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DOCTORAL DISSERTATION

Title of doctoral dissertation: Studies on the application of acoustic cavitation as a pre-treatment technique for the fermentation of food waste-based feedstocks to produce lactic acid

Title of doctoral dissertation (in Polish): Badania nad zastosowaniem kawitacji akustycznej jako techniki wstępnego przetwarzania wsadu na bazie odpadów spożywczych do procesu fermentacji w celu produkcji kwasu mlekowego

Supervisor *signature*
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Gdańsk, 2025

GDAŃSK UNIVERSITY
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Description of Doctoral Dissertation

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Summary of doctoral dissertation in English

This thesis explored the use of ultrasounds (US), inducing acoustic cavitation, as a pre-treatment strategy to enhance lactic acid production from the fermentation of food waste-based feedstocks. Two ultrasonic reactors, one operating at a low frequency of 24 kHz and the other working at a higher frequency of 120 kHz, were compared based on their ability to improve soluble chemical oxygen demand (sCOD). Then, the influence of power density on sCOD and the subsequent production of lactic acid and volatile fatty acids (VFAs) through fermentation processes (FPs) was investigated. Subsequently, the effect of the US in combination with chemical agents, including an acid (HCl), an alkali (NaOH), and an oxidant (H_2O_2), was studied. The study progressed to the fermentation of actual food waste using the most effective reactor and power density. The effect of pre-treatment duration and food waste concentration in the ultrasonic reactor on sCOD and the production of lactic acid and VFAs was analyzed. The experimental results clearly demonstrated the strong potential of acoustic cavitation as an effective pre-treatment method to intensify lactic acid production from the fermentation of food waste. Finally, an economic evaluation was conducted to determine the financial and practical viability of combining this ultrasonic pre-treatment into FPs.

Summary of doctoral dissertation in Polish

Niniejsza rozprawa doktorska dotyczy zastosowania ultradźwięków do indukowania zjawiska kawitacji akustycznej do wstępnego przetworzenia wsadu do procesu fermentacji odpadów spożywczych w celu zwiększenia produkcji kwasu mlekowego. Porównano dwa rodzaje reaktorów ultradźwiękowych (częstotliwość 24 kHz lub 120 kHz) do podwyższenia parametru rozpuszczalnego chemicznego zapotrzebowania na tlen (ang. *soluble Chemical Oxygen Demand*, sCOD) wsadu do fermentacji. Wykonano badania wpływu gęstości mocy ultradźwięków na sCOD i późniejszą produkcję kwasu mlekowego i lotnych kwasów tłuszczowych w procesach fermentacji. Zbadano również wpływ ultradźwięków w połączeniu z odczynnikami chemicznymi, w tym kwasem (HCl), zasadą (NaOH) i utleniaczem (H_2O_2). Dla wytypowanych warunków procesu, z zastosowaniem wsadu w postaci rzeczywistych odpadów żywnościowych, przeanalizowano wpływ czasu trwania procesu w reaktorze ultradźwiękowym na wzrost sCOD oraz późniejszą produkcję kwasu mlekowego i lotnych kwasów tłuszczowych. Wyniki badań jednoznacznie wykazały duży potencjał kawitacji akustycznej jako skutecznej techniki wstępnej obróbki wsadu, zapewniającej intensyfikację produkcji kwasu mlekowego w procesie fermentacji odpadów żywnościowych. Na koniec przeprowadzono analizę ekonomiczną w celu określenia opłacalności i zasadności zastosowania wstępnej obróbki ultradźwiękowej do procesów fermentacji.

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Abbreviations

AcA	Acetic acid
AOPs	Advanced oxidation processes
BuA	Butyric acid
°C	Degrees Celsius
CCUS	Carbon capture, utilization, or storage
cm	Centimeter
cm^2	Square centimeter
CSTR	Continuously-stirred tank reactor
Eq.	Equation
FP	Fermentation process
FPs	Fermentation processes
GC	Gas chromatography
g/L	Gram per liter
HPLC	High-performance liquid chromatography
ISR	Inoculum to substrate ratio
kg/L	Kilogram per liter
kHz	Kilohertz
kWh	Kilowatt-hour
LAB	Lactic acid bacteria
min	Minutes
mg	Milligram
mL/min	Milliliter per minute
mm	Millimeter
mM	Millimolar

µm	Micrometer
nm	Nanometer
*p*CBA	*Para*-chlorobenzoic acid
PHAs	Polyhydroxyalkanoates
PrA	Propanoic acid
rpm	Revolutions per minute
sCOD	Soluble chemical oxygen demand
sono-HF	High-frequency reactor
sono-LF	Low-frequency reactor
TS	Total solids
TSS	Total suspended solids
UHPLC	Ultra-high performance liquid chromatography
US	Ultrasounds
UV	Ultraviolet
V	Volt
VaA	Valeric acid
VFAs	Volatile fatty acids
VSS	Volatile suspended solids
W	Watt
W/L	Watt per liter
w/v	Weight per volume

1. Introduction

The global increase in food waste over the years has become a serious issue. Studies indicate that food waste now accounts for over one-third of the total food produced worldwide, contributing not only to economic losses but also to environmental concerns [1, 2].

The conversion of waste into valuable products helps reduce overall waste generation, aligning with the principles of the circular economy. The top priority in effective waste management is to minimize waste production at its source through smarter design, sustainable consumption, and efficient resource use. The next best steps involve recycling and reusing materials to extend their lifecycle, reduce environmental impact, and promote a more sustainable and resource-efficient economy [3].

One effective method of recycling food waste is using it as a feedstock in biotechnological processes such as anaerobic digestion, where microorganisms break down organic matter in the absence of oxygen. This process can yield high-value products such as volatile fatty acids (VFAs) and lactic acids, which have applications in industries ranging from pharmaceuticals to biodegradable plastics. The potential of food waste can also be employed for the production of biofuels as sustainable alternatives to fossil-based resources [1, 4]. In **Figure 1**, some important compounds, including biofuels (e.g., hydrogen and methane), organic acids (e.g., lactic acids and VFAs), and alcohols (e.g., ethanol and n-propanol), produced from the conversion of food waste through anaerobic digestion, are presented.

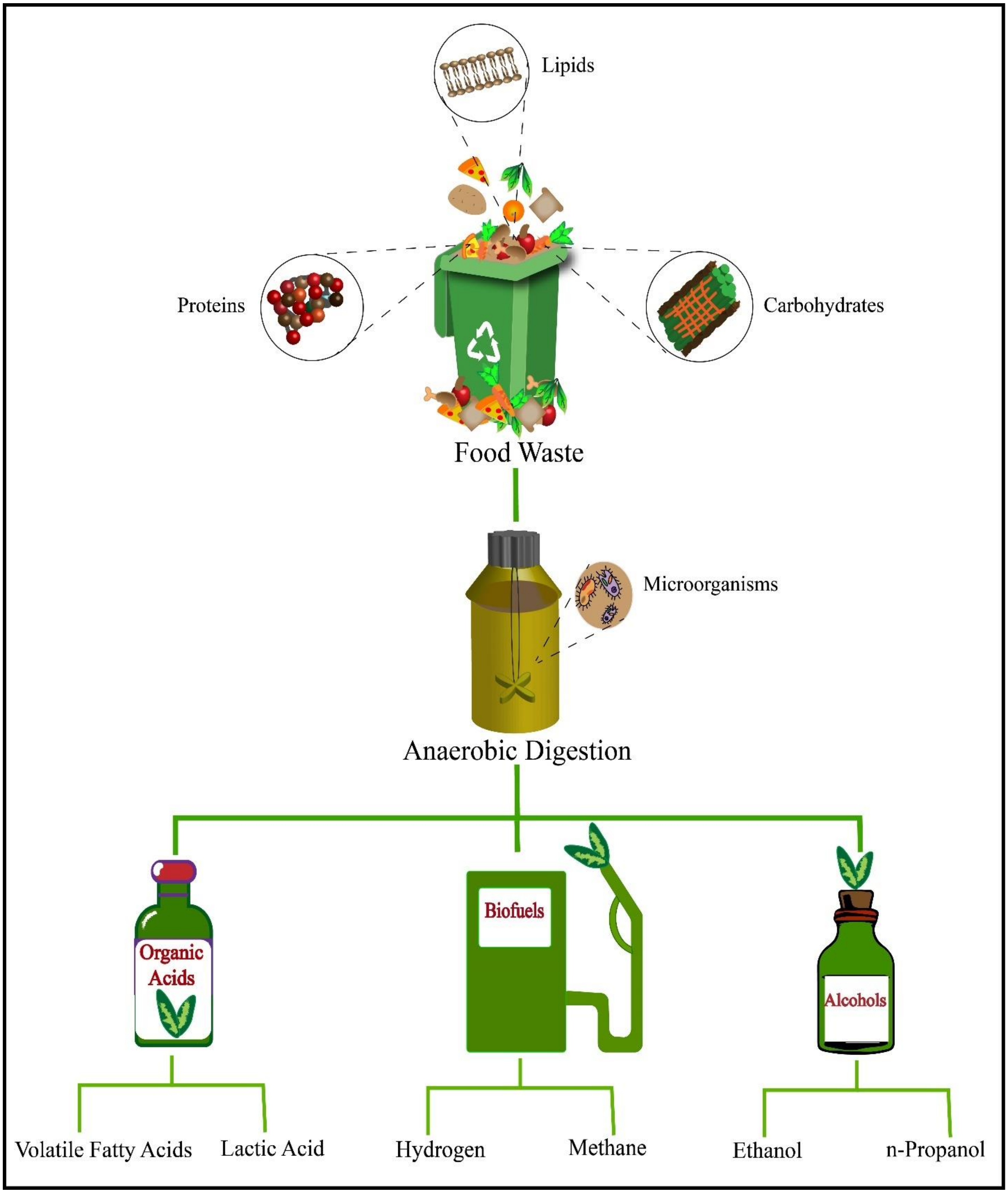


Figure 1. Valuable compounds produced through the anaerobic digestion of food waste.

The disintegration of feedstocks during the hydrolysis stage plays a key role in anaerobic digestion, as it is the first and often rate-limiting step in the process of converting organic matter into valuable compounds [5]. During this stage, complex macromolecules such as carbohydrates and proteins are converted into simpler, more accessible forms by hydrolytic enzymes, enabling subsequent microbial fermentation. However, if feedstocks are resistant to hydrolysis due to their structural complexity or composition, it can significantly hinder the overall efficiency of the process. This resistance may lead to slower reaction rates, incomplete degradation, and ultimately lower yields of desired products [6]. The utilization of a pre-treatment stage to modify the properties of feedstocks is a strategy to enhance the hydrolysis process in fermentation. This pre-treatment can involve chemical or physical methods, or their combination to break down complex organic structures, making them more available to enzymatic actions [7].

Cavitation pre-treatment techniques facilitate the breakdown of larger particles into finer ones, resulting in an increase in surface area [8-12]. The physical (e.g., shock waves and shear wall stress) and chemical impacts (e.g., hydroxyl radicals) generated by cavitation processes can assist in the breakage of chemical bonds within large, complex molecules, leading to their breakdown into smaller, lighter, and more bioavailable molecular structures. This alteration enhances enzyme-substrate interactions, leading to a more efficient and accelerated hydrolysis process [5].

1.1. Food waste

Food waste refers to organic matter that can be produced throughout various phases of the food system. This includes waste generated on farms during crop cultivation and harvesting, during transportation and storage where food may spoil or be damaged, in factories and processing plants where unusable by-products are created, throughout distribution networks and retail environments where unsold or expired goods accumulate, and finally at the consumer level at homes, in restaurants, and other food service establishments where leftovers and discarded food contribute to the overall volume of waste [13]. The continuous rise in the global population, along with urban and economic development, is significantly contributing to the growing volume of food waste, causing additional problems in waste management systems and environmental resources. Current estimates indicate that global waste generation could reach approximately 3.4 billion tons per year by 2050. This represents a substantial increase from the current level of around 2 billion tons, highlighting the urgent need for more sustainable waste management practices and policies [14]. In Central and South Asia, approximately 21% of food is lost or discarded, representing a significant portion of food waste in these regions. This amount is higher than the combined food waste rate of North America and Europe, which together account for 16% food loss. On the other hand, New Zealand and Australia exhibit the lowest food loss, with only 6% of food being wasted in these countries [15].

In most cases, food waste is simply discarded as trash, with only a small portion occasionally reused by farmers as organic fertilizer to enrich the soil. However, the large amount of this waste creates serious problems. It contributes to environmental problems such as greenhouse gas emissions from landfills, soil and water pollution, and the unnecessary consumption of resources used in the first place [16].

Food waste contains valuable nutrients, mainly composed of carbohydrates (30–60%), proteins (5–20%), and lipids (15–40%), making it an excellent raw material for various biological processes [17]. The proportion between the main components of food waste can vary depending on its composition. If the waste contains a higher proportion of vegetables and rice, the carbohydrate content will be greater. In contrast, meat and eggs are the primary sources of protein in food waste [18].

1.2. Valorization of food waste

The serious problem of pollution caused by the growing amounts of waste has attracted attention, and many scientists have been focusing on how to use these waste materials instead of simply throwing them away. This approach, known as waste valorization, involves finding efficient ways to turn waste into useful products, such as biofuels, fertilizers, or other valuable resources, to reduce environmental damage while also creating economic and sustainable benefits [14, 19].

1.2.1. Incineration

Incineration as a waste management method offers multiple benefits, including significant reduction of waste volume and the production of usable energy. It involves the controlled burning of organic materials to create heat, which is then used to produce electricity through steam turbine systems [20]. Incineration also produces flue gas and ash as secondary outputs. Flue gas contains compounds like nitrogen, sulfur dioxide, and carbon dioxide (CO_2) [21]. Carbon capture, utilization, or storage (CCUS) technologies can be integrated with incineration to lower CO_2 emissions [22]. Carbon capture includes approaches at pre-combustion, oxy-fuel combustion, and post-combustion stages [23]. In pre-combustion, fuel is processed to produce synthetic gas, from which CO_2 is removed before combustion. In Oxy-fuel combustion, oxygen is used instead of air as an oxidizer, producing a flue gas rich in CO_2 [24]. In post-combustion, CO_2 is captured from flue gases after combustion, by methods such as membrane technology, absorption, or adsorption. CO_2 can be utilized in various ways: to boost plant growth in greenhouses, as a refrigerant, and to harden concrete. It also enhances oil and gas extraction, and is used as a raw material in producing chemicals, polymers, and synthetic fuels like methane and methanol. CO_2 can also be permanently stored underground in rock formations or deep in the ocean, requiring careful monitoring to reduce environmental risks [22]. Sulfur obtained from sulfur dioxide is widely used in the production of fertilizers, polymers, and batteries, and is also useful for thermal energy storage since it is cheap and abundant [25]. However, combustion without sufficient air can cause the formation of harmful substances such as carbon monoxide and dioxins [26]. Additionally, harmful ash particles must be effectively removed from flue gas using dedicated filters. The emission control systems are complex and require regular maintenance. Failures can lead to pollution, and leftover ash residues cause further environmental and management difficulties [21]. Several investigations have used incineration as a method of food waste management [27-29].

1.2.2. Gasification

Gasification is a thermochemical process carried out at high temperatures (800–1200 °C) through four key stages: drying, pyrolysis, oxidation, and reduction [30]. Gasification converts carbon-rich solids into synthetic gas using limited oxygen or other agents like air, steam, or CO_2 [31]. The primary product, synthetic gas, which includes hydrogen, methane, carbon monoxide (CO), and CO_2, can be utilized as a fuel or for chemical synthesis. Other byproducts include char, tar, ash, and oils, with their proportions depending on the feedstock and process conditions [32]. As gasification converts solid biomass into usable synthetic gas under limited oxygen conditions, it helps minimize the creation of harmful dioxins [33]. The gasification of food waste has been reported as an effective method for recovering energy in various studies [34-37].

