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Enzymatic hydrolysis of food waste for bioethanol production

Hidrólise enzimática de resíduos alimentares para a produção de bioetanol

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ABSTRACT

The concern for environmental sustainability and the rational use of natural resources drives the development of new technologies to better utilize energy sources, culminating in the use of waste for biofuel production. This approach is strategic, as the use of agro-industrial and food waste aligns with the concept of circular bioeconomy and food security, allowing for value addition to waste and reducing environmental liabilities. Bioethanol stands out as the most promising biofuel derived from food waste, considering its chemical composition rich in carbohydrates and fermentable sugars. The biotechnological conversion of biomass into bioethanol requires pretreatment steps to facilitate enzyme action during the hydrolysis process, a crucial stage for sugar release. However, it underscores the need to optimize enzymatic processes, especially regarding pH and temperature ranges for enzyme activity, to ensure efficiency in converting biomass into bioethanol. The aim is to understand the processes involved in the enzymatic hydrolysis of organic waste. The literature review included studies with recent advances on the enzymatic hydrolysis of food waste for the sustainable production of bioethanol, using the keywords "Biomass," "Enzymatic hydrolysis," "Bioethanol," and "Food waste" or "Food residues". The hydrolysis of food waste for bioethanol production highlights the necessity of selecting the most efficient and sustainable pretreatment techniques, aiming to minimize byproduct generation while fully utilizing the raw material. Additionally, the use of different classes of enzymes in consortium during the production processes is emphasized.

Keywords: bioenergy; biofuels; circular economy; food residues.

RESUMO

A preocupação com a sustentabilidade ambiental e o uso racional dos recursos naturais impulsiona o desenvolvimento de novas tecnologias para melhor utilizar fontes de energia, culminando no uso de resíduos para a produção de biocombustíveis. Essa abordagem é estratégica, pois o uso de resíduos agroindustriais e alimentares está alinhado ao conceito de bioeconomia circular e segurança alimentar, permitindo a valorização dos resíduos e a redução das responsabilidades ambientais. O bioetanol destaca-se como o biocombustível mais promissor derivado de resíduos alimentares, considerando sua composição química rica em carboidratos e açúcares fermentáveis. A conversão biotecnológica da biomassa em bioetanol requer etapas de pré-tratamento para facilitar a ação enzimática durante o processo de hidrólise, estágio crucial para a liberação de açúcares. No entanto, ressalta-se a necessidade de otimizar os processos enzimáticos, especialmente em relação aos intervalos de pH e temperatura para a atividade enzimática, a fim de garantir eficiência na conversão da biomassa em bioetanol. O objetivo é compreender os processos envolvidos na hidrólise enzimática de resíduos orgânicos. A revisão da literatura incluiu estudos com avanços recentes na hidrólise enzimática de resíduos alimentares para a produção sustentável de bioetanol, utilizando as palavras-chave "Biomassa", "Hidrólise enzimática", "Bioetanol" e "Resíduos alimentares" ou "Resíduos alimentares". A hidrólise de resíduos alimentares para a produção de bioetanol destaca a necessidade de seleção das técnicas de pré-tratamento mais eficientes e sustentáveis, visando minimizar a geração de subprodutos enquanto utiliza totalmente a matéria-prima. Além disso, destaca-se o uso de diferentes classes de enzimas em consórcio durante os processos de produção.

Palavras-chave: bioenergia; biocombustíveis; economia circular; resíduos alimentares.

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Introduction

Food waste constitutes a significant portion of municipal solid waste, encompassing agricultural and industrial food waste, as well as commercial and domestic waste (Prasoulas et al., 2020). Waste occurs when produced food is not utilized for consumption, stemming from issues in production, logistics, and supply, involving the reduction of food quality or quantity as a result of decisions and conclusions made by retailers, food service providers, and consumers (Lahiri et al., 2023).

Additionally, it is recognized that proper disposal is essential for food waste, with a common practice being its disposal in landfills or through incineration (Dhiman and Mukherjee, 2021; Batool et al., 2023). According to Esteban and Ladero (2018), food waste includes peels, bones, cooking oil, and discarded portions of food.

In the context of the circular economy and bioeconomy, the application of food waste is observed in different routes, and Karthikeyan et al. (2018) consider the application in 4 viable options: a. for bioenergy and biofuels; b. to produce biofertilizers; c. to produce new biomaterials and industrial biochemicals; and d. to produce animal feed. More recently, Said et al. (2023) studied the concept of food waste bioeconomy intending to integrate different pathways for valorization through the Internet of Things, Artificial Intelligence, and Machine Learning strategies.

Food waste is promising from an economic and environmental point of view to produce biofuels such as bioethanol, due to the high carbohydrate content in its composition (Panahi et al., 2022), which can be starches, but also cellulose fibers, hemicellulose, and lignin (Ávila et al., 2018). In the process of converting food waste into bioethanol, it is necessary to break down more complex molecules than starch, such as cellulose (Ogeda and Petri, 2010; Panahi et al., 2022); in this way, the production of bioethanol from food waste will involve first and second-generation technologies.

