase, and enhancing the enzymatic hydrolysis of cellulose. The main phenolic compounds in all substrates are chlorogenic and caffeic acids, which serve as a natural surfactant and improve cellulase activity, so not every polyphenol is toxic for biodegradation of lignocellulosic substrate and biosurfactants production.

For a better understanding of this complicated subject, further researches need to be done. The study of the effect of lipids content in substrates on cellulase and biosurfactants productions can clarify the relationship between the studied parameters. To understand the role and effect of lipids on bioactive compounds production; a comparative study can be done between lipids rich substrates and free lipids substrates, by extracting them from the same substrates. The extracted lipidic fraction can be added to the fermentation medium, to see their direct effect.

Another comparative study can be done with non-lipidic lignocellulosic substrates like starch-rich substrates such as potato or banana peels, or sugars rich substrates like grape pomaces and molasses. Such a study can show the effect of substrate nature on bioactive product yields.

Chromatography techniques like HPLC (High-Performance Liquid Chromatography) can be used to reveal phenolic compounds profiles of each substrate before and after pretreatment, to understand and prove the exact effect of treatments on each component and how those profiles can affect the yields of different bioactive compounds (cellulase, polyphenols, and biosurfactants).

At the molecular level, it would be interesting to search if chlorogenic and caffeic acids can also regulate microbial lipids metabolism genes, by defining signaling pathways activated by these phenolic compounds leading to promote beta-oxidation genes. Also, a screening of cellulase and biosurfactants machinery genes in the used strain could help to understand genes regulation by different repressors and inductors. Molecular tools like directed evolution method can be used to improve cellulase structure, catalytic efficiency, binding, and tolerance to inhibitors.

For industrial purposes, a new method needs to be developed to valorize lignocellulosic wastes to different value-added bioactive products. First, the removal of the inhibitory effect of polyphenols without losing them or altering their properties, selectively and inexpensively, by using rich lipids carbon sources like oleaginous lignocellulosic wastes and genetically modified bacterial strains that gives high productivity. Lipids need to be recycled and reused after pretreatments to enhance biosurfactants production, thereby the recovery of released phenolic compounds; cellulase and fermentable sugars can be recovered at the end of biohydrolysis. Such a new approach can provide more productivity of cellulase, polyphenols, and biosurfactants using low coast, eco-friendly materials.

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Annexes

Table 01: PH of substrates after pretreatment

Pretreatment	**PH**
Coffee grounds	
NaOH	9,90
H_2SO_4	3,43
H_2O_2	4,48
Ethanol/methanol	6,50
Tea waste	
NaOH	10,77
H_2SO_4	2,85
H_2O_2	4,14
Ethanol/ methanol	6,55
Banana peel	
NaOH	10,33
H_2SO_4	3,14
H_2O_2	5,52
Ethanol/methanol	6,49
Olive pomace	
NaOH	10,95
H_2SO_4	3,07
H_2O_2	5,62
Ethanol/methanol	7,13
Potato peel	
NaOH	11
H_2SO_4	2,95
H_2O_2	6,63
Ethanol/methanol	6,97
Orange peel	
NaOH	10,57
H_2SO_4	3,79
H_2O_2	4,72
ethanol/methanol	5,66
Soybean waste	
NaOH	10,40
H_2SO_4	2,68
H_2O_2	5,29
Ethanol/methanol	7,68

Table 02: moisture content of substrates

Substrate	Loss percentage
Coffee grounds	56,75 %
Tea waste	75,2 %
Banana peel	89,32 %
Olive pomace	54,97 %
Potato peel	85,5 %
Orange peel	74,57 %
Soybean waste	8,9 %

Note. *Initial weight of substrates 400g*

Table 03: loss percentage after pretreatment

Pretreatment	Loss percentage
Coffee grounds	
NaOH	60 %
H_2SO_4	50 %
H_2O_2	23 %
Ethanol/methanol	35 %
Tea waste	
NaOH	74 %
H_2SO_4	43 %
H_2O_2	37 %
Ethanol/methanol	27 %
Banana peel	
NaOH	81 %
H_2SO_4	69 %
H_2O_2	69 %
Ethanol/methanol	63 %
Olive pomace	
NaOH	59 %
H_2SO_4	39 %
H_2O_2	36 %
Ethanol/methanol	42 %
Potato peel	
NaOH	76 %
H_2SO_4	92 %
H_2O_2	83 %
Ethanol/methanol	63 %
Orange peel	
NaOH	83 %
H_2SO_4	82 %
H_2O_2	84 %
Ethanol/methanol	64 %
Soybean waste	
NaOH	94 %
H_2SO_4	64 %
H_2O_2	51 %
Ethanol/methanol	47 %

Note. *Initial weight of substrates 10g*