1.2.3. Composting

Composting represents an advanced form of resource recovery that transforms organic solid waste into a valuable end product. Utilizing microorganisms to break down waste in the presence of oxygen has emerged as a highly efficient and eco-friendly strategy [38]. This biological process helps in a significant reduction of organic waste, such as food waste, and leads to the creation of safe, non-toxic byproducts. These byproducts can be reused as nutrient-rich biofertilizers and soil enhancers, contributing to sustainable agricultural practices and improving soil health [15]. By converting waste materials through controlled biological processes, composting simplifies the challenges of waste management and disposal and reduces landfill methane emissions [39]. Food waste from various resources, including hotels, restaurants, schools, and bazaars, was utilized for composting [40-43].

1.2.4. Production of biodiesel

Food waste oils can be reused to create biodiesel, a renewable fuel made from the fatty components of plants and animals. In the core process, called transesterification, a catalyst such as potassium hydroxide (KOH) is used to convert fats into fatty acid methyl esters by reacting them with alcohols like methanol or ethanol. Biodiesel can also be made without a catalyst, but this requires higher temperatures or supercritical conditions [44, 45]. Oils containing a high level of free fatty acids, often from non-edible sources, can cause soap formation if reacted with alkaline catalysts. To handle this, a two-step method or an acid-catalyzed synthesis is employed to lower the acid levels before producing biodiesel [46]. Oily food waste such as chicken grease, beef-lamb grease, and other animal fat has been employed in several studies for the production of biodiesel through transesterification [45, 47, 48].

1.2.5. Production of adsorbents

A variety of waste ranging from groundnut and coconut shells to rice bran, hazelnut, and walnut shells, wheat and maize residues, as well as fruit peels like those of bananas and oranges, have been extensively studied and identified as effective raw materials for the development of low-cost, eco-friendly adsorbents in wastewater treatment systems. These organic waste materials possess natural properties, such as high surface area and porosity, making them promising alternatives to conventional, often more expensive, synthetic adsorbents in the treatment of contaminated water sources [49]. This category of waste can be chemically treated by agents such as sodium hydroxide (NaOH), ferric chloride ($FeCl_3$), zinc chloride ($ZnCl_2$), and phosphoric acid (H_3PO_4) to significantly enhance their adsorption capacity and overall pollutant removal efficiency [15, 50, 51].

1.2.6. Production of biopolymers

Natural polysaccharides are renewable, high molecular weight polymers made from plant and marine sources. Common types include cellulose, starch, chitin, and alginate. They are widely used in applications like heavy metal adsorption due to their biodegradability, affordability, and biocompatibility [52]. Vegetable and seafood waste are rich sources of polysaccharides, which can be used to produce sustainable plasticizers and valuable biopolymers like chitosan [53-55]. Food waste from plant and animal sources, rich in proteins like collagen, gelatin, keratin, casein, and lactoferrin, can be used to produce protein-based biopolymers. These materials are suitable for packaging and industrial uses due to their strength, thermal stability, and water resistance [56]. Protein-rich waste, including chicken feathers, dairy products, and fruit pomace (like grape and apple), has also been used to produce bioplastics such as polyhydroxyalkanoates (PHAs), offering sustainable alternatives for industrial and pharmaceutical applications [57-61]. Biopolymers produced from food waste are still in the early stages of research, which should be considered when discussing the challenges of making them from waste materials.

1.2.7. Enzyme recovery

Enzymes are essential biological catalysts that accelerate a wide range of metabolic reactions, enabling these processes to proceed more efficiently and under milder conditions [62]. Commercially, enzymes are employed in applications such as enhancing juice extraction, refining plant-derived oils, improving the brightness of paper during bleaching, and facilitating the fermentation of products like tea and coffee. They also play a critical role in the production of alcoholic beverages and are essential in many processes within the broader food industry [15]. Food waste such as wheat bran, rice bran, banana residues, corn gluten meal, citrus peels, potato peels, watermelon rind waste, and grape pomace are rich in cellulose, hemicellulose, and protein, making them cost-effective and nutrient-rich feedstocks for microbial cultivation. These materials have demonstrated strong potential in supporting the growth of enzyme-producing microorganisms, including *Aspergillus* species, *Bacillus* species, *B. amyloliquefaciens* species, and *Trichoderma* virens species, particularly under solid-state fermentation conditions. These microorganisms have successfully been used to produce a variety of industrially relevant enzymes such as α-amylase, cellulase, and laccase [15, 63-67].

1.2.8. Anaerobic digestion

Anaerobic digestion has emerged as an effective method for managing both solid waste and wastewater [68]. The anaerobic digestion of organic waste is gaining significant attention as a promising solution for several critical challenges. It provides an effective way to minimize the volume of organic materials requiring disposal, helps decrease negative environmental effects, and contributes to the broader goal of advancing a cleaner, more sustainable energy future [69].

In the case of wastewater treatment plants, anaerobic digestion is the most commonly applied for the treatment of high-concentrated wastewater and sewage sludge, where its main objectives are to stabilize the sludge and generate energy through biogas production. The biogas generated during digestion is a valuable energy resource that can be used for multiple purposes. It can be combusted in burners to help sustain the necessary operating temperatures inside the digester. Alternatively, biogas can be used to power combined heat and power systems, simultaneously generating electricity and useful heat. Moreover, after biogas is purified to improve its methane concentration, it can be supplied into natural gas pipelines or used directly as a clean fuel for vehicles, helping to promote more environmentally friendly transportation [70].

Moreover, anaerobic digestion is a sustainable and efficient method for managing animal manure, converting it into two valuable products: biogas and digestate [71]. The digestate can be utilized as a nutrient-rich organic fertilizer, returning essential elements to the soil. Therefore, the application of anaerobic digestion in manure management offers environmental and economic benefits by capturing methane emissions, reducing odors, minimizing water pollution risks, and promoting waste recycling into energy and fertilizers [72].

Anaerobic digestion is also regarded as a highly efficient and sustainable method for managing food waste, leading to the production of renewable energy in the form of methane and hydrogen, and also generates several commercially important compounds such as lactic acid and VFAs [73, 74]. Therefore, anaerobic digestion contributes to resource recovery and supports the transition toward a circular economy by transforming waste into useful materials rather than allowing it to contribute to landfill overflows and environmental pollution [5, 75].

Anaerobic digestion can process a wide range of complex feedstocks to produce the desired end products [76]. The diagram of an anaerobic digestion process is presented in **Figure 2**. Anaerobic digestion typically involves four stages. The first stage is hydrolysis, in which hydrolytic bacteria break down complex substances like carbohydrates, proteins, and lipids into simpler, soluble units such as sugars, amino acids, and fatty acids through the use of specialized enzymes. The breakdown rate of various polymers is influenced by their type, structure, and complexity. The hydrolysis of proteins and lipids may require several days, and lignocellulose and lignin degrade slowly and often incompletely [77]. It is an important step in converting food waste into valuable products, and any resistance to hydrolysis can lead to a reduction in the production of the desired outputs. In the second stage, acidogenesis, in which the soluble compounds formed in the first step are converted into organic acids, which are mainly composed of one to five carbon atoms, by acidogenic bacteria, along with the formation of additional compounds such as hydrogen and CO_2. The third stage, acetogenesis, the products formed during the acidogenic phase are utilized as feedstocks by acetogenic bacteria to produce acetic acid, hydrogen, and CO_2. Homoacetogenic bacteria produce acetic acid through the reduction of hydrogen and CO_2. Finally, in methanogenesis, hydrogenotrophic methanogens convert CO_2 and hydrogen into methane, while acetotrophic methanogens transform acetic acid into methane [7, 77, 78]. The two initial stages of anaerobic digestion (hydrolysis and acidogenesis) are recognized as a fermentation process (FP) [79]. The valuable compounds produced through the different stages of anaerobic digestion have been discussed in the following paragraphs.

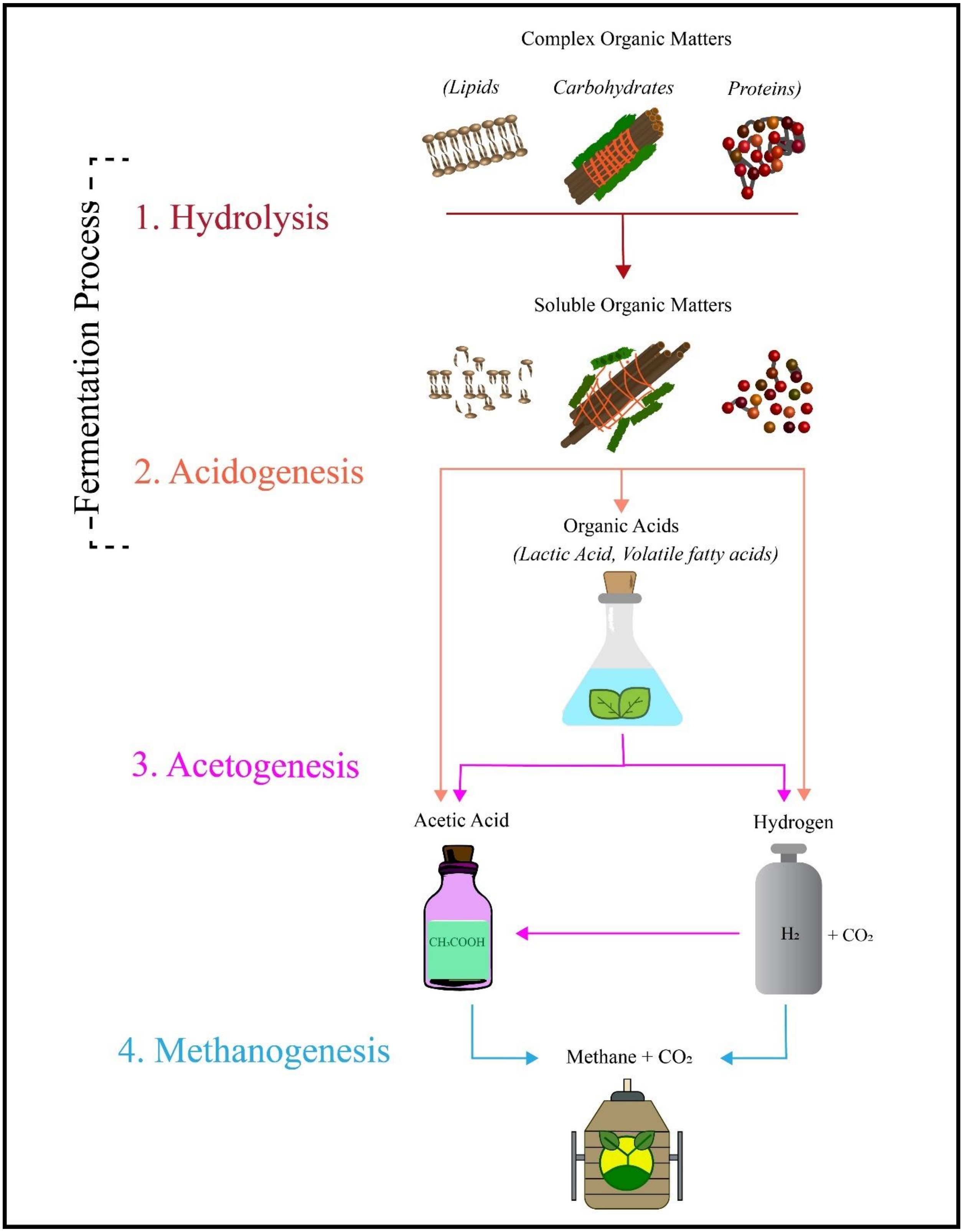


Figure 2. Diagram of a conventional anaerobic digestion process and the products of each stage.

1.2.8.1. Lactic acid

Lactic acid ($C_3H_6O_3$) is a valuable compound widely used across multiple industries, including pharmaceuticals, food, chemicals, and textiles. It is primarily employed as a flavoring agent or as a building block for polymers used in food packaging, grafts, and other applications [80, 81]. Lactic acid can be produced through chemical synthesis or a FP, however, a FP is considered a more eco-friendly approach [82]. During fermentation processes (FPs), lactic acid bacteria (LAB) thrive in oxygen-free environments, where they break down sugars and other fermentable carbohydrates, producing lactic acid [80, 81, 83]. A mixture of isomers, mostly L-lactic acid and D-lactic acid, can be produced through the biological fermentation of organic matter [84].

1.2.8.2. Volatile fatty acids

VFAs, including acetic acid (AcA), propanoic acid (PrA), butyric acid (BuA), and valeric acid (VaA) can be generated from the disintegration of complex carbohydrates during anaerobic fermentation. VFAs are valuable products that can improve the biosynthetic value of PHAs and be employed as raw substances for manufacturing biodegradable thermoplastics [85, 86]. Like other products, the initial phase involving solubilization and hydrolysis is a key limitation in the production of VFAs [87, 88].

1.2.8.3. Biohydrogen

Hydrogen is considered the fuel of the future, with the potential to create a cleaner economy compared to fossil fuels. It is recognized as one of the most efficient and safe energy carriers, particularly for transportation [89]. Food waste is an ideal feedstock for biohydrogen production because of being widely available, rich in carbohydrates, and cost-effective [90]. Nutrients in food waste can be utilized to produce renewable biohydrogen through FPs, in which fermentable sugars and organic acids are used to generate biohydrogen [5].

1.2.8.4. Biomethane

Biomethane produced through anaerobic digestion is regarded as one of the most efficient and environmentally friendly biofuels currently available, offering a cleaner energy source alternative to conventional fuels. Sugar and starch-based wastes are the primary feedstocks being commercially cultivated for biomethane production [91]. Methanogenesis is the slowest biochemical reaction in anaerobic digestion [68].

1.3. Pre-treatment processes

The modification of food waste properties through pre-treatment approaches is a useful step that breaks down complex materials into simpler forms, making them easier and faster for microbes or enzymes to digest [92]. This initial processing significantly enhances the efficiency of subsequent steps, such as fermentation or biogas production, by accelerating the hydrolysis phase [93].

Pre-treatment of food waste is typically done to break down complex components like lignin, loosen the structure of cellulose, and help hydrolyze hemicellulose, and as a result, improve the solubility of materials. Common pre-treatment methods include biological pre-treatment, chemical pre-treatment (e.g., using acids or alkalis and oxidants), physical pre-treatment (e.g., grinding, heating, microwave, and cavitation), and combined techniques that use two or more of these approaches together [5, 15].

1.3.1. Grinding/ milling

The main purpose of grinding processes is to break down (disintegrate the structure) food waste into much finer particles. By decreasing the particle size, this method increases the overall surface area of the material, which enhances the efficiency of subsequent biological processes. Smaller particles allow for better contact with enzymes, speeding up decomposition or conversion processes and improving the overall yield of valuable products [15, 94]. Various mechanical milling methods, such as hammer, knife, ball, jet, bead, and centrifugal mills, are employed to break down biomass into fine particles [95-97]. Reducing food waste to a fine particle size of approximately 2.5 mm greatly enhanced methane generation through anaerobic digestion by improving microbial accessibility and accelerating the breakdown of organic matter [98]. Ultra-fine milling of wheat straw had no effects on the total methane yield but enhanced the rate of methane production, especially for particles around a size of 200 µm [94].