The route based on enzymatic hydrolysis is considered efficient (Zhou et al., 2023) if the conditions for using and producing the enzymes are optimized (Chen et al., 2021). Studies addressing the enzymatic hydrolysis of various food waste biomasses focus on different biotechnological routes for the comprehensive utilization of diverse organic compounds (Caldeira et al., 2020). They emphasize the necessity of an enzymatic cocktail, incorporating accessory enzymes to enhance yields during the enzymatic hydrolysis of biomass (Álvarez et al., 2016; Chen et al., 2017; Du et al., 2020). Given the heterogeneous composition of food waste, subjected to pretreatment processes, complex chains are made available for enzyme hydrolysis. It is crucial to understand the chemical composition of raw materials to use specific enzymes tailored to each substrate (Zou et al., 2020).

Using food waste can reduce greenhouse gas emissions and global warming, as biofuels like bioethanol emit fewer greenhouse gases than conventional fuels (O'Driscoll et al, 2018; Abdullah et al., 2019). The production of biofuels from waste aligns with Sustainable Development Goals 7, 12, and 13 — focusing on affordable and clean energy, responsible consumption and production, and climate action. In the context of the circular economy and bioeconomy, reusing food waste is crucial in waste logistics and for building a more sustainable energy matrix through enzymatic hydrolysis for biofuel conversion.

Therefore, the present study aims to analyze recent advancements in the enzymatic hydrolysis of food residues as a sustainable treatment method for bioethanol production. The objective is to comprehend the processes involved in the enzymatic hydrolysis of food residues, spanning physical-chemical steps to the application of enzymes on various substrates of food waste.

Literature review

The study utilized Scopus, a major peer-reviewed literature database, to search for articles on enzymatic hydrolysis of food residues for sustainable bioethanol production (Calof et al., 2022). The search included keywords like "Biomass," "Enzymatic hydrolysis," "Bioethanol," and "Food waste" or "Food residues." The survey of articles was limited to the last 10 years to assess the primary advancements and technologies related to the enzymatic hydrolysis of food residues more comprehensively.

Out of 37 texts found, 36 were analyzed, revealing six main clusters in a literature review. The primary clusters emphasized keywords such as "bioethanol" and "enzymatic hydrolysis," with a focus on biomass pretreatment for bioethanol production. The secondary clusters highlighted enzymes like "cellulase," which are crucial for hydrolysis in food residues with lignocellulosic characteristics. Additionally, scientific productions predating the research were incorporated to provide further context and support for the topics addressed in this literature review.

Bioethanol from food waste

Biofuels are categorized into four generations based on the origin of the raw material used in conversion processes (Abdullah et al., 2019; Hafid et al., 2021). First-generation biofuels, derived from traditional food sources, like sugar and starch, pose risks to food security (Sun, 2024). Second-generation biofuels utilize less expensive and more abundant raw materials, including crop residues, lignocellulosic materials, and food waste (Kordala et al., 2024). The current industrial production of bioethanol often relies on first-generation raw materials like sugar cane or cereal grains, raising ethical concerns regarding global hunger and malnutrition (Mussatto et al., 2010; Pesce et al., 2020). However, it is important to note that some cereals, unfit for human or animal consumption, can be repurposed for ethanol production as waste. Additionally, there are underutilized agricultural areas worldwide that could contribute to bioenergy production (Lubis and Parashakti, 2019). These considerations highlight the need for a comprehensive discussion on the ethical implications and the balance between food and bioenergy production to meet global societal needs.

According to Osman et al. (2021), second-generation biofuels can be produced from carbon-neutral biomass. Using food waste as a raw material for second-generation biofuel production involves an application based on recovery through material reuse (Dhiman and Mukherjee, 2020). The food waste chain proves versatile in terms of applications and can be contextualized within the biorefineries utilizing the raw material (Dahiya et al., 2018) (Figure 1). Among the options for food waste disposal, reuse stands out as the most sustainable alternative, either through recovery (biofuels, enzymes, biopolymers) or through direct use as animal feed. The concept of biorefinery has gained significance, and can be defined as the use of refinery schemes to extract value from biomass through an extensive range of interconnected processes and products (Karmee, 2016).

Regardless of their origin, food waste is generally rich in carbohydrates, but the proportion of soluble (readily fermentable) and complex carbohydrates (requiring hydrolysis) can vary significantly (Angelo et al., 2017; Di Bitonto et al., 2018; Panahi et al., 2022; Rehman et al., 2023). Thus, depending on the eating habits of each country, food waste can contain large amounts of starch and cellulose, which need to be saccharified to be efficiently fermented. Saccharification can be carried out chemically (Ulbrich et al., 2020), thermochemically (Hashem et al., 2019), or enzymatically by adding, in the latter case, amylolytic and/or cellulolytic enzymes under appropriate conditions (Atitallah et al., 2019).

The production of bioethanol from waste is strongly dependent on the waste composition (Matsakas et al., 2014; Prasoulas et al., 2020). In general, food waste is predominantly composed of carbohydrates (30 to 60%), proteins (5 to 20%), and lipids (15 to 40%), with the content of these components varying according to the biomass composition (Xue et al., 2019).

In food waste, lignocellulosic compounds are found in cereals (Apprich et al., 2014; Caldeira et al., 2020), consisting of the cellulosic component containing structural carbohydrates like cellulose, hemicelluloses, and heterogeneous polymeric lignin as primary components. The content of these compounds fluctuates depending on different species (Das et al., 2021). Cellulose, due to its arrangement in linear and parallel microfibrils, is formed by extensive interchain and intrachain hydrogen bonds between individual strands. Cellulose exhibits high structural stability and is insoluble in water and most organic solvents.

For the conversion of these compounds through biotechnological routes for bioethanol generation, it becomes necessary to break down more complex compounds into those that are easily assimilated (Robak and Balcerek, 2018). Therefore, the pretreatment of food residues aims to make the carbohydrate content available in the medium for saccharification to occur and obtain bioproducts with high added value (Banu et al., 2020).

Pretreatments of food waste biomass

Biomass pretreatments are essential for breaking down lignocellulosic structures, modifying indirect factors, and improving direct factors that impact cellulose accessibility. These treatments facilitate cellulose and hemicellulose digestion and enhance hydrolysis rates of hydrolytic enzymes and chemicals (Dawson and Boopathy, 2007; Jørgensen et al., 2007; Arumugam et al., 2021). Various pretreatment methods, categorized as physical, chemical, physical-chemical, and biological, have been explored to increase saccharification and bioethanol production (Rezania et al., 2018; Banu et al., 2020; Shukla et al., 2023).

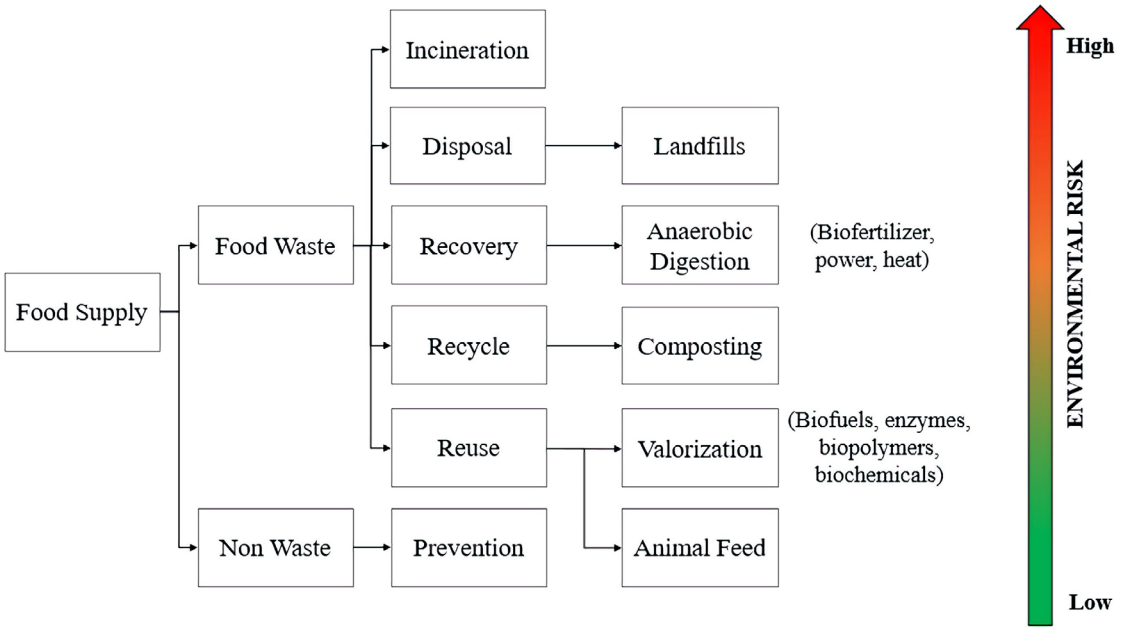

Figure 1 – Application of the Food Waste Chain in the Context of Biorefineries.

Chemical pretreatments are widely applied to lignocellulosic residues and substrates to remove lignin and/or hemicelluloses, thereby decreasing the cellulose crystalline structure and increasing pore size and surface area. This makes cellulosic biomass more susceptible to cellulolytic enzyme action for efficient degradation into sugar monomers (Behera et al., 2014; Das et al., 2021). At an industrial level, acid pretreatment, especially with sulfuric acid (H_2SO_4) and hydrochloric acid (HCl), is prevalent due to its efficiency in breaking down lignocellulosic materials, primarily degrading hemicellulose. However, acid pretreatment may not be as effective in lignin breakdown (Arumugam et al., 2021). Acid pretreatment breaks down some food waste components into monomeric sugars, converting polysaccharides into oligosaccharides and maltodextrins (Hafid et al., 2017). Kitchen waste pretreated with sulfuric or hydrochloric acid can increase ethanol fermentation yield, mainly due to enhanced sugar production during enzymatic saccharification (Panahi et al., 2022).