1.3.2. Biological pre-treatment

Biological pre-treatment is an environmentally safe method that uses microbes to break down food waste and support enzymatic hydrolysis, without relying on heat, pressure, or harsh chemicals. While it is energy-efficient, it involves a long treatment time [15]. This method uses various enzymes, such as amylase, protease, cellulase, etc., along with fungi like *Monascus* species and *Aspergillus* species, to break down food waste. During pre-treatment, fungi release numerous carbohydrases which help convert complex organic materials into simpler, more digestible compounds [99]. An improvement in the production of fermentation products, including butanol, VFAs, and propionic acid, from food waste made by biological pre-treatment was reported in the literature [100, 101].

1.3.3. Heating

Thermal pre-treatment is commonly used to soften and disrupt the dense structural framework of food waste, enhancing its suitability for subsequent biochemical conversion processes. The main objective is to break down cell walls and membranes, which facilitates the quicker release and hydrolysis of organic compounds into simpler molecules [102]. Heat is generally supplied using equipment like autoclaves, conventional heaters, or through hydrothermal processing methods. Temperature is a key factor in this method, as higher heat enhances the solubilization of complex substances like proteins, carbohydrates, and lipids [15]. It was observed that heating food waste to 72°C for 22 minutes enhanced the solubility of its compounds and, as a result, increased the methane production [103].

1.3.4. Microwave

Microwave pre-treatment of food waste works by causing intracellular components to absorb microwave energy and rapidly vibrate. This vibration generates heat throughout the material [93, 98]. The heat helps break down complex biopolymers like proteins and carbohydrates, making them easier to process in later stages. Microwaves can reach effective temperatures without damaging structural components like hemicellulose and lignin. This is beneficial because excessive heat from traditional methods can release compounds such as phenols, which can inhibit microbial activity and lower methane production during anaerobic digestion [104]. Microwave irradiation on rice straw at 190 °C significantly enhanced the availability of soluble organic compounds, which led to a high methane yield during the subsequent anaerobic digestion [105].

1.3.5. Acidic or alkaline pre-treatment

Acid-based pre-treatment involves the application of acidic solutions to alter and disintegrate the complex structure of lignin within biomass. The acid reduces the structural integrity and chemical stability of lignin, facilitating its structural breakdown. This chemical environment promotes the solubility of lignin and enhances the hydrolysis of hemicellulose and other polymers [100, 106]. Studies have utilized sulfuric acid (H_2SO_4) and hydrochloric acid (HCl) for the pre-treatment of food waste, leading to the intensification of FPs [100, 107].

Alkaline pre-treatment of food waste helps create conditions that make it easier to break down complex organic substances. This process breaks apart larger, more complicated molecules and converts them into simpler, smaller ones that are easier to process [108]. This can be done by disrupting the chemical bonds, such as ether and ester linkages, that connect lignin with carbohydrate structures in the biomass [109]. By loosening these bonds, the materials become more accessible for later steps like enzymatic digestion or fermentation, improving the overall efficiency of bioresource recovery. The alkalis, such as calcium oxide (CaO), sodium hydroxide (NaOH), and potassium hydroxide (KOH), were investigated for the pre-treatment of food waste, enhancing biogas production [108, 109].

Dilute acid pre-treatment typically demands elevated temperatures (over 100 °C) and prolonged reaction durations, potentially leading to the generation of inhibitory compounds. Alkaline pre-treatment also usually operates within a high-temperature range of 100–180 °C. Combining acid or alkaline methods with other pre-treatment methods, such as cavitation, can allow the process to proceed under milder conditions [110-112].

1.3.6. Oxidative pre-treatment

Oxidative pre-treatment can be an effective strategy for enhancing the breakdown of biomass by enzymes. This process primarily works by targeting and removing lignin as a complex component in plant cell walls that typically hinders enzymatic access. The use of oxidizing agents facilitates this removal by the cleavage of lignin structure, thereby making the cellulose more accessible [113]. This pre-treatment method was fully explained in section 1.4.

1.3.7. Cavitation

Cavitation can be considered an effective pre-treatment technique for the modification of the properties of food waste used as the feedstock of anaerobic digestion. Cavitation offers several advantages, including low processing costs and the potential to be combined with other physical or chemical pre-treatment techniques [5, 114]. Cavitation refers to a dynamic phenomenon characterized by the rapid formation, expansion, and subsequent collapse of microscopic bubbles within a liquid medium over a very short period. This process occurs due to localized pressure fluctuations, often induced by ultrasonic waves or hydrodynamic forces [73]. Cavitation can be classified into four main types based on how it is produced: hydrodynamic cavitation, acoustic cavitation, particle-induced cavitation, and optical cavitation. Among these, only hydrodynamic cavitation and acoustic cavitation are relevant for practical applications like biomass pre-treatment. In contrast, particle and optical cavitation are typically used for controlled single-bubble generation in research settings and are not suitable for large-scale pre-treatment processes [115].

Hydrodynamic cavitation is typically produced using specially designed mechanical systems such as venturi tubes [116], orifice plates [117], vortex diodes [118], or rotating devices [119]. These setups create zones where fluid velocity rapidly accelerates, leading to a sharp drop in static pressure, following Bernoulli’s principle. While venturi and orifice reactors are commonly employed due to their ability to generate optimal cavitation intensity for pollutant breakdown and simple structure, they exhibit greater pressure losses compared to other reactor designs. Additionally, there is a high possibility of erosion and cognition in a venturi or orifice happening by waste containing solid particles [5, 120]. Rotating devices involve the disadvantages of high energy consumption and maintenance expenses, as well as a complex structure. Vortex diodes were reported to be suitable for lab-scale research and have scaling-up problems [5].

When the local pressure drops below the liquid’s vapor pressure, microbubbles form. Interestingly, even when the pressure doesn’t fall below the vapor pressure, intense turbulence and pressure fluctuations in the flow can still trigger cavitation events due to localized instabilities in the fluid dynamics [5]. The cavitation number (C_V), represented in **Eq. 1**, is a dimensionless parameter that characterizes the intensity of cavitation within a fluid system [121].

$$C_v = \frac{P_2 - P_v}{\frac{1}{2} \times \rho \times U_{th}^2} \quad (1)$$

In this context, P_2, P_v, ρ, and U_{th} refer to the downstream pressure after recovery, vapor pressure, fluid density, and linear velocity of the fluid at the throat of the cavitation device, respectively. Typically, cavitation initiates and bubble formation occurs when the cavitation number (C_V) is less than or equal to one [122, 123]. Nevertheless, due to the presence of cavitation nuclei such as dissolved gases and suspended particles, cavitation can occur even when the cavitation number exceeds one [124]. Lower cavitation numbers typically produce more bubbles but reduce the strength of their collapse. Extremely low values (close to zero) can cause supercavitation, which further lowers the collapse energy [125].

In acoustic cavitation, microbubbles form, expand, and collapse through the exposer of liquid medium to ultrasonic waves [126]. The cycles of compression and expansion of ultrasonic waves create regions of positive and negative pressure within a liquid. The expansion phase creates conditions that facilitate the formation and growth of microbubbles. While in the compression phase, pressure increases, leading to the collapse of microbubbles. [127]. Acoustic cavitation was comprehensively explained in section 1.5.

Each pre-treatment method has its own set of advantages and limitations. However, by combining multiple pre-treatment strategies, it is possible to mitigate the associated drawbacks while amplifying their individual benefits. Specifically, the combination of physical and chemical pre-treatments has been shown to enhance the overall efficiency of the process. This combined approach can boost the effectiveness of biomass processing and reduce both operational costs and energy consumption, leading to a more sustainable and cost-efficient technique [15].

1.4. Advanced oxidation processes

Advanced oxidation processes (AOPs) represent an effective method for breaking down organic pollutants [128, 129]. AOPs rely on chemical, electrochemical, or photochemical methods to produce reactive radicals, having the potential to interact with organic compounds and break them down to simpler ones [130]. A key advantage of AOPs is their ability to convert and even completely mineralize numerous organic pollutants that are resistant to biological treatments, significantly reducing their environmental and human health risks [131]. AOPs, including persulfate-based processes [132], percarbonate-based processes [133], and photocatalytic processes [134], have been mainly utilized for wastewater treatment. The synergistic application of cavitation with external oxidizing agents or catalytic systems has been extensively investigated to enhance the oxidative degradation processes in advanced wastewater treatment [135, 136].

AOPs can also be employed as a pre-treatment method of biomass in biological processes to enhance enzymatic hydrolysis [113]. There are a variety of AOPs, such as hydrogen peroxide-based processes [137, 138], ozone-based processes [139, 140], Fenton processes [141, 142], and cavitation-based processes [7, 143], which have been applied for both wastewater treatment and waste pre-treatment.

1.4.1. Hydrogen peroxide-based processes

Hydrogen peroxide (H_2O_2) is recognized as a compound that can be activated to form reactive hydroxyl radicals ($^{\bullet}OH$) [141]. Besides its significant oxidation potential ($E° = +1.77$ V), H_2O_2 can be activated by methods, including ultraviolet (UV) light, cavitation, Fenton process, as well as their combinations, to generate $^{\bullet}OH$, which has an even higher oxidation potential ($E° = +2.80$ V) [137, 141, 144].

Alkaline H_2O_2 as an advanced oxidation process (AOP) generating $^{\bullet}OH$ has been applied for the pre-treatment of lignocellulosic compounds to speed up the typically slow process and enhance overall efficiency [145-147]. $^{\bullet}OH$ can degrade resistant macromolecular organic compounds into smaller, more soluble organic molecules, which enhances the efficiency of hydrolysis. $^{\bullet}OH$ can specifically break down cellulose in the amorphous regions, leading to an increase in the content of carbonyl and carboxyl groups [148]. This radical damages microbial cell membranes, leading to the breakdown of cell walls and the release of both extracellular and intracellular components into the solution [149]. These soluble organics are then readily consumed by bacteria and enzymes during the acidification and methanogenesis stages, leading to a significant increase in the desired products [150].

1.4.2. Ozone-based processes

Ozone, recognized as a powerful oxidizing agent, has been utilized in several studies as the pre-treatment of lignocellulosic biomass to enhance the efficiency of cellulose breakdown during enzymatic hydrolysis [140, 151-153]. Ozone-based oxidation processes operate through two primary mechanisms: one involves the direct reaction of ozone molecules with target compounds, while the other proceeds indirectly by the formation of $^{\bullet}OH$ through the activation of ozone [154, 155]. Ozone can be activated under alkaline conditions or through the use of catalysts, H_2O_2, UV, or cavitation [156, 157]. Over the past several decades, ozonation has gained significant interest as a pre-treatment method, mainly because it operates under mild conditions and effectively targets lignin.

1.4.3. Fenton process

Fenton oxidation, widely applied in wastewater treatment, is also emerging as a promising approach for lignocellulosic biomass pre-treatment. Its advantages include environmentally friendly chemistry and relatively mild operating conditions, making it a sustainable alternative for biomass processing [158]. Several studies have applied Fenton processes for pre-treatment purposes [159-162]. The conventional Fenton process involves the reaction between ferrous ions (Fe^{2+}) and H_2O_2 under acidic conditions to generate $^{\bullet}OH$ [163]. Fe^{2+} can act as a catalyst as it can be regenerated through the subsequent reaction between H_2O_2 and generated ferric ions (Fe^{3+}). Consequently, as long as H_2O_2 is available in the solution, the formation of $^{\bullet}OH$ can continue [141].

This radical can effectively break down lignin and hemicellulose, leading to a significant improvement in cellulose accessibility for subsequent enzymatic hydrolysis [92].

1.5. Acoustic cavitation

Ultrasounds (US) refer to sound waves that have frequencies exceeding the range of human hearing (>16 kHz) [164]. When a liquid is exposed to ultrasonic waves, numerous microscopic bubbles form. This happens during the rarefaction phase of the sound wave, when the wave induces a temporary expansion of the liquid medium, lowering the local pressure. If the sound wave pressure amplitude exceeds the ambient pressure, it can create a temporary negative pressure within the liquid. This negative pressure creates conditions where dissolved gases are no longer stable and start to come out of solution, forming bubbles [165]. In the rarefaction phase, microbubbles within the liquid grow in size because the pressure inside the bubble exceeds the surrounding liquid pressure. When the wave shifts into its compression phase, the pressure rapidly increases, and some of these bubbles implode with great intensity, releasing significant energy [166]. In **Figure 3**, the schematic of the formation and collapse of cavities through ultrasonic waves is illustrated.

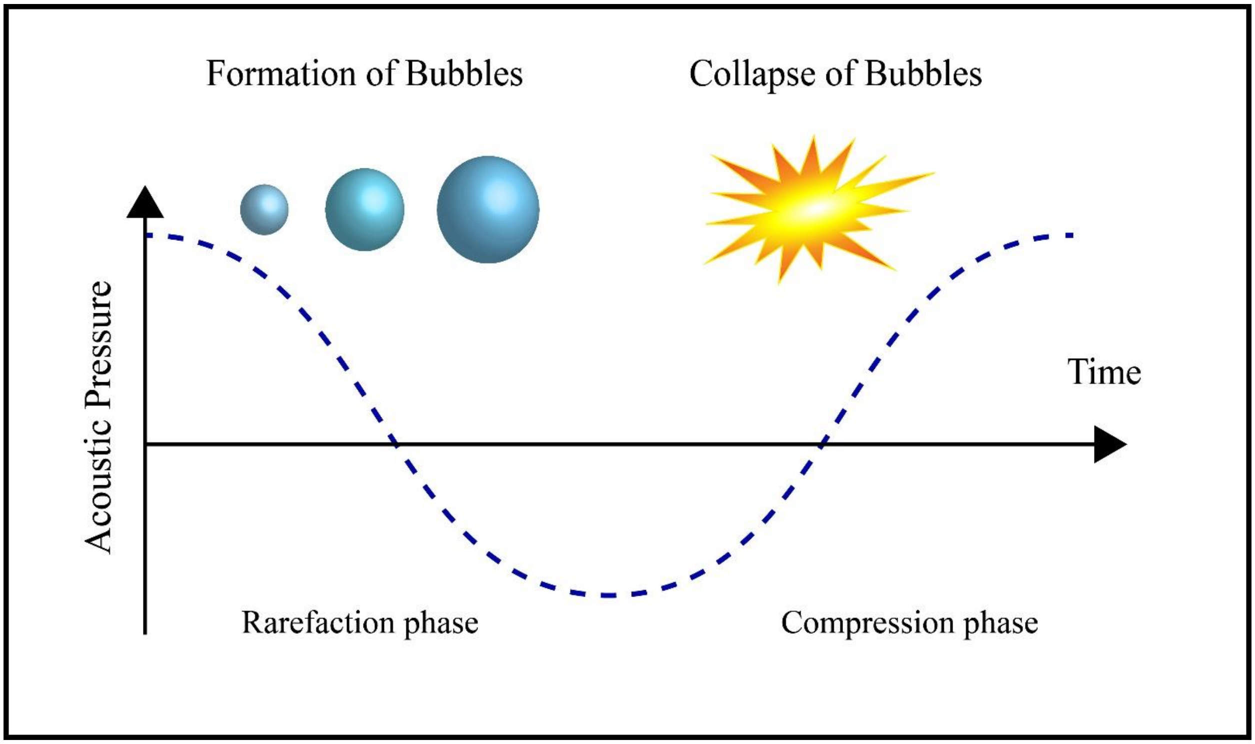


Figure 3. Schematic of formation and collapse of bubbles during acoustic cavitation.