Like acid treatment, controlling the medium's pH can break certain lignocellulosic material chains. Alkaline pretreatment chemicals like sodium hydroxide (NaOH) and potassium hydroxide (KOH) have been applied to hydrolyze hemicellulose by saponifying intermolecular ester bonds of lignin and hemicellulose, preventing hemicellulose polymerization (Arumugam et al., 2021). Although chemical pretreatments are widely used for waste sludge and lignocellulosic substrates, limited studies have focused on the organic fraction of municipal solid waste (MSW), as chemical pretreatment may not be suitable for easily biodegradable substrates with high carbohydrate content (Ariunbaatar et al., 2014; Hafid et al., 2017).

Öner and Nazan (2018) compared acid and alkaline pretreatment methodologies in kitchen waste for bioethanol production. They utilized hydrochloric acid (HCl) and sodium hydroxide (NaOH) for acid and alkaline pretreatment, respectively, in concentrations ranging from 0 to 5%, with incubation times of 30, 60, and 90 minutes, and temperatures of 30°C and 60°C. The optimal conditions for pretreatment involved incubating samples in 1% HCl for 90 minutes at 60°C, producing 638.24 mg carbohydrates/g of fermentable sugars in the dry sample. Pretreatment with 3% NaOH for 90 minutes at 30°C yielded 414.35 mg of carbohydrates/g of fermentable sugars and a 61.66% glucose recovery. Acid or alkaline pretreatment, along with increasing temperatures, enhanced glucose yield from kitchen waste compared to untreated organic material.

Physical treatment involves breaking down material structures, including milling and hydrothermal treatment of biomass. Smaller particle sizes from milling increase the surface area accessible to enzymes, leading to faster hydrolysis. Hydrothermal treatment aims to alter the structure of the insoluble fraction, making it more biodegradable (Hafid et al., 2017; Gao et al., 2021; Zhang et al., 2023). However, fine granulometry in biomass can lead to lumps during pretreatments and enzymatic hydrolysis, negatively impacting total sugar production (Sarkar et al., 2012; Hafid et al., 2017).

Among the most widely used pretreatments currently are thermochemical methods, utilizing acids and bases with high temperatures to produce low molecular weight products like fermentable sugars (Zabed et al., 2019). However, there is a shift in the industry towards more sustainable methodologies, incorporating biological means such as microorganisms and enzymes in bio-product production (Saha et al., 2016; Shukla et al., 2023).

In the biological process, microorganisms utilize free and readily accessible carbohydrates as the main carbon source during the pretreatment process. Maintaining a pure culture of bacteria and optimizing their growth conditions for food waste pretreatment is usually challenging due to microbial competition with native microorganisms (Hafid et al., 2017; Panahi et al., 2022). Biological pretreatment methods using microorganisms, such as white, brown, or soft rot fungi, can be employed for delignification and sugar production from lignocelluloses (Tabatabaei et al., 2020). For instance, fungi of the *Basidiomycetes* genus are cellulase and xylanase producers, while the oxidative-ligninolytic system consists of laccases, ligninases, and peroxidases that degrade lignin and phenyl components (Hatakka, 1983; Ilić et al., 2021). Extracellular ligninolytic enzymes degrade lignin, making the substrate more easily degradable without producing inhibitors commonly found in other pretreatments (Salvachúa et al., 2011). Specifically, the white-rot basidiomycete *Irpex lacteus* demonstrates a high capacity for biodegradation (Mezule and Civzele, 2020; Salvachúa et al., 2011).

Moreover, crude enzymes produced from biomass lysate are directly used for pretreatment to reduce costs (Kiran et al., 2014; Yin et al., 2016). Biological pretreatment offers advantages such as low energy requirements and mild conditions. However, there is an inevitable trade-off between lignin removal and sugar consumption, as the fungal strategy involves degrading lignin to access cellulose and hemicellulose more easily. Another main disadvantage, compared to physicochemical pretreatments, is the extended time required to achieve similar digestibility improvements, often ranging from 4 to 8 weeks (Sarkar et al., 2012). This time can be reduced to 2 to 3 weeks by combining biological treatment with an alkaline wash under mild conditions and optimizing operating conditions (Salvachúa et al., 2011; López-Abelairas et al., 2013).

Potumarthi et al. (2013) studied simultaneous pretreatment and saccharification of rice husks by *Phanerochaete chrysosporium*. Effective delignification was achieved by cultivating the fungus on rice husks, and the pretreated biomass underwent enzymatic hydrolysis. Enzymes such as cellulase, xylanase, lignin peroxidase, glyoxidase, and alcohol aryl oxidase were produced during fungal pretreatment. The highest reducing sugar content (895 mg/mL) was observed on the eighteenth day of fungal treatment. This method avoids operating costs associated with washing and removing inhibitors during conventional pretreatment.

Enzymatic hydrolysis

Enzymatic hydrolysis has emerged as a primary method in most biological processes for treating and valorizing food waste, serving as a precursor to bioethanol production from food waste (Salimi et al., 2019; Zhang et al., 2020; Zou et al., 2020). Notably, enzymatic hydrolysis offers advantages such as the absence of toxic compounds generated for yeast during fermentation compared to chemical hydrolysis processes (Sagar et al., 2024). Additionally, it exhibits low corrosion when compared to chemical methods (Sarkar et al., 2012; Zhang et al., 2012; Lv et al., 2024).