The changes in pressure in the external interface of cavities generate intense effects, including the formation of high-speed microjets [167]. The violent collapse of vapor-filled cavities also generates powerful shock waves [168]. In addition, the collapse of these cavities generates significant shear stress, which can reach a high pressure of approximately 3.5 kPa, causing a rapid and intense transfer of energy to the surroundings [169]. The extreme effects mentioned above, known as the mechanical effects of cavitation, can cause the breakage of molecular bonds in organic materials, leading to their direct decomposition [120]. Furthermore, the compression of gas and vapor within the bubbles creates intensely high pressure and temperature, leading to localized hotspots [170]. This intense energy released by collapsing cavities can thermally break down a wide range of compounds [171]. Moreover, cavitation is also regarded as a form of AOPs since the elevated temperature and pressure can cause the decomposition of water molecules, creating reactive species like $^{\bullet}OH$, $HOO^{\bullet}$, and H_2O_2 with considerable redox potential, recognized as the chemical effect of cavitation. These radicals trigger chain reactions that break down complex molecular structures into smaller ones [5]. Cavitation effects typically occur in three key areas:

1) Within the cavities, the high temperature and pressure supply the activation energy needed to break down vaporized solvents and solutes;

2) On the interface, as hot spots inside the bubbles heat up the interface of vapor-filled cavities, both pyrolysis and radical reactions can take place there;

3) In the bulk of the solution, radicals and byproducts formed inside may diffuse through the cavity walls and participate in chemical reactions in the bulk medium [172].

Cavitation-based pre-treatment of lignocellulosic food waste is achieved through a synergistic interaction of physical forces and chemical reactions. The physical effects of cavitation, including shear wall stress and shock waves, can break down the feedstock and reduce particle size [5, 173]. Cavitation can disrupt structural integrity and alter surface morphology, causing a loosening effect that expands the overall surface area [174, 175]. Furthermore, the chemical impact of cavitation, like the formation of $^{\bullet}$OH, can facilitate the oxidation of lignin components, enhancing the solubility of organic matter, represented by soluble chemical oxygen demand (sCOD) [176, 177]. The physical impact of cavitation has an important role in breaking down proteins through a disruption in hydrogen bonds within large protein polymers, leading to the disintegration of these structures and the creation of smaller protein molecules [178, 179]. Hence, cavitation can make the organic matter more accessible for microorganisms, enhancing the subsequent formation of desired products.

Li et al. [180] investigated US (frequency: 20 kHz, power density: 500 W/L, pre-treatment duration: 80 minutes) as a pre-treatment technique for the modification of waste activated sludge, and obtained 2300% increase in sCOD. Mao et al. [181] utilized US (20 kHz, 4000 W/L, 20 minutes) to pre-treat primary sludge and secondary sludge. They reported a particle reduction of 71% (primary sludge) and 85% (secondary sludge), as well as a sCOD increase of 290% (primary sludge) and 685% (secondary sludge). Bundhoo [182] employed low-frequency US (20 kHz) to modify food and yard waste as the feedstock of a FP and achieved 159% increase in sCOD. Elbeshbishy et al. [183] used US as the pre-treatment stage for improving the properties of food waste, and reported 9% increase in sCOD, 17% increase in soluble carbohydrate, and 20% increase in soluble protein. In another study, Elbeshbishy et al [184] studied the application of US for the pre-treatment of food waste and reached 21.9% decrease in particle size, 22% increase in sCOD, 30% increase in soluble carbohydrate, and 14% increase in soluble protein in 30 minutes. Jiang et al. [185] investigated US (20 kHz, 480 W/L, 15 minutes) for the pre-treatment of food waste at a TS of 100 g/L and 40 g/L and obtained an enhancement in sCOD by 210% and 179% and protein content by 424% and 374%, respectively.

Joshi and Gogate [7] found that applying low-frequency US (20 kHz, 400 W/L, 10 minutes) led to a 62.8% rise in sCOD and nearly doubled biogas output. However, when the power density was raised to 1000 W/L, sCOD was only improved by 55.5% relative to its initial level without ultrasonic pre-treatment. Cho et al. [186] observed that methane output rose by 39% under ambient conditions when low-frequency US (20 kHz) was applied at a power density of 2.5 W/L. Cho et al. [187], in another study, utilized low-frequency US to boost methane production and also reported a 213% increase in the activity of enzymes.

Elbeshbishy et al. [184] analyzed hydrogen production with and without US, finding that pre-treating pulp waste led to a 77% rise in hydrogen production during an anaerobic FP. Additionally, they achieved 70% increase in VFAs through the application of US in the pre-treatment stage. This group, in another research, [89] evaluated biohydrogen and VFAs production across three setups: (A) a continuously-stirred tank reactor (CSTR) processing food waste, (B) a CSTR utilizing pre-treated food waste by US, and (C) a biological hydrogen reactor featuring a CSTR with an ultrasonic producer probe at its base. Their findings showed that hydrogen production and VFAs production increased by 27% and 7%, respectively, in system B. In the case of system C, they also reported an increase of 85% and 18% in hydrogen and VFAs production, respectively, compared to system A. Dinesh et al. [1] examined the impact of ultrasonic pre-treatment on biohydrogen production and reported an increase of over 80% in hydrogen output.

Ma et al. [188] applied US (28 kHz, 30 minutes) as a pre-treatment technique to improve food waste as a feedstock for a FP and observed 62% increase in lactic acid production. Đukić-Vuković et al. [80] utilized US (20 kHz, 10 minutes) in order to pre-treat the feedstock and reached 15% increase in the concentration of lactic acid.

Acoustic cavitation is typically formed using one of two main types of ultrasonic devices: a direct-contact ultrasonic probe (often called a horn) or an ultrasonic bath, in which indirect irradiation of US is usually applied.

The determination of the optimal operating conditions of US is important for process intensification, as it directly impacts the effectiveness and efficiency of the system.

1.5.1. Ultrasonic frequency

Frequency has an inverse relationship with bubble size and the energy produced during bubble collapse. High frequencies usually result in a greater number of ultrasonic cycles, which can lead to more frequent cavitation occurrences. However, they provide shorter bubble growth periods, meaning that bubbles reach smaller sizes and collapse with less force [189, 190]. Frequencies above 100 kHz may increase bubble quantity while reducing bubble size. Thus, they can weaken the physical effects [189]. A high-frequency cavitation phenomenon is effective for breaking down various compounds in wastewater treatment and chemical synthesis, especially when used alongside different wastewater treatment techniques such as AOPs [191, 192]. However, employing high irradiation frequencies comes with several disadvantages, including the erosion of transducer surfaces due to prolonged operation [193].

Conversely, a low-frequency cavitation phenomenon allows bubbles to grow for an extended period, resulting in fewer but larger bubbles. When these large bubbles collapse, they release substantial energy, intensifying the physical effects of cavitation, such as intense shear stress and powerful shock waves [5]. Generally, low frequencies ranging from 20 to 50 kHz can amplify the physical impacts of cavitation, which may cause the breakdown of complex molecules into simpler forms, and ultimately, can improve both solubility and biodegradability [46].

1.5.2. Ultrasonic power density

This parameter can influence the performance of cavitation phenomena through affecting the quantity, size, and lifespan of bubbles [191]. Ultrasonic power density that exceeds a certain level induces cavitation. While this level is quite high for pure liquid, it can significantly drop in the presence of impurities [194]. Increasing the irradiation intensity to an optimal level can enhance cavitation activity [195, 196]. A further increase in power intensity can lead to the formation of clouds, which weaken cavitation activity [197]. The determination of an optimal power density can also lower the operational costs associated with a specific process [198]. An optimal level of power density can significantly improve sCOD and bioproducts yield by maximizing activity [199].

1.5.3. Ultrasonic pre-treatment duration

In the case of the application of US generating acoustic cavitation as a pre-treatment technique followed by FPs, the size of particles usually reduces rapidly [200]. However, food waste containing high lignin levels, such as fruits and vegetables, requires more time for effective solubilization of organic compounds [7, 201].

1.5.4. Feedstock concentration in ultrasonic reactors

Feedstock concentration in ultrasonic reactors, as a pre-treatment stage, also has a role in the effectiveness of cavitation phenomena. Feedstock particles can act as nuclei for bubble formation. Therefore, an increase in the concentration can improve cavitation efficiency by promoting bubble generation and intensifying its impact [202]. However, it should be taken into account that too high density of slurry can cause the resistance of bubbles towards expansion and collapse, hence, negatively impact cavitation efficiency and decrease the effectiveness of cavitation [203].

As a result of literature review for this thesis, a comprehensive review article on the application of cavitation as a pretreatment technique has been published [5].

1.6. Summary

Food waste management is becoming an increasingly critical issue because of its serious effects on the environment, economy, and society. Every year, large amounts of food are thrown away, which wastes valuable natural resources, increases greenhouse gas emissions, and causes economic losses. Therefore, managing food waste in an efficient and sustainable way is important to reduce environmental damage and improve food system sustainability. After reviewing food waste management methods, anaerobic digestion is recognized as an efficient and sustainable approach. It produces renewable energy like biomethane and biohydrogen, along with commercially valuable compounds such as organic acids. This process supports resource recovery and promotes a circular economy by converting waste into useful products instead of contributing to landfill overflow and pollution. However, most research has been focused primarily on hydrogen and methane production, with less attention given to other valuable products like lactic acid. A key challenge in anaerobic digestion is the slow hydrolysis stage, highlighting a need for pre-treatment techniques. Acoustic cavitation as a pre-treatment method presents several benefits, including low costs and the potential to be combined with other chemical or physical methods. Its comprehensive impact on lactic acid production remains underexplored, offering a promising area for study. Additionally, the combined use of US, generating acoustic cavitation, with chemicals such as acids, alkalis, and oxidants during pre-treatment is rarely examined. This combination might facilitate milder processing conditions, minimizing the reliance on harsh chemicals, which may improve efficiency and reduce environmental impact.

2. Aim and objectives

The comprehensive investigation of an acoustic cavitation phenomenon as an effective pre-treatment method of food waste-based feedstocks, leading to the intensification of lactic acid production through the subsequent FPs has been rarely reported in the literature.

Hence, this research focuses on the application of US to induce acoustic cavitation as a pre-treatment technique to intensify the production of lactic acid through the fermentation of rice as a model food waste as well as actual food waste. The investigation involved the study of the effect of various parameters on sCOD and the concentrations of lactic acid and VFAs.

The aim was to study:

- The effectiveness of high- and low-frequency ultrasonic reactors in enhancing sCOD.
- The effect of ultrasonic power density on sCOD and the production of lactic acid and VFAs.
- The effect of ultrasonic pre-treatment duration on sCOD and the production of lactic acid and VFAs.
- The effect of feedstock concentration in the ultrasonic reactor on sCOD and the production of lactic acid and VFAs.
- The effect of combining US with acid (HCl) and alkali (NaOH) in the pre-treatment stage on sCOD and the production of lactic acid and VFAs.
- The effect of combining US with H_2O_2 as an oxidant in the pre-treatment stage on sCOD and the production of lactic acid and VFAs.

3. Methodology

3.1. Materials

3.1.1. Chemicals

Hydrogen peroxide (30%), potassium iodide (pure), sodium hydroxide (99%), cupric sulphate (pure), and methanol were bought from POCH (Poland). Hydrochloric acid (30%) was obtained from Sigma-Aldrich (USA), and 4-chlorobenzoic acid (99%) was purchased from Thermo Fisher Scientific (USA).

3.1.2. Feedstock

Rice, as a model food waste, used as the feedstock of the FPs, was purchased from a local grocery store in Gdansk, Poland. The rice was first boiled to ensure the uniform consistency.

The actual food waste used as the feedstock of the FPs was sourced from the canteen of Gdańsk University of Technology. The composition of the collected food waste was approximately 40% pork, 50% potatoes, and 10% vegetables, including cucumber, beetroot, and mayonnaise. This specific mixture was selected to represent a realistic and commonly generated type of food waste, providing a balanced combination of carbohydrates, proteins, and fats.

The feedstocks were blended using a Mandine 600 W hand mixer to create a homogenized mixture. They were subsequently stored at 4 °C to preserve their stability until further processing.

3.1.3. Inoculum

Aerobic sludge was obtained from a secondary treatment facility, while anaerobic sludge was collected from the digester of a large municipal wastewater treatment plant in Gdynia, Poland. To maintain the viability and activity of the microbial community, the inoculums were stored at a controlled temperature of 20°C before being used in the fermentation system. These storage conditions were carefully maintained to ensure optimal microbial performance during the experimental process.

TSS (total suspended solids) and VSS (volatile suspended solids) of anaerobic sludge used for the fermentation of rice were 30.26 g/L and 23.96 g/L, respectively.

Anaerobic sludge employed for the fermentation of food waste possessed a TSS of 38.76 g/L and a VSS of 31.45 g/L. Additionally, aerobic sludge utilized for the fermentation of food waste had TSS and VSS of 26.87 g/L and 22.34 g/L, respectively

3.2. Apparatus

Two reactors were applied in the pre-treatment stage. A majority of the experiments were conducted in a low-frequency reactor (*sono*-LF), Hielscher UP400th, possessing a sonotrode (probe). The sonotrode had an immersion depth of 45 mm, a tip diameter of 14 mm, and a radiating surface area of 2.2 cm^2 (designated by the producer as s24d14D). The diagram of the *sono*-LF is illustrated in **Figure 4**.

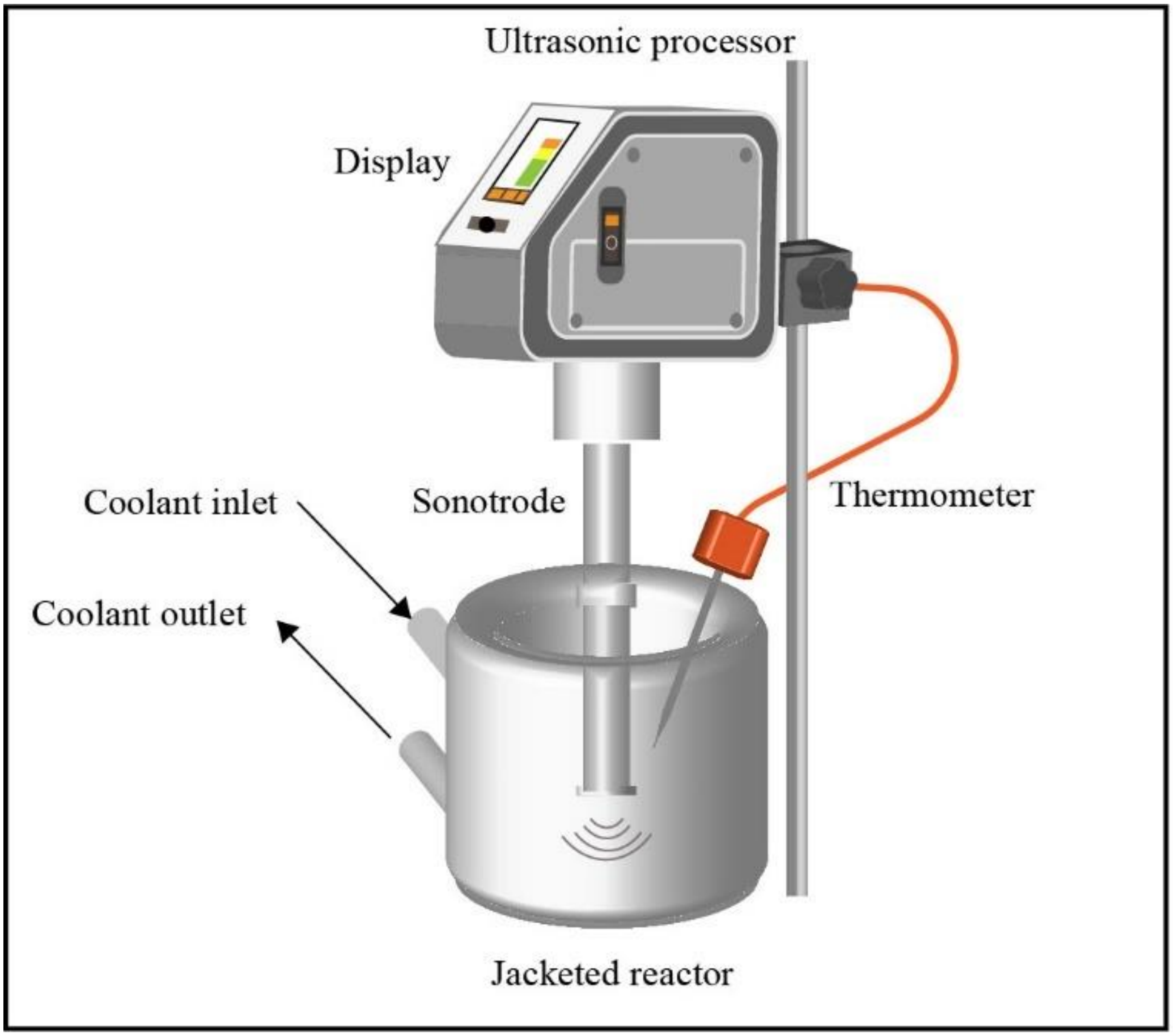


Figure 4. Diagram of *sono*-LF operating at a low frequency of 24 kHz [204].