Enzymatic hydrolysis is employed to break down polysaccharides in food waste, releasing simple sugars like glucose, xylose, fructose, galactose, and ribose. While yeasts of the genus *S. cerevisiae* easily utilize sugars like glucose and fructose, sugars derived from hemicellulose hydrolysis, such as pentoses, may require other microorganisms or even genetically modified organisms (Qaseem et al., 2021). The concentration and productivity of bioethanol can vary based on the fermentable sugar concentration in the hydrolyzed broth. Consequently, selecting an appropriate enzymatic formulation that aligns with the composition of food waste is crucial to enhancing the enzymatic hydrolysis process (Anwar Saeed et al., 2018; Salimi et al., 2019), as different matrices may require specific or combined enzyme application configurations (Esteban and Ladero, 2018) (Figure 2).

In Table 1, enzymes used in the enzymatic hydrolysis process of food waste for bioethanol production are presented, highlighting the main use of cellulolytic and amylolytic enzymes, as well as commercial enzymes in the hydrolysis process. According to Anwar Saeed et al. (2018), the dominant enzyme used in the hydrolysis of food waste to produce ethanol is glucoamylase, also known as amyloglucosidase. Since food waste is rich in carbohydrates and starches, this enzyme is responsible for breaking long-chain glycosidic bonds into glucose units, which are then consumed by yeast and transformed into ethanol during the fermentation process.

Jarunglumlert et al. (2021) assessed the enzymatic hydrolysis of cafeteria food waste using the commercial enzyme α-amylase derived from *Aspergillus oryzae* (Sigma–Aldrich). They determined the optimal enzyme concentration and hydrolysis duration for achieving the highest reducing sugar content. The enzyme concentration ranged from 1 to 5% w/w (g enzyme/g dry MSW), and hydrolysis occurred for 1 to 9 hours at 60°C, maintaining the pH of the food waste biomass within the ideal range for enzymatic activity (4.0–6.5), as indicated by the supplier. With an increased enzyme concentration (5%), the production of reduced sugar increased, reaching a maximum of 0.49 g/g of food waste.

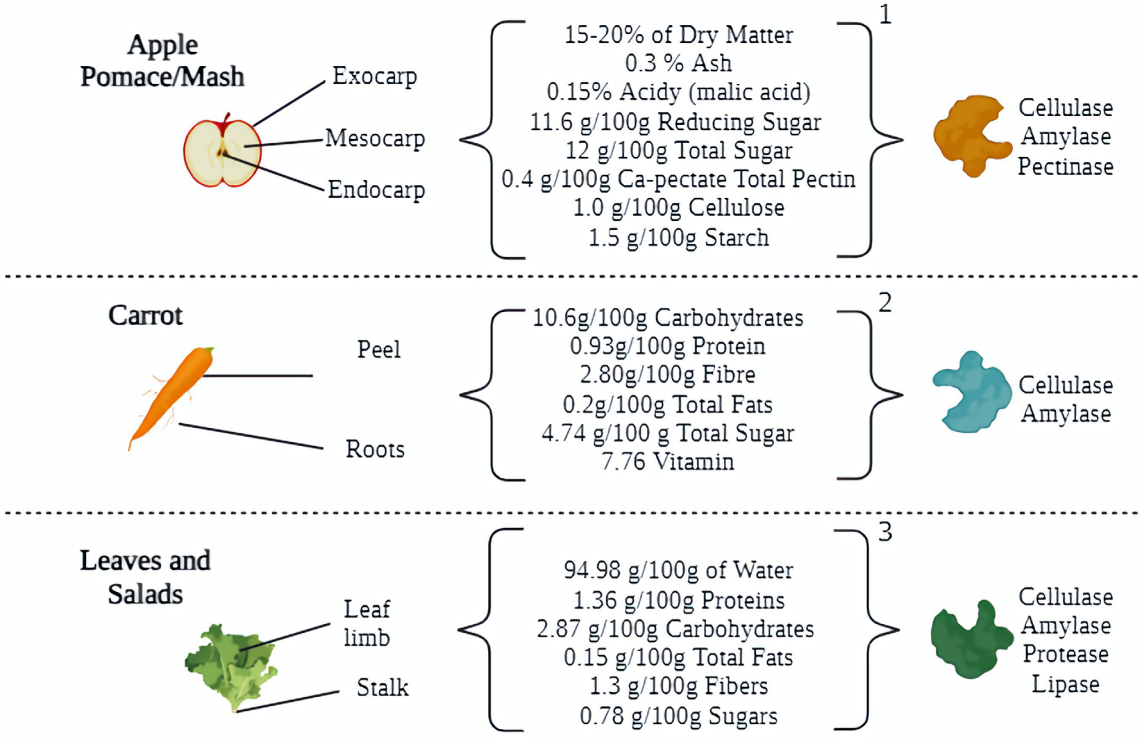

Figure 2 – Food residues and enzyme classes for hydrolysis.
Source: (1) Savatović et al. (2009); (2) Sharma et al. (2012); (3) USDA National Nutrition Database.

Table 1 – Use of enzymes in the enzymatic hydrolysis processes for obtaining bioethanol from food waste.

Food waste type	Enzymes	Origin of enzymes	Hydrolysis process	Efficiency	References
Municipal solid waste (MSW)	α-amylase	Commercial enzyme produced by *Aspergillus oryzae*	10% (w/v) of MSW, enzyme concentration of 1, 3, 4, and 5% w/w (g enzyme/g dry FW). Hydrolysis was carried out to 1, 2, 3, 4, 5, and for 9h at 60°C and pH of 4.0–6.5.	After 120h of fermentation, almost all reducing sugars in the hydrolyzate were converted to ethanol, yielding 0.43–0.50 g ethanol/g reducing sugar, or 84.3–99.6% of the theoretical yield	Jarunglumlert et al. (2021)
Household food waste	Cellulase, amylase, amyloglucosidase	Commercial enzymes	pH 4.8 and 50°C, a solid load of 10% ST biomass/v, supplementation with three enzymes (2 to 30 FPU/g ST)	Maximum fermentation yield reaching 83%. Enzymatic mixture with the highest amount of cellulolytic and amylolytic enzymes, with ethanol yields reaching 141.06±6.81 g ethanol/kg residue	Ntaikou et al. (2021)
Kitchen food waste	Amylase	Enzyme produced by *Bacillus licheniformis*	40% w/v of dry material for 8h with 15 IU/g of amylase at 50°C with pH at 7.5	The process developed in the present study leads to 0.129 g/mL, that is, 0.32 g/g of ethanol production biomass.	Sondhi and Kaur (2020)
MSW	Endo- and exoglucanase, xylanase, cellulase, amylase, β-glucosidase, β-xylosidase and glucoamylase	Commercial glucoamylase and other enzymes produced in the laboratory by the fungus *Fusarium oxysporum*	Enzymatic cultivation supplemented with pretreated MSW (cultivation to RS ratio, 1/10 w/w) and commercial glucoamylase, hydrolysis was carried out at 50±1°C on a rotary shaker (250 rpm)	Supplementation of the mixed culture with glucoamylase resulted in 30.3 g/L of ethanol with a volumetric productivity of 1.4 g/L/h	Prasoulas et al. (2020)
MSW	Amylase and cellulase	Amylolytic (NS22109) and cellulolytic (NS22177) produced by Novozyme	Starch in MSW was hydrolyzed by amylase (36 µL/g starch) at 65°C for 1 h. Followed by hydrolysis of cellulose with cellulase (304 µL/g cellulose) at 50°C for 5 h	Saccharification yielded between 16.43-17. 31 g of glucose per 100 g of raw material	Taheri et al. (2020)
Hamburger waste	α-amylase	Commercial enzyme supplied by Ningxia Chemicals Ltda.	50g of ground residue and various enzyme loading volumes (0.02 mL/L, 0.08 mL/L and 0.14 mL/L) named B1, 2 and 3. The temperature of B1 and B3 was 90°C, while the temperature of B2 was set to 95°C	Reducing sugar (RS) production was 39.2 g/L, and hydrolysis efficiency of 0.784 g AR/g RH could be achieved with B3, with the highest ethanol production of 27.4 g/L	Han et al. (2020)
Canteen waste (rice, meat and vegetables)	Glucoamylase	Commercial enzyme produced by *A. niger*	Glycoamylase (85 U/mL), hydrolysis was performed at 100 rpm for additional 6h (Hafid et al., 2015)	Hydrolysis efficiency of 86.8% was observed via acid-enzymatic pretreatment. The ethanol yield was 0.42 g/g with a conversion efficiency of 85.38%	Hafid et al. (2017)
Fruit waste	Pectinase, glucanase and xylanase	Enzymes produced by microorganisms *A. citrisporus* and *Trichoderma longibrachiatum*	Enzymes were added with concentrations of 12–16 and 10–25 mg protein/g fruit waste, respectively, 1% matter (w/v) at pH 4.8 for 48h at 45°C	Enzymatic conversion rates of fruit residues into fermentable sugars were approximately 90% after 48 h. Ethanol concentrations (14.4–29.5 g/L) and yields (90.2–93.1%)	Choi et al. (2015)
Kitchen food waste	α-amylase and amyloglucosidase-AMG	Commercial enzymes - Megazyme	Hydrolysis was performed at combined enzyme dosage levels (0–3.6% v/v α-amylase and 0–3.2% v/v amyloglucosidase-AMG) at pH 5.0, 50°C for 30 min	The glucose concentration increased by about 300% after pretreatment with acid or KOH in combination with enzymatic hydrolysis when compared to the untreated residues	Vavouraki et al. (2014)
Kitchen food waste	α - amylase, amyloglucosides, cellulase and glycosidase	Commercial enzymes (*Aspergillus oryzae*, A6211-1MU; *Aspergillus niger*, AMG; *Trichoderma viride*, C1794-10KU, and almonds, 49290)	To liquefy the starchy portion, α-amylase was added (120 U/g dry substrate (SS)) at 95°C for 1h and pH 5.5, the oligosaccharides and the cellulosic portion were processed with AMG (120 U/g SS), cellulase (8 FPU/g SS), and β-glucosidase (50 U/g SS).	The highest and lowest glucose production rates were found to be 0.644 and 0.128 (h−1). Fermentation results indicated that final ethanol concentrations are not significantly improved by nutrient addition (17.2–23.3 g/L)	Cekmecelioglu and Uncu (2013)