Sono-LF operated at a constant ultrasonic frequency of 24 kHz. It had a total working capacity of 500 mL, within which the US was applied at power densities of 100 W/L, 300 W/L, and 400 W/L. These specific power levels were chosen based on an optimal range identified in our previous review, ensuring relevance and effectiveness for the experimental conditions [5]. It was also impossible to investigate higher power densities due to the technical limitation.

Additionally, at the initial stage of the research, a comparison experiment was conducted using a custom-built bath ultrasonic reactor operating at a higher frequency of 120 kHz. The layout of this high-frequency reactor (*sono*-HF) is shown in **Figure 5**.

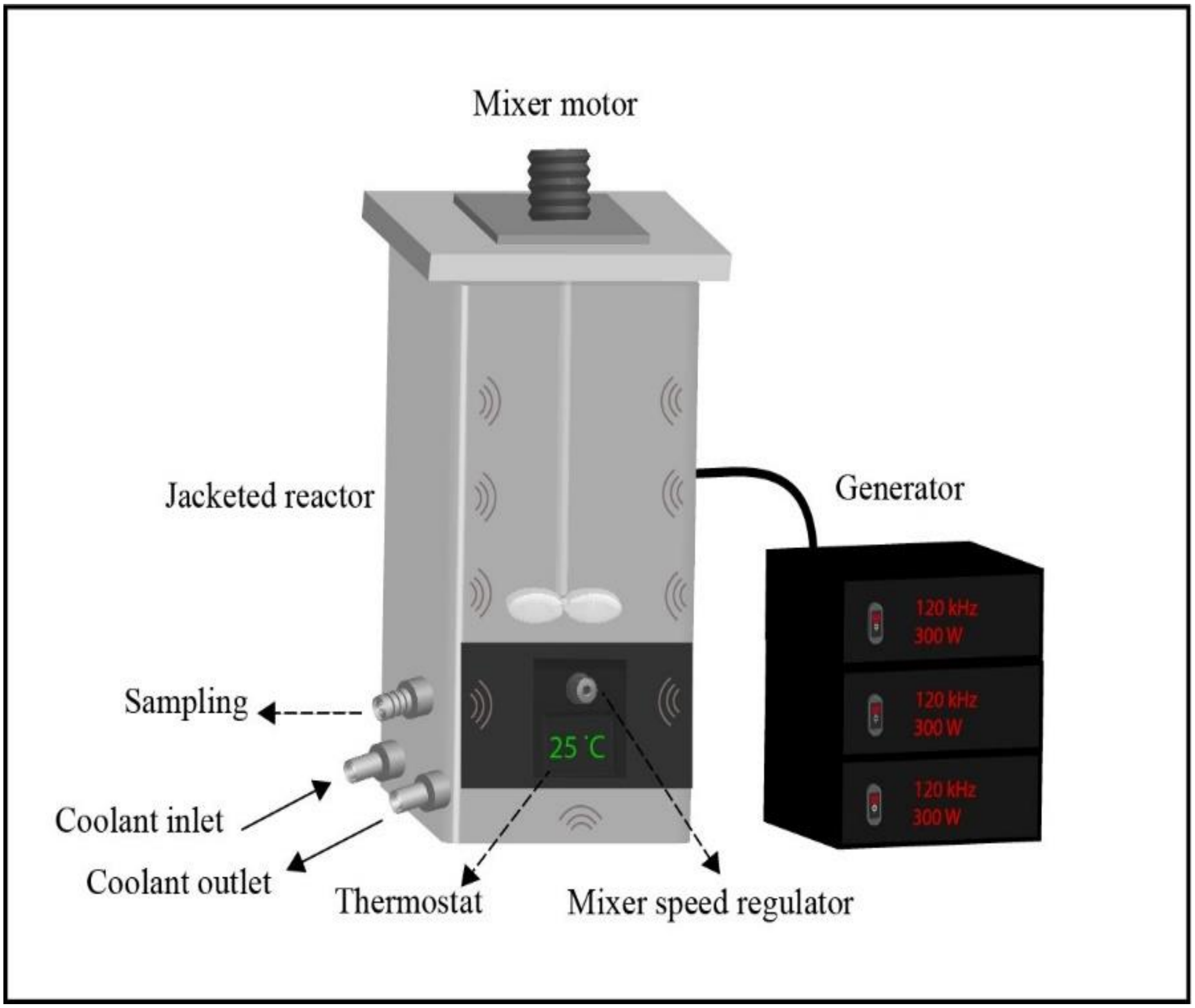


Figure 5. Diagram of *sono*-HF operating at a high frequency of 120 kHz [204].

S*ono*-HF was designed with a rectangular form (280 mm in height and 130 mm in width). It included an internal mixer and multiple transducers that generate high-frequency waves, which were installed along the reactor walls, providing uniform wave propagation. The reactor was designed to process a volume of 3 liters and was powered by a total of 900 W (300 W/L), provided by three separate generators, each delivering 300 W. This reactor was specifically used to investigate the impact of a higher operating frequency of 120 kHz on the pre-treatment process.

In both reactor setups, a cooling water system was employed to regulate and maintain the temperature within a controlled range of 25±3°C, warranting stable experimental conditions and preventing excessive heat buildup that could affect the results.

3.2.1. Detection of hydroxyl radicals in the ultrasonic reactors

The generation of $^{\bullet}$OH was confirmed in both reactors, using *para*-chlorobenzoic acid (*p*CBA), as a probe compound for this radical. A solution containing 5 ppm of *p*CBA in deionized water sourced from a Direct-Q® Water Purification System (Merck Millipore, Germany) was processed using the two types of ultrasonic reactors. Both reactors were set to deliver the same power density of 300 W/L and run for 30 minutes. The samples were taken every 10 minutes.

Additionally, a potassium iodide (KI) solution was employed to verify the generation of $^{\bullet}$OH due to its known reactivity with these highly oxidative species. KI at a concentration of 5 mM in deionized water was processed in these reactors working at the same power density of 300 W/L for 30 minutes. Samples were taken at the end of the processes.

3.3. Pre-treatment experiments

In the pre-treatment experiments, the rice was mixed with deionized water using a magnetic stirrer (MS-H-Pro produced by Chemland) at 500 rpm for 15 minutes, obtaining a feedstock concentration of 5% w/v in every experiment. Before the pre-treatment, the rice slurry (5% w/v) exhibited a VSS content of 16.75 g/L.

First, a comparison was made between the reactors (*sono*-LF and *sono*-HF). Rice slurry at a concentration of 5% w/v was pre-treated in these reactors at a power density of 300 W/L for 30 minutes. The initial sCOD as well as the sCOD of pre-treated feedstocks were determined to select a suitable reactor for the rest of the experiments.

Afterwards, the rest of the experiments were performed in the *sono*-LF. To investigate the effect of power density on sCOD and later on the concentrations of the fermentation products (lactic acid and VFAs), rice slurry at a concentration of 5% w/v was pre-treated at power densities of 100, 300, and 400 W/L for 30 minutes. The sCOD of the feedstock slurry was analyzed before the start and at the end of the pre-treatment experiments to measure the enhancement made in sCOD by the US in the different power densities. Subsequently, the rice slurry pre-treated under the mentioned conditions was employed as the feedstock in the FPs to investigate the effect on the concentrations of D-lactic acid, L-lactic acid, and VFAs.

Once the most effective power density was established, a subsequent set of experiments was conducted to explore the effect of combining the US with specific chemical agents on sCOD and the fermentation products. The chemical agents employed in these trials comprised (1) hydrochloric acid (HCl) to provide acidic conditions (pH 3) or sodium hydroxide (NaOH) to obtain alkalinity (pH 11) and (2) H_2O_2 (500 ppm) as an oxidative agent. In the combination of the US with acid or alkali, firstly, the pH of the slurry (5% w/v of rice) was adjusted, and it was pre-treated by the US in *sono*-LF at a power density of 400 W/L for 30 minutes. In the combination of the US and H_2O_2, 500 ppm H_2O_2 was added to the slurry (5% w/v of rice), and then it was pre-treated using the US in *sono*-LF at a power density of 400 W/L for 30 minutes. In the pre-treatment process using only H_2O_2, the slurry (5% w/v of rice) was combined with H_2O_2 (500 ppm) and stirred at 500 rpm for 30 minutes. Later, the pH of rice pre-treated under different combined conditions was adjusted to 7 before the start of the FPs, and then the pre-treated rice was employed as the feedstock in the FPs to explore the combination effect on the concentrations of D-lactic acid, L-lactic acid, and VFAs.

Food waste was also mixed with deionized water to obtain a slurry of 5% w/v to investigate the effect of pre-treatment duration of 10 minutes, 20 minutes, and 30 minutes in *sono*-LF at a power density of 400 W/L. Before the pre-treatment processes, food waste had a VSS content of 17.26 g/L. The sCOD of the samples was measured before and after the pre-treatment processes to assess the improvements achieved under different treatment durations. Following this analysis, pre-treated food waste was utilized as the fermentation feedstock to evaluate its influence on the production of D-lactic acid, L-lactic acid, and various VFAs.

Then, food waste was mixed with deionized water to achieve the slurry with different values of 3%, 5%, and 7% w/v to study the effect of feedstock concentration in the ultrasonic reactor. Experiments were performed in *sono*-LF at a power density of 400 W/L for 30 minutes. The sCOD of each sample was determined, and subsequently, food waste pre-treated in the mentioned conditions was employed as the fermentation feedstock to investigate the effect on the formation of D-lactic acid, L-lactic acid, and VFAs.

3.4. Fermentation experiments

Batch fermentation experiments were carried out to produce lactic acid and VFAs and assess the impact of ultrasonic parameters on the formation of these products. The FPs were conducted in 500 mL flasks, which were continuously agitated at 100 rpm to ensure proper mixing. Model food waste (rice) was utilized for the investigation of parameters, including ultrasonic frequency, ultrasonic power density, as well as the combined process of the US with H_2O_2, HCl, and NaOH.

In these experiments, fermentation was performed by anaerobic sludge, and the inoculum-to-substrate ratio (ISR) was maintained at 1:4.

To achieve more realistic results, actual food waste was also employed for more experiments investigating the influence of ultrasonic pre-treatment duration and feedstock concentration in the ultrasonic reactor on the products of the FPs done by anaerobic and aerobic sludge at an ISR of 1:6.

The temperature was kept constant at 35 °C throughout the fermentation period. At the beginning of the FPs, the initial pH of all samples was set to 7, but no pH control was applied as the process progressed. To track the fermentation dynamics over time, daily samples were collected.

Experiments were performed in duplicate, and the mean values were reported.

3.5. Analytical methods

The generation of $^{\bullet}$OH in *sono*-HF and *sono*-LF was investigated using *p*CBA and potassium iodide (KI). Ultra-high performance liquid chromatography (UHPLC) and a spectrophotometer were used for the analysis of these two types of experiments, respectively. The details of the instruments were provided in sections 3.5.1 and 3.5.2.

TSS and VSS were measured according to the standardized methods recommended by the American Public Health Association [205]. Total nitrogen was measured using a Shimadzu TOC-Vcsn total organic carbon analyzer. pH measurements were conducted using test strips produced by Merck, Germany.

Other analyses were conducted to measure sCOD, L-lactic acid, D-lactic acid, and various VFAs (AcA, PrA, BrA, and VaA). These measurements provided insights into the effectiveness of the pre-treatment method. Before these analyses, samples were centrifuged using MPW-352 centrifuge (MPW Med. Instruments) for 30 minutes at 10000 rpm. The supernatant was then filtered using a 0.45 μm hydrophilic cellulose acetate membrane filter (Alchem, Poland). Where needed, it was subsequently diluted with deionized water. The details of these analyses were provided in sections 3.5.3, 3.5.4, and 3.5.5.

3.5.1. *Para*-chlorobenzoic acid analysis

A Nexera XS UHPLC system, produced by Shimadzu, Japan, was utilized to detect *p*CBA. The system was equipped with an Agilent Zorbax C-18 chromatographic column (3.5 µm particle size and dimensions of 4.6 × 150 mm). The mobile phase consisted of 10 mM phosphoric acid and methanol in a 45:55 ratio, while a flow rate of 1.0 mL/min was maintained and the column oven was set at 30 °C. Each sample was automatically injected into the system in 10 µL volumes using the SIL-40C XS autosampler. *p*CBA was detected by a SPD-M40 photodiode array detector for monitored wavelength of 239 nm.

3.5.2. Triiodide ion analysis

A UV-1900i spectrophotometer (Shimadzu, Japan) was employed to monitor the formation of triiodide ions (I_3^-) by measuring absorbance at 352 nm. This detection was done as an indirect assessment of the oxidation process, where KI was oxidized by $^{\bullet}OH$ generated in both *sono*-HF and *sono*-LF. By the measurement of the absorbance at this specific wavelength, the experiment effectively demonstrated the oxidative potential in these two reactors.

3.5.3. Soluble chemical oxygen demand analysis

The measurement of sCOD was performed using Hach-Lange cuvette test kits vials LCK 214 (0-1000 mg O_2) and LCK 914 (5-60 g/L O_2). The samples were added to the vials by micropipette and digested in HACH HT200S Temperature Thermostat (High Speed Digestion Digester), and after cooling to the room temperature, sCOD was measured by Hach-Lange spectrophotometer DR3900.

Increase in sCOD% was calculated according to **Eq. 2**.

$$Increase\ in\ sCOD\% = \frac{sCOD_{after\ treatment} - sCOD_{before\ treatment}}{\text{sCOD}_{before\ treatment}} \times 100 \tag{2}$$

3.5.4. Lactic acid analysis

Lactic acid was analyzed using high performance liquid chromatography (HPLC) with a Hitachi LaChrom Elite system. The analysis was performed on an Astec CLC-D column (15 cm × 4.6 mm (ID), particle size 5 µm,) supplied by Sigma Aldrich. A 5 mM cupric sulphate ($CuSO_4$) solution prepared in deionized water was employed as the mobile phase. The autosampler injected 10 µL of each sample into the system.

g/L lactic acid was converted to g COD/L lactic acid as follows:

First, the theoretical oxygen demand of lactic acid for complete mineralization was obtained through **Eq. 3**.

$$C_3H_6O_3 + 3O_2 \rightarrow 3CO_2 + 3H_2O \tag{3}$$

Then, COD factor was calculated through **Eq. 4**.

$$COD\ factor = \frac{\text{g } O_2 \text{demand per mole of lactic acid}}{\text{molar mass of lactic acid}} \approx \frac{3 \times 32}{90} \approx 1.07 \tag{4}$$

Final conversion was performed through **Eq. 5**.

$$g\ COD/L\ lactic\ acid = COD\ factor \times g/L\ lactic\ acid \tag{5}$$

The same procedure was applied for the conversion of g/L of each VFA to g COD/L (COD factors: AcA ≈ 1.07, PrA ≈ 1.51, BuA ≈ 1.82, VaA ≈ 2.04).

3.5.5. Volatile fatty acids analysis

VFAs were analyzed using Shimadzu Nexis GC-2030 gas chromatography (GC). The instrument possessed a flame ionization detector (FID) and was equipped with an Agilent J&W DB-FFAP column (30 m length, 0.25 mm internal diameter, and 0.25 µm film thickness) for precise separation and detection. A 1 µL portion of each sample was injected into the system using an autosampler. Both the injector and detector were maintained at 250 °C. The oven temperature was programmed as follows: initially held at 50 °C for 5 minutes, then increased at a rate of 5 °C per minute up to 120 °C with a 5-minute hold, followed by a ramp of 25 °C per minute reaching 200 °C, where it was held for 20 minutes.

3.5.6. Microbiological analysis

Microbial composition analysis was conducted on four fermented samples, one pre-treated by the sole US, and the rest pre-treated by the US combined with different external additives before the FPs. Furthermore, the analysis was conducted on the anaerobic sludge, used as the inoculum in the fermentation of rice. Immediately after sampling, fermented biomass was frozen at -20°C. DNA extraction was performed using the FastDNA Spin Kit for Soil (MP Biomedicals, USA) following thawing at room temperature. A phosphate buffer was used to resuspend 200 mg of semi-dry biomass, and bead beating was carried out at maximum speed for 2 minutes using a Uniequip device (Uniequip, Planegg, Germany). The quality of the extracted DNA was assessed through agarose gel electrophoresis, while DNA concentration was fluorometrically determined using the Quant-iT BR DNA Assay (Thermo Fisher Scientific, USA).

For 16S rRNA sequencing, the 16S Barcoding Kit (SQK-16S114.24) from Oxford Nanopore Technologies was used to prepare the 16S meta-library via the MinION sequencing platform. DNA was combined with LongAmp 2X Master Mix (New England Biolab, Herts, UK) and barcoded with 16S primers based on the manufacturer’s instructions. Amplification was carried out using an Eppendorf Mastercycler (Eppendorf, Hamburg, Germany) under the following PCR conditions: initial denaturation at 95°C for 1 minute, followed by 30 cycles of denaturation at 95°C for 20 seconds, annealing at 55°C for 30 seconds, and extension at 65°C for 2 minutes, concluding with a final extension at 65°C for 5 minutes.

Sequencing was performed using an R10.4.1 (FLO-MIN114) flow cell, with live base calling executed via Guppy v3.2.10 within the MinKNOW software (version 5.8.7, corresponding to version 23.11.5) (Oxford Nanopore Technologies). Taxonomic classification was conducted using the EPI2ME platform (Oxford Nanopore Technologies) [206]; which processes 16S rRNA sequences by demultiplexing and assigning bacterial taxonomy to the genus level based on the National Center for Biotechnology Information (NCBI) 16S rRNA bacterial database. Sequencing reads underwent quality filtering with a minimum Q-score threshold of 9 before classification at the bacterial phylum and genus levels.

Shannon diversity index (H) is a measure of biodiversity according to the number of bacterial groups (richness) as well as their relative abundance (evenness) [207]. This index is calculated according to **Eq. 6** [208].

$$H = -\sum_{i=1}^{n} p_i \, ln p_i \tag{6}$$

In this context, p_i refers to the proportion of DNA sequences related to i genus, and n is the total number of genera. PAST software (version 4.03) was used to calculate this index.

4. Results and Discussion

In an ultrasonic reactor, several key factors can significantly impact the efficiency of acoustic cavitation as a pre-treatment method for improving the properties of feedstocks, and as a result, the production of lactic acid and VFAs through the subsequent FPs. These factors include ultrasonic frequency, ultrasonic power density, feedstock concentration, and ultrasonic pre-treatment duration. Furthermore, the combination of the US with external additives in the pre-treatment stage can have influences on sCOD and the production of the desired products. Following sections explore the influence of these parameters.

4.1. Comparison of high-frequency and low-frequency ultrasonic reactors effectiveness

A cavitation phenomenon can be applied as an effective pre-treatment technique due to its mechanical and chemical effects [209]. Two ultrasonic reactors, *sono*-HF working at a high frequency of 120 kHz and *sono*-LF working at a lower frequency of 24 kHz, were utilized under a constant power density of 300 W/L. It was aimed to enhance the solubility of the feedstock (rice) by physio-chemical processing under cavitation conditions, represented by a rise in sCOD.

The chemical effect of cavitation includes the production of highly reactive species, such as $^\bullet$OH, produced according to **Eq. 7**, which occurs due to the pyrolysis of water within extremely high-temperature and high-pressure zones. These radicals can lead to the oxidative degradation of complex molecules, hence, aiding in the disintegration of food waste components [176, 210].

$$H_2O + \textit{cavitation} \rightarrow {}^\bullet OH + {}^\bullet H \qquad (7)$$

This highly reactive radical is capable of attacking and breaking the side chain bonds of complex molecules such as lignin. Therefore, the depolymerization of the polymer structure of lignin can happen, resulting in the formation of smaller and simpler molecules. These simpler compounds are generally more suitable for further biodegradation [211]. Additionally, adding hydroxyl groups to these molecules can enhance their water affinity, making them more soluble and, subsequently, more accessible for microbial metabolism [212].

To evaluate and compare the production of $^{\bullet}$OH in the two reactors, *p*CBA was employed as a probe compound. *p*CBA is highly effective for this purpose because it reacts with $^{\bullet}$OH at a very high second-order rate constant of 5×10^{9} M^{-1} s^{-1} [213, 214]. This rapid reaction provides the possibility to monitor the generation of $^{\bullet}$OH, demonstrating the oxidative potential within each of the reactors. Thus, monitoring the reduction in the concentration of *p*CBA can be evidence of $^{\bullet}$OH formation in the ultrasonic reactors. In this experiment, a 5 ppm solution of *p*CBA was treated in both *sono*-HF and *sono*-LF, each operating at a power density of 300 W/L for a duration of 30 minutes. The results, as an indirect sign of radical generation, are illustrated in **Figure 6**.

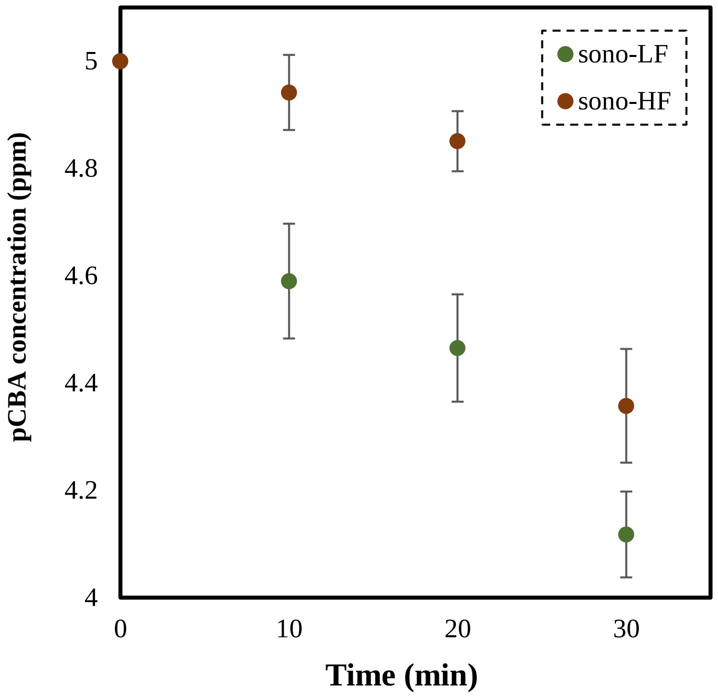


Figure 6. Degradation of *p*CBA in *sono*-LF and *sono*-HF (power density: 300 W/L, [*p*CBA]: 5 ppm) [204].

It is evident from the results that the initial concentration of 5 ppm *p*CBA reduced to 4.12 ppm in the *sono*-LF, while the *sono*-HF decreased the *p*CBA level to 4.36 ppm.

Moreover, a 5 mM solution of potassium iodide (KI) was employed to verify the formation of $^{\bullet}OH$. When $^{\bullet}OH$ reacts with iodide ions (I^-), they are expected to induce a series of subsequent reactions (**Eq. 8 to Eq. 11**) [215].

$$^{\bullet}OH + I^- \rightarrow I + HO^- \quad (8)$$

$$I + I^- \rightarrow I_2^- \quad (9)$$

$$2I_2^- \rightarrow I_2 + 2I^- \quad (10)$$

$$I_2 + I^- \rightarrow I_3^- \quad (11)$$

I^- oxidized by $^{\bullet}OH$ leads to the formation of triiodide ions (I_3^-), visually indicated by the light-yellow color of the solution at the end of the reaction. This color change was further validated through spectrophotometric analysis. Specifically, the absorbance peak of I_3^- was detected at a wavelength of 350 nm, as presented in **Figure 7**.

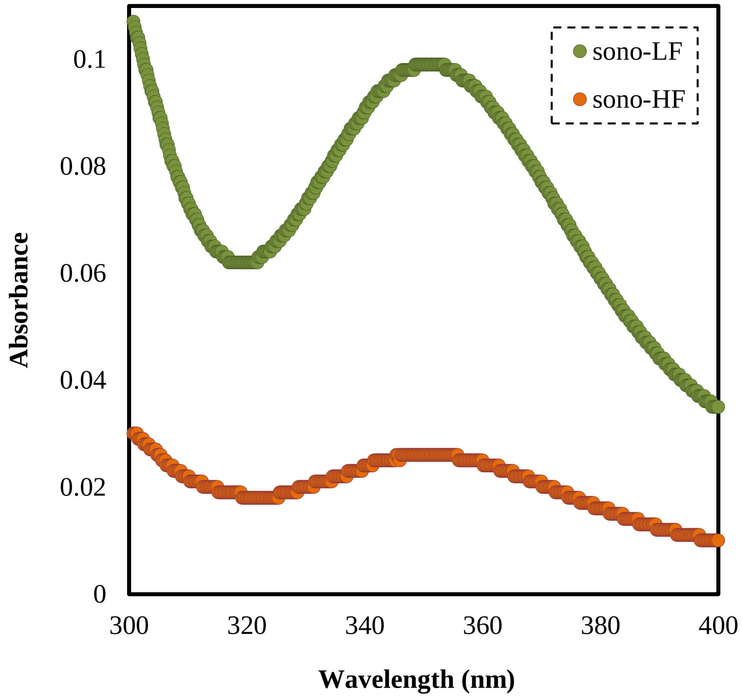


Figure 7. Absorption spectrum of the generated I_3^- in *sono*-LF and *sono*-HF (power density: 300 W/L, [KI]: 5 mM, process duration: 30 minutes) [204].

As the results show, *sono*-LF produced a greater quantity of $^{\bullet}OH$ compared to *sono*-HF, highlighting its superior oxidative capacity under the tested conditions.

The improvement made in sCOD by the pre-treatment with the two ultrasonic reactors is illustrated in **Figure 8**. Both reactors were run under the same power density of 300 W/L and a feedstock concentration of 5% w/v for 30 minutes.

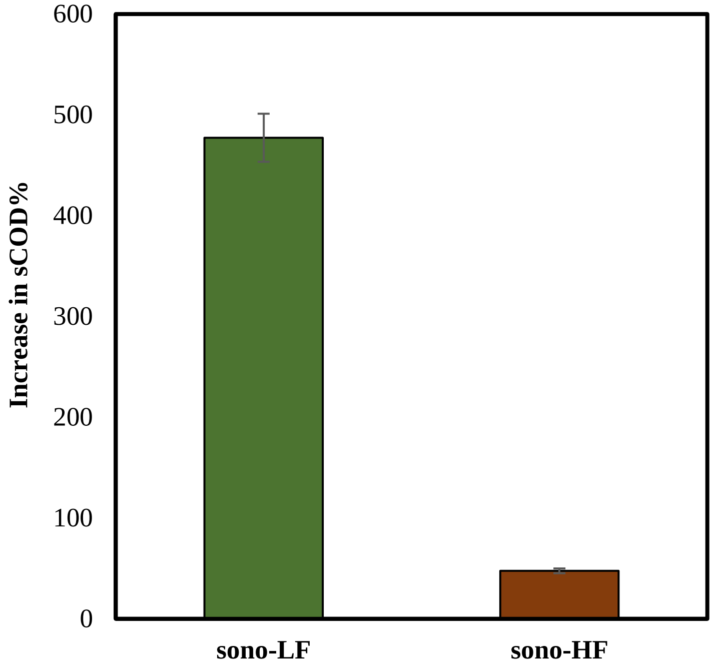


Figure 8. Increase in sCOD obtained in *sono*-LF and *sono*-HF (power density: 300 W/L, rice concentration: 5% w/v, pre-treatment duration: 30 minutes) [204].

As the figure reveals, *sono*-LF led to a significant increase in sCOD, 477% rise, indicating a significant breakdown of complex organic matter into more soluble forms. In contrast, *sono*-HF exhibited only a small effect, with a sCOD increase of just 48%, which is comparatively minimal.

This large contrast in the performance can be attributed not only to the different amounts of $^{\bullet}OH$ generated by each reactor but also to the mechanical forces associated with their operating frequencies.

The mechanical effects of cavitation include intense shear wall stress and powerful shock waves, both of which can contribute to the disintegration of solid particles and cellular structures within the waste material.

Ultrasonic reactors operating at low frequencies allow bubbles to have a longer time for growth before collapsing, which results in the formation of larger bubbles compared to those in high-frequency systems. When these larger bubbles collapse, they release a greater amount of localized energy, producing intense mechanical forces within the liquid medium.

These extreme mechanical effects of cavitation can play a key role in the physical disintegration of complex organic structures. For instance, they can contribute to the disintegration of large, insoluble carbohydrate polymers like starch, producing sugars or oligosaccharides that are easier to dissolve in water and more accessible for subsequent biochemical or microbial processes. These forces facilitate the release of intracellular components, making the organic matter more accessible for microbial fermentation. These mechanical effects can be a primary factor in the superior solubilization efficiency observed in reactors operating in low frequencies [5].

The literature also supports these findings, reporting that low-frequency US can lead to even more impressive increases in sCOD, with some studies showing an enhancement of up to 2300% [5]. This evidence highlights the effectiveness of low-frequency cavitation as a pre-treatment technique, suggesting that it can significantly improve the disintegration of organic materials, making it a highly valuable method for enhancing subsequent FPs and bioconversion processes [5, 180].

Hence, the formation of lactic acid and VFAs was examined using the feedstock pretreated in the *sono*-LF under different power densities to assess how energy input influences their production.

4.2. Effect of ultrasonic power density

Power density is an important parameter influencing the efficacy of cavitation as a pre-treatment stage before FPs, since it directly affects the intensity of cavitation and the subsequent breakdown of organic materials. In this study, the impact of three power densities, 100 W/L, 300 W/L, and 400 W/L, was examined on sCOD and the production of lactic acid and VFAs.