Sondhi and Kaur (2020) employed amylase produced in the laboratory by *Bacillus licheniformis* for the enzymatic hydrolysis of household food waste, using 15 IU/g of amylase and a pH of 7.5. The process resulted in the production of ethanol at a rate of 0.32 g/g of biomass. The waste primarily consisted of potato peels, onions, and other vegetables, rich in starch. Amylase acted to break the α-(1–4) bonds of starch.

Viscosity is a crucial parameter in enzymatic processes, and α-amylase enzymes, by releasing soluble sugars, can reduce the viscosity of hydrolysis media. Amylase treatment in the enzymatic saccharification of kitchen waste effectively reduced viscosity and facilitated fermentation by converting starchy sugars to glucose (Sondhi and Kaur, 2020).

Cekmecelioglu and Uncu (2013) conducted enzymatic hydrolysis of kitchen food waste using four enzymes. The process involved liquefaction of the starchy portion with α-amylase, followed by simultaneous processing of starch-based oligosaccharides and the cellulosic fraction using amyloglucosidase, cellulase, and β-glucosidase. The highest glucose concentration achieved was 64.8 g/L, calculated as 0.70 g glucose/g dry residue (or 70% of non-pretreated samples) after 6 hours of hydrolysis.

Taheri et al. (2020) performed enzymatic hydrolysis of household food waste using amylolytic and cellulolytic formulations from Novozymes. The hydrolysis occurred in two stages, with amylase hydrolyzing starch at an optimum pH of 4.8 and a temperature of 65°C in the first stage, followed by cellulase hydrolysis of cellulose at 50°C in the second stage. The highest glucose concentrations were observed in food waste hydrolysates subjected to hexane oil extraction, yielding 21.66 g of glucose per 100 g of raw material, with 90.30 and 53.75% degradation of starch and cellulose, respectively.

Enzymatic hydrolysis offers many advantages over the chemical process. However, one of the challenges associated with using enzymatic hydrolysis for bioethanol production is the current cost of pretreatment and enzymes, which are significant obstacles to large-scale ethanol production (Berlin et al., 2006).

To make enzymatic hydrolysis of food waste more economical, it is preferable to produce enzymes on-site from less expensive raw materials. Solid-state fermentation (SSF) stands out as a promising approach, offering several biotechnological advantages such as greater fermentation capacity, stability of the final product, less catabolic repression, and economical technology (Behera and Ray, 2016; Sadh et al., 2018; Arumugam et al., 2021). SSF is particularly suitable for the cultivation of filamentous fungi, as solid substrates mimic the fungi's natural habitat, resulting in better growth and secretion of a wide range of enzymes. The selection of an appropriate substrate is crucial for the process, with the medium acting as physical support and a nutrient source (De Castro and Sato, 2015; Prasoulas et al., 2020).

Choi et al. (2015) evaluated the activities of pectinase, endo- and exoglucanase, and xylanase produced by *Aspergillus citrisporus* and *Trichoderma longibrachiatum* in the enzymatic hydrolysis process of fruit residues (citrus fruits, apples, bananas, and pears). Two unknown enzymes, referred to as internal enzyme A (produced by *A. citrisporus*) and internal enzyme B (produced by *T. longibrachiatum*), were assessed for endo and exoglucanase, pectinase, and xylanase activities. The enzymatic activities for enzyme A were 8.41, 0.18, 170.95, and 17.90 U/mg of protein for endoglucanase, exoglucanase, pectinase, and xylanase, respectively. Enzyme B exhibited enzymatic activities of 13.22, 1.26, 4.34, and 1.11 U/mg of protein for endoglucanase, exoglucanase, pectinase, and xylanase, respectively. Enzymes A or B were added to fruit waste at concentrations of 12–16 and 10–25 mg protein/g fruit waste, respectively. Enzymatic hydrolysis was carried out at 1% matter (w/v) with a pH of 4.8 at 45°C. The enzymatic conversion rates of fruit residues to fermentable sugars were approximately 90% for all raw materials after 48 h. Fruit waste proves to be an attractive biomass alternative for bioethanol production due to its high levels of fermentable sugars, such as sucrose, glucose, and fructose (Choi et al., 2015).

In addition to the factor of polysaccharide hydrolysis, another important consideration for the bioconversion of food waste into valuable bioproducts is the solid-liquid ratio of the substrates. A low solids load increases the energy demand to raise ethanol concentration during fermentation, while a high load causes substrate inhibition (Uncu and Cekmecelioglu, 2011). However, a high proportion of solids to liquid can pose challenges in terms of homogeneous mixing, heat and mass transfer problems, and diffusion limitations of enzymes and final products. Therefore, careful attention should be paid to optimizing the mixing system in a bioreactor and/or enzymatic hydrolysis operation methods (e.g., using fed-batch instead of a batch) to address both the relatively high enzyme digestibility index and the reduction of sugar concentration during batch enzymatic hydrolysis of food waste (Yan et al., 2012).