As shown in **Figure 9**, increasing the power density consistently resulted in higher sCOD values, indicating that higher power densities led to more efficient disintegration of the feedstock.

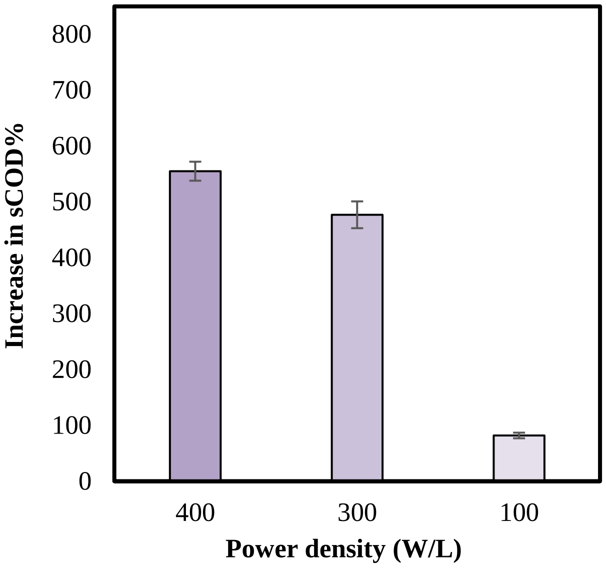


Figure 9. Effect of power density on sCOD (*sono*-LF, rice concentration: 5% w/v, pre-treatment duration: 30 minutes) [204].

At power densities of 100 W/L, 300 W/L, and 400 W/L, the US led to remarkable increases in sCOD, with values rising by 82%, 477%, and 555%, respectively. These results indicate that higher power densities have a significant impact on breaking down complex organic materials. At lower power densities, the cavitation effect may not be intense enough to effectively break the chemical bonds within starch molecules present in rice. This results in a relatively modest increase in feedstock solubility and nutrient availability for microbial fermentation. However, as the power density increases, the intensity of cavitation also increases, leading to more powerful mechanical forces that decompose the starch molecules more effectively. At a suitable power density, a cavitation process can reach the maximum decomposition of the feedstock, thus improving the overall performance of FPs by making more nutrients available to the microorganisms [195, 196].

It is important to emphasize that while increasing power density can enhance cavitation intensity, excessively high power densities may lead to the formation of cavitation clouds. These clouds consist of clustered bubbles that can collapse inefficiently, reducing the overall effectiveness of a cavitation process. Consequently, it can weaken the mechanical and chemical effects generated by cavitation phenomena, which in turn limits the breakdown of the feedstock and the subsequent increase in nutrient availability. Therefore, it is helpful to identify the suitable power density that balances the intensity of cavitation without causing the formation of cavity clouds. This ensures that the cavitation process remains efficient and maximizes the decomposition of organic matter, leading to more effective fermentation and higher yields of desired products [197]. In this study, although it was observed that an increase in power density increased the disintegration of feedstock, the difference between the enhancements made by power densities of 300 W/L and 400 W/L was almost small. It is useful to consider other factors, such as economic considerations. This includes evaluating the cost-effectiveness of increasing power density and finding a balance that maximizes efficiency while minimizing operational costs.

Figure 10 illustrates how the power density in the pre-treatment stage impacted the amount of lactic acid produced by the subsequent FPs, highlighting how adjustments in power density can enhance the production of this compound during the process.

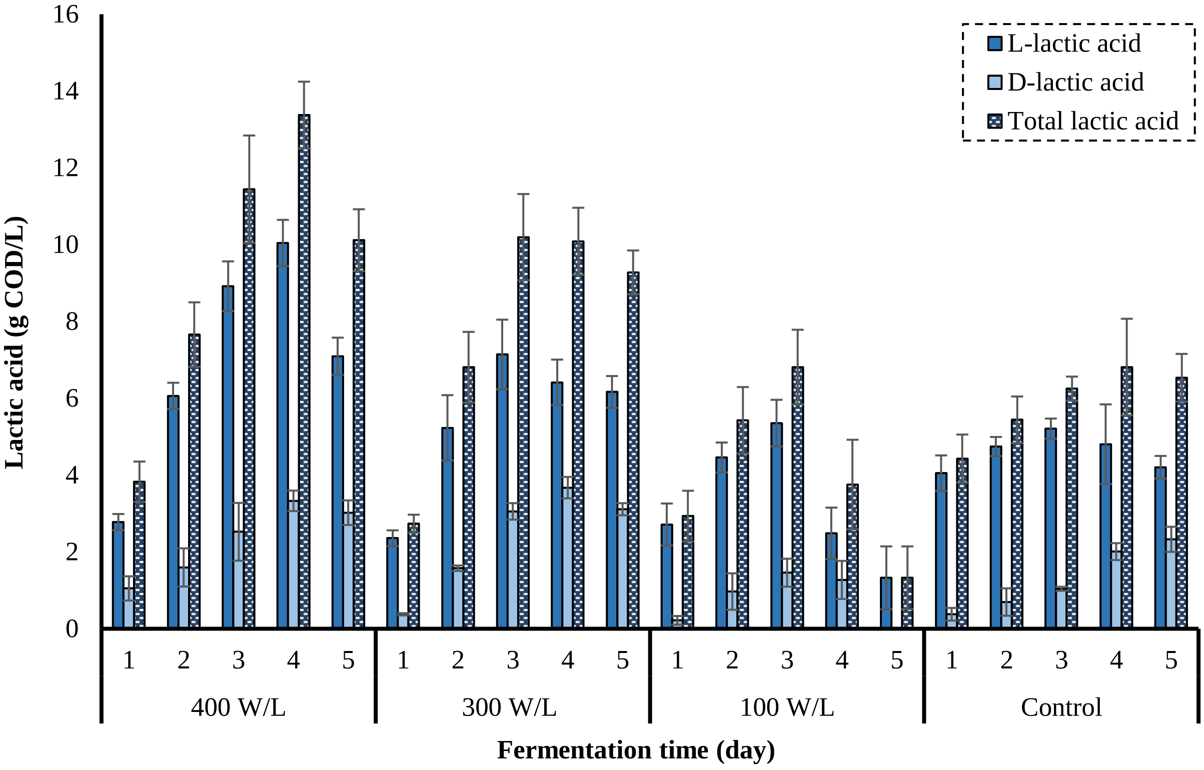


Figure 10. Effect of power density on the amount of lactic acid (*sono*-LF, rice concentration: 5% w/v, pre-treatment duration: 30 minutes) [204].

L-Lactic acid was produced in greater quantities than D-lactic acid during food waste fermentation, likely due to the metabolic pathways employed by lactic acid bacteria (LAB). LAB predominantly follow a metabolic route that favors the production of L-lactic acid, especially under the conditions commonly found in fermentation environments. These conditions tend to support the growth of homofermentative bacteria, which are specialized in converting sugars into lactic acid and typically produce only the L-isomer. Homofermentative LAB utilize a pathway that involves the conversion of glucose into L-lactic acid, with little production of other byproducts. As a result, the overall yield of L-lactic acid is higher compared to D-lactic acid, which is mainly produced by heterofermentative bacteria. These heterofermentative strains typically produce both L-lactic acid and D-lactic acid, along with other byproducts like AcA and ethanol [216]. Additionally, these bacteria have adapted to primarily produce L-lactic acid because it is less harmful to their cells than D-lactic acid. This preference provides them with a survival benefit, allowing them to thrive more effectively in both natural environments and industrial FPs where lactic acid production is required [217].

Hydrolysis can significantly restrict the biochemical conversion of lignocellulosic food wastes into valuable products, limiting the efficiency of the process. This step often presents challenges, as it involves breaking down complex lignocellulosic structures, and any inefficiency in this phase can reduce the yield of desirable compounds [6].

In *sono*-LF**,** at a rice concentration of 5% w/v and a pre-treatment duration of 30 minutes, an increase in power density in the pre-treatment stage led to higher concentrations of L-lactic acid produced through the subsequent FPs. It can demonstrate that the pre-treatment done by the US did not produce any inhibitor compounds, which are harmful to the microorganisms responsible for the production of L-lactic acid. The highest amount of L-lactic acid, reaching 10.0 g COD/L, was obtained from the feedstock modified by the US at a power density of 400 W/L, produced on the fourth day of the FP. On the other hand, from the feedstocks pre-treated at lower power densities of 300 W/L and 100 W/L, the concentrations of L-lactic acid reached 7.1 g COD/L and 5.3 g COD/L, respectively. These values were observed on the third day of the FPs.

In terms of D-lactic acid production, the highest concentration (3.7 g COD/L) was obtained from the feedstock pre-treated by the US at a power density of 300 W/L, occurring on the fourth day of the subsequent FP. The feedstock pre-treated at a power density of 400 W/L led to a production of 3.3 g COD/L D-lactic acid. The amount of D-lactic acid produced from the feedstock pre-treated at a lower power density of 100 W/L was significantly lower, reaching 1.5 g COD/L. The control process (a conventional FP without ultrasonic pre-treatment) reached 5.2 g COD/L L-lactic acid and 2.3 g COD/L D-lactic acid.

Total lactic acid exhibited a similar trend to L-lactic acid due to the dominant presence of L-lactic acid in the process. This dominance reflects the preference of the microorganisms for producing L-lactic acid over D-lactic acid. The results demonstrate that power density is a key parameter for making an enhancement in FPs for lactic acid production. **Figure 11** displays how different power densities affected the production of VFAs.

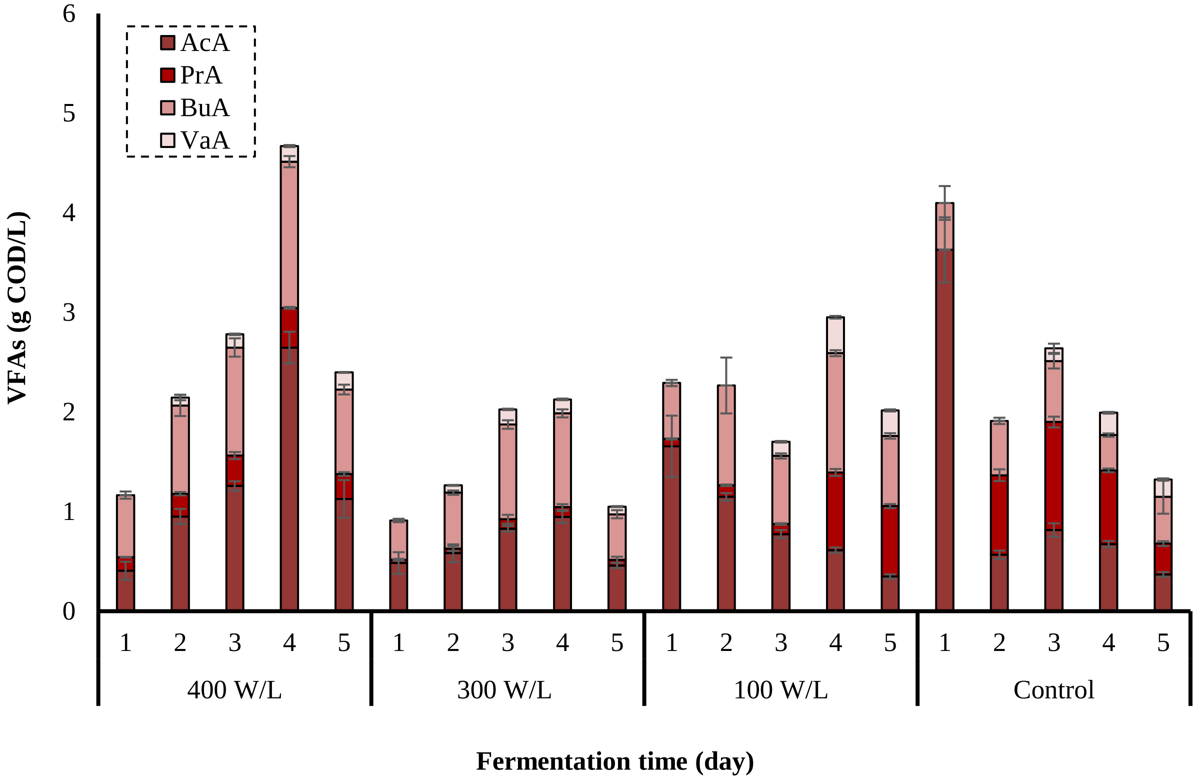


Figure 11. Effect of power density on the amount of VFAs (*sono*-LF, rice concentration: 5% w/v, pre-treatment duration: 30 minutes) [204].

In the case of total VFAs, the highest concentration of 4.7 g COD/L was achieved from the feedstock pre-treated through the application of the US at a power density of 400 W/L, observed on the fourth day of the FP. On the other hand, from the feedstocks pre-treated at lower power densities of 300 W/L and 100 W/L, the total VFAs reached their maximum values of 2.1 g COD/L and 2.9 g COD/L, respectively, both occurring on the fourth day of the FPs.

VFAs analysis revealed that AcA and BuA were the predominant compounds formed when the feedstock was modified through ultrasonic pre-treatment. For the control process and the feedstock pre-treated by a lower power density of 100 W/L, PrA was also present in high concentrations. The highest levels of AcA (3.6 g COD/L) and PrA (1.1 g COD/L) were observed in the control process, occurring on the first and third days of the FP, respectively. No VaA was detected on the first day of the FP in any of the conditions.

The application of the US significantly altered the feedstock composition, likely enriching the medium in a way that favored LAB. This microbial shift probably created competitive pressure, as LAB rapidly consumed available sugars, consequently, suppressing other microorganisms involved in the production of VFAs. As a result, lactic acid levels rose, but overall VFAs generation declined when compared to the control.

However, when the power density increased to 400 W/L, resulting in a higher rise in sCOD, a different dynamic emerged. The abundance of soluble carbohydrates likely reduced the competition, allowing both LAB and VFA-producing microbes to thrive simultaneously. Consequently, the production of both lactic acid and VFAs increased when the feedstock was pre-treated by the US at 400 W/L compared to the control process.

4.3. Enhancing pre-treatment performance by the combined application of ultrasounds and external additives

After finding the most effective reactor and power density, a series of additional experiments were carried out to further enhance the production of desired fermentation products. This strategy focused on combining the US with the external chemical additives to assess their potential impact on feedstock properties and fermentation efficiency. The selected additives included acid (HCl), alkali (NaOH), and an oxidant (H_2O_2).

HCl and NaOH were employed to obtain pH values of about 3 and 11, respectively, while the feedstock without any additives (sole US) had a neutral pH of ~7. It is worth mentioning that these pH adjustments were applied exclusively during the ultrasonic pre-treatment stage to investigate their influence on biomass decomposition and solubilization. To ensure consistency in fermentation conditions across all experiments, the pH was subsequently readjusted to neutral (pH ~7) before the start of the FPs.

By implementing these modifications, the study aimed to explore how these combined processes affected the release of soluble organic compounds, microbial metabolism, and the subsequent formation of the valuable fermentation products.

Microbiological examinations were carried out on the fermented feedstocks, which had been pre-treated using the sole US as well as its combination with the external additives before the FPs. The purpose of these analyses was to investigate the composition of microbial species present in the fermented feedstock and to evaluate their specific role in facilitating the production of the desired end products.