Furthermore, Choi et al. (2015) note that higher concentrations of food residues correspond to increased enzymatic activity with both enzymes. Studies highlight that enzymatic hydrolysis of food waste is affected by factors like enzyme concentration, specificity, and substrate characteristics (Patria et al., 2022).

Similar findings were observed by Ntaikou et al. (2021), who studied the enzymatic hydrolysis of food waste, varying the enzymatic concentration of a mixture of cellulolytic and amylase enzymes from 2 to 30 FPU/g TS. They found that higher enzyme concentrations (30 FPU/g TS) led to nearly 95% conversion rates of residues to reducing sugars and the highest ethanol conversion rate. The authors emphasized that higher enzyme concentrations enhanced enzymatic hydrolysis, especially with the simultaneous action of cellulolytic and amylase enzymes on starches and cellulose, increasing conversion rates. Higher enzyme concentrations can accelerate hydrolysis, but considerations for enzyme stability and cost-effectiveness are crucial when balancing enzyme concentration.

Enzymes with high specific activity are more effective in substrate conversion, yielding higher sugar yields during food waste hydrolysis (Torres-León et al., 2021; Padhan et al., 2023). Selecting enzymes with high substrate-specific activity in food waste can enhance biomass conversion into fermentable sugars (Hafid et al., 2017; Han et al., 2020).

Achieving a balance in enzyme concentration is essential to avoid high operational costs and ensure process efficiency (Choi et al., 2015; Ntaikou et al., 2020).

Nevertheless, Yu et al. (2013) mention the competitive adsorption behavior of enzymes at high concentrations and their sensitivity to active binding site availability, crucial for enzyme/substrate interaction. To address this, appropriate enzyme concentration or increased exposure of active binding sites on substrates through pH/temperature adjustments, cofactor use, protein engineering, and other methods are necessary (Zhang et al., 2021; Sun et al., 2023). They also note that substrate availability and accessible surface properties change throughout the hydrolysis process, leading to dynamic equilibrium between bound and free enzymes (Yu et al., 2013).

Enzymatic hydrolysis primarily relies on bound enzymes, while free enzymes play a minor role after a certain hydrolysis period (Desai et al., 2021). Yang et al. (2019) achieved high storage stability (82.5%) and recycling rate (53.6%), and improved the stability/durability of immobilized lipase enzymes with magnetic dialdehyde starch nanoparticles. Free enzymes offer simplicity and flexibility in operation but face challenges like instability, recovery/reuse difficulties, and susceptibility to inhibition by reaction products (Zhang et al., 2021).

Research on free enzymes focuses on recycling strategies to enhance their value in industrial applications (Xin et al., 2020; Zhang et al., 2021). Despite decreased activity in recycled enzymes, efficiency can be improved with high enzyme concentrations and moderate temperatures (Xin et al., 2020; Cai et al., 2023).

Regarding the use of enzymes, advances in enzyme development and optimization of enzymatic hydrolysis processes for bioethanol production have been significant. Strategies include using immobilized enzymes, genetic engineering for improved activity, and specific enzyme cocktails for diverse food waste, aiming at efficiency and cost reduction (Anwar Saeed et al., 2018; Torres-León et al., 2021; Panahi et al., 2022).

Conclusion

In the production of bioethanol through the enzymatic hydrolysis of food waste, various factors must be considered, including the characteristics of the raw material, production costs, and the demand for advanced technology. When discussing scale-up, the mechanisms for bioethanol production through the enzymatic route can only be ensured if they are based on the bioconversion of abundant, renewable, and low-cost (or even zero-cost) biomass types, preferably those requiring minimal pretreatment for exploitation.

This literature review ensures that the explored routes align with the mentioned constraints, with further advancements needed, particularly in the 'homemade' production of enzymes. One of the significant challenges in enzymatic hydrolysis is achieving a more cost-effective enzyme production. Additionally, there is a need for the development of suitable pretreatment protocols to enhance enzymatic activity. Due to the diverse characteristics of food waste, studies commonly employ different pretreatment techniques, necessitating standardization and adaptation of these protocols. Strengthening various routes is also essential to achieve integrated biorefineries using food waste.

Moreover, numerous studies emphasize the prominent use of the enzyme amylase in the enzymatic hydrolysis process, given its proficiency in breaking down carbohydrates (starch) into fermentable sugars. The application of enzymes in a consortium, such as enzyme cocktails, allows for simultaneous interactions, facilitating the degradation of carbohydrate chains, which become available for fermentation and bioethanol production. Additionally, the potential use of different enzymes in the same hydrolysis process broadens the applicability in food waste systems with varied compositions.

Authors' contributions

FAGUNDES, V.D.: conceptualization, formal analysis and investigation, writing – original draft preparation. FREITAG, J.F.: conceptualization, formal analysis and investigation, writing – original draft preparation. SIMON, S.: writing – original draft preparation, writing - review and editing. COLLA, L.M.: writing – review and editing, supervision.

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