A total of 555,850 high-quality reads were obtained, ranging from 55,298 to 197,701. The results revealed distinct bacterial compositions across the fermented feedstocks, with the inoculum displaying significantly greater diversity than the fermented feedstocks. The inoculum exhibited the highest species richness, with an effective species count of 99.4, while the fermented feedstocks showed lower diversity, ranging from 7.9 in the feedstock pre-treated by the US at pH 7 to 10.2 in the feedstock pre-treated by the US combined with H_2O_2. The Shannon diversity index (H) was also highest in the inoculum (4.6), while fermented feedstocks had lower values, H: 2.1 in the feedstocks pre-treated by the US at pH 7 and pH 3, H: 2.2 in the feedstock pre-treated by the US at pH 11, and H: 2.3 in the feedstock pre-treated by the combined application of the US and H_2O_2.

As illustrated in **Figure 12**, the bacterial composition of the inoculum was dominated by four major phyla. *Firmicutes* accounted for the largest proportion at 40.43%, followed by *Proteobacteria*, which constituted 23.06% of the community. *Bacteroidota* made up 21.88% of the total bacterial population, while *Actinobacteria* represented 7.43%. The remaining bacterial phyla were present in much smaller quantities, each contributing less than 5% to the overall microbial composition of the inoculum.

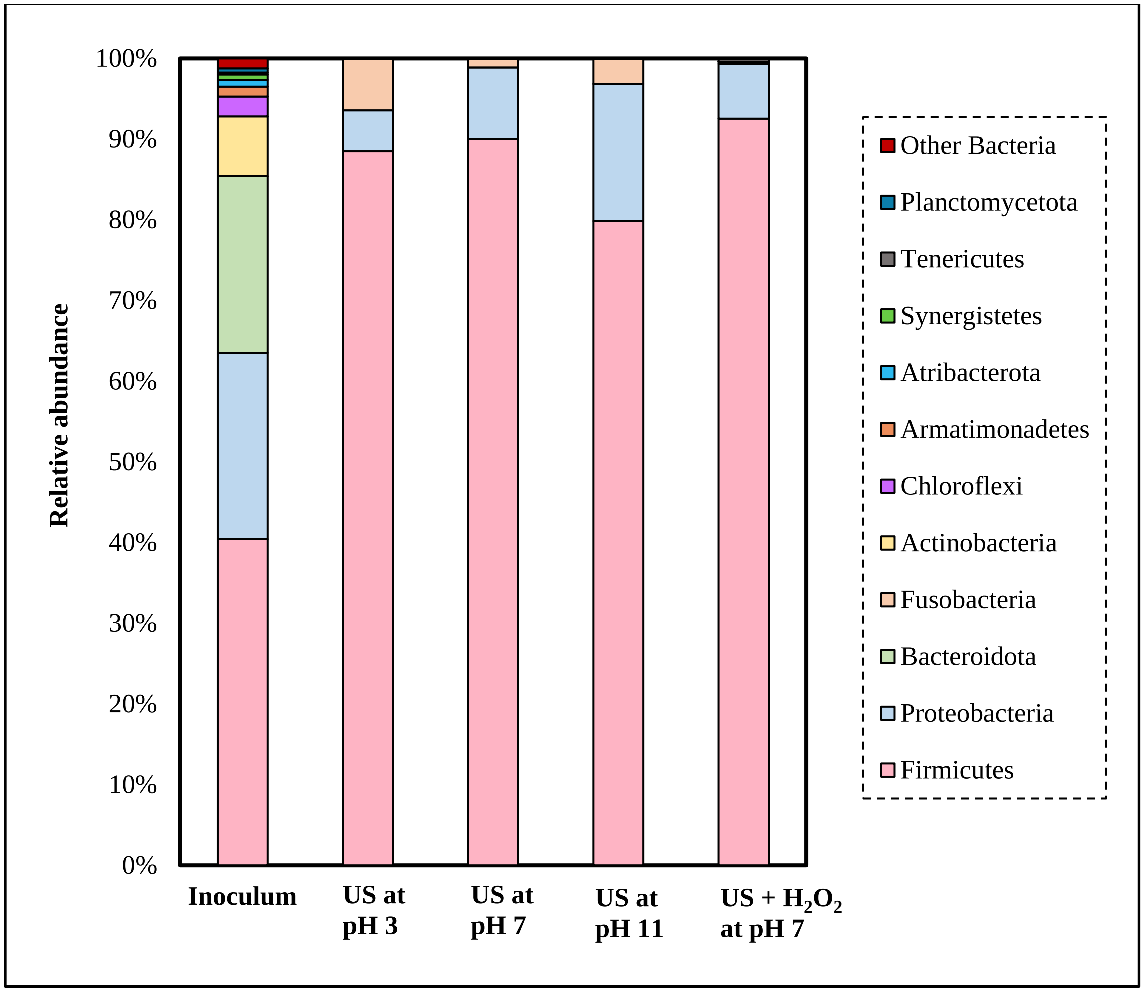


Figure 12. Average relative abundance of the sequences identified at phylum level in the feedstocks pre-treated by the US (*sono*-LF, power density: 400 W/L, rice concentration: 5% w/v, [H_2O_2]: 500 ppm, pre-treatment duration: 30 minutes) [204].

Firmicutes were the most abundant bacterial phylum observed in the fermented feedstocks, maintaining relatively consistent levels across the different pre-treatment processes. However, their presence was the lowest in the feedstock pre-treated by the US at pH 11. Specifically, *Firmicutes* dominated the microbial community in the feedstock pre-treated by the US combined with H_2O_2, comprising 92.53% of the total bacterial population. Similarly, they constituted 90% of the community in the feedstock pre-treated by the US at pH 7 and 88.49% in the feedstock pre-treated by the US at pH 3. The lowest proportion of *Firmicutes*, 79.85%, was recorded in the feedstock pre-treated by the US at pH 11.

In contrast, *Proteobacteria* were found in smaller quantities. Their abundance rose from 5.07% in the feedstock pre-treated by the US at pH 3 to 16.96% in the feedstock pre-treated by the US at pH 11. Meanwhile, in the feedstock pre-treated by the combined application of the US and H_2O_2, *Proteobacteria* accounted for 6.79% of the bacterial composition.

Additionally, members of the *Fusobacteria* phylum were detected in different proportions. Their presence was recorded at 1.05% in the feedstock pre-treated by the US at pH 3, while a significantly higher proportion of 6.37% was observed in the feedstock pre-treated by the US at pH 11. However, *Fusobacteria* were nearly undetectable in the feedstock pre-treated by the US at pH 7 and that pre-treated by the US in combination with H_2O_2.

As illustrated in **Figure 13**, the bacterial community structure at the genus level in the fermented feedstocks appears to be largely consistent across the various pre-treatments. This similarity is especially evident in the feedstocks that were pre-treated by the US at pH levels 3, 7, and 11, where the microbial composition shows little variation between these conditions.

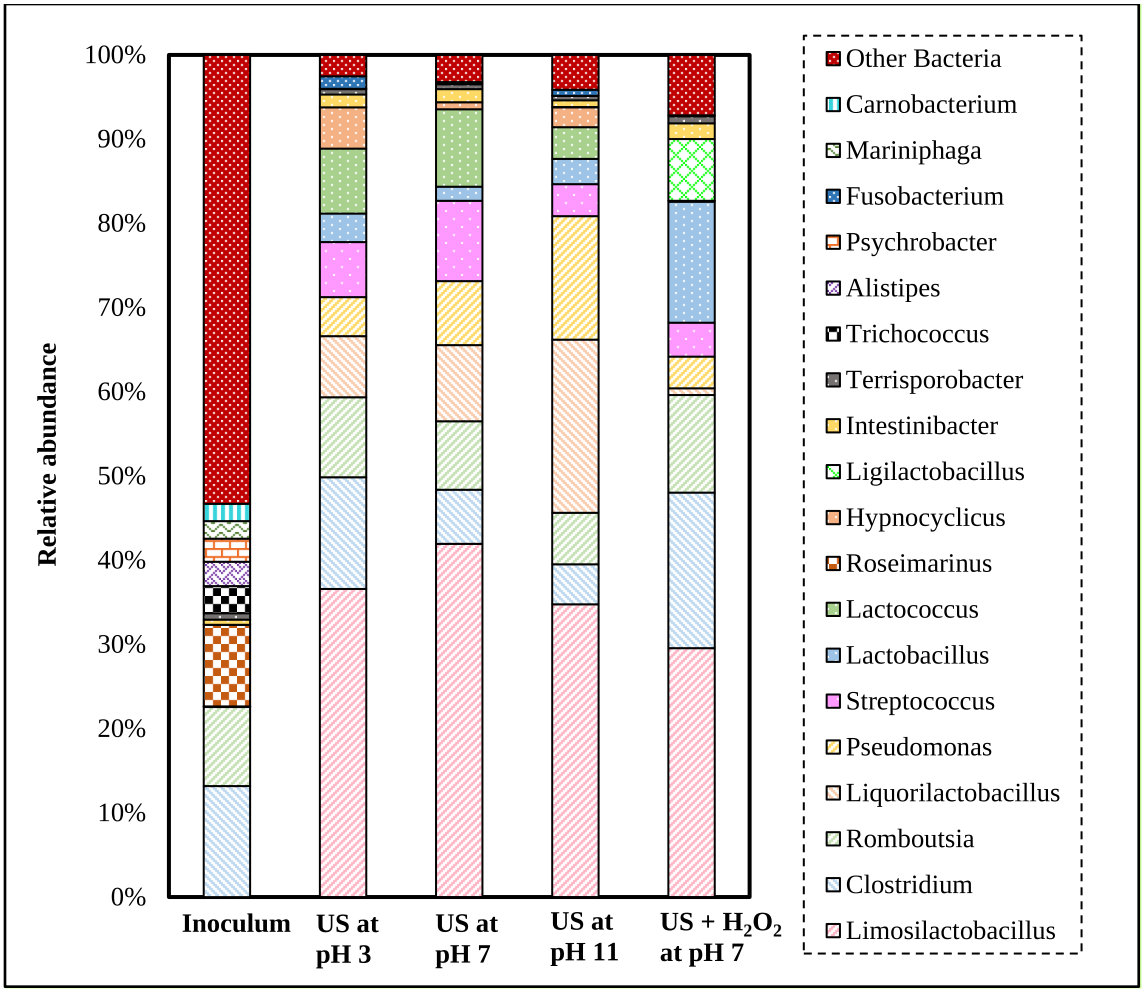


Figure 13. Average relative abundance of the sequences identified at genus level in the feedstocks pre-treated by the US (*sono*-LF, power density: 400 W/L, rice concentration: 5% w/v, [H_2O_2]: 500 ppm, pre-treatment duration: 30 minutes) [204].

In the inoculum, more than half (53%) of the bacterial genera were classified as rare or unidentified, reflecting a significant portion of the community that could not be easily identified or was present in low numbers. Among the identified genera, several were found in abundance greater than 2%, with the most notable being *Clostridium* species, which made up 13.18% of the community. Other prominent genera included *Romboutsia* species (9.35%), *Trichococcus* species (3.22%), *Alistipes* species (2.87%), *Psychrobacter* species (2.75%), *Mariniphaga* species (2.07%), and *Carnobacterium* species. These genera represented the most significant portions of the microbial community in the inoculum.

In the fermented feedstocks, the overall microbial composition followed a similar pattern, especially in the terms of the dominant genera. *Limosilactobacillus* species were the most prevalent in these samples, with an average abundance of 35.71% ± 5.10%, highlighting their major role in the microbial community. Other important genera included *Clostridium* species (10.73% ± 6.34%), *Romboutsia* species (8.82% ± 2.30%), *Liquorilactobacillus* species (9.41% ± 8.23%), *Pseudomonas* species (7.66% ± 4.94%), *Streptococcus* species (5.97% ± 2.66%), *Lactobacillus* species (5.59% ± 5.87%), and *Lactococcus* species (5.21% ± 4.09%). The abundance of other genera was relatively low, remaining below 5%.

The highest proportion of *Limosilactobacillus* species was detected in the feedstock pre-treated by the US at pH 7, where no additives were used, while the lowest abundance of this genus was found in the feedstock pre-treated by the combined application of the US and H_2O_2. The high and consistent presence of *Limosilactobacillus* species in the fermented feedstocks across all treatment variants indicates its importance in the overall process of biomass conversion.

Some genera, such as *Liquorilactobacillus* species and *Pseudomonas* species, exhibited higher abundances in the feedstocks pre-treated at higher pH levels, while others, including *Clostridium* species and *Romboutsia* species, showed a decrease in abundance under these conditions. Despite these variations, the total abundance of Lactic acid bacteria (LAB) remained relatively consistent across all the fermented feedstocks, ranging from 67.81% in the feedstock pre-treated by the US at pH 11 to 71.44% in the feedstock pre-treated by the US combined with H_2O_2. This consistency highlights the dominant role of LAB in the microbial communities of the fermented feedstocks, regardless of the different pre-treatment conditions.

The abundance of *Lactobacillus* species and *Ligilactobacillus* species was significantly higher in the feedstock pre-treated by the combined application of the US and H_2O_2, suggesting that this pre-treatment positively influenced the growth of these specific lactic acid bacteria. The other LAB, *Liquorilactobacillus* species also showed a high presence in the feedstocks. However, its abundance exhibited a noticeable trend in the feedstock pre-treated at higher pH levels. In contrast, *Liquorilactobacillus* species were found in much lower abundance in the feedstock pre-treated by the combined application of the US and H_2O_2. The results imply that the addition of H_2O_2 in the pre-treatment stage later might have led to a microbial shift, favoring the proliferation of other LAB species, such as *Lactobacillus* species and *Ligilactobacillus* species over *Liquorilactobacillus* species.

Clostridium species are also bacteria known for their ability to produce lactic acid [218]. A significant increase in their abundance was observed in the feedstock pre-treated by the combined application of the US and H_2O_2. This suggests that the presence of H_2O_2 in the pre-treatment stage, reacting with the content of the feedstock, may have created favorable compounds for the growth and proliferation of *Clostridium* species in the FP, potentially enhancing its metabolic activity related to lactic acid production.

4.3.1. Ultrasounds combined with acid or alkali in the pre-treatment stage

The utilization of either HCl or NaOH can modify the effectiveness of acoustic cavitation by enhancing the solubility of organic matter, which in turn, can facilitate the production of more favorable compounds. To further understand the extent of these effects, the influence of combining the US with acid (US+HCl) and alkali (US+NaOH) on sCOD was examined. The results of these investigations are presented in **Figure 14**, highlighting the variations observed under different conditions.

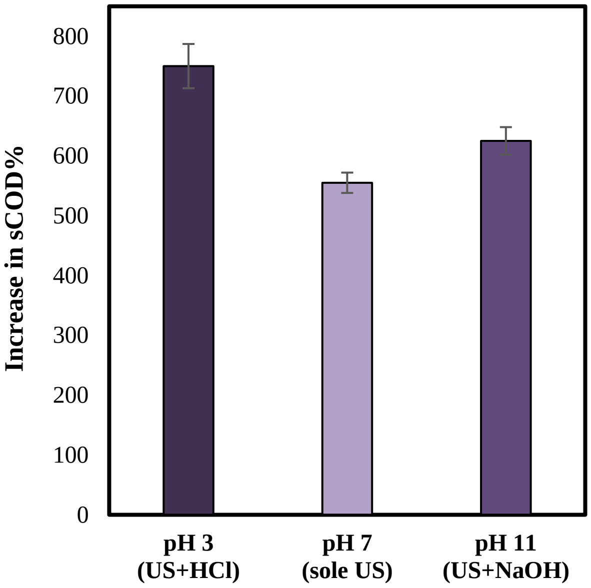


Figure 14. Effect of the combined application of the US with acid and alkali in the pre-treatment stage on sCOD (*sono*-LF, power density: 400 W/L, rice concentration: 5% w/v, pre-treatment duration: 30 minutes) [204].

The combination of the US with acidic and alkaline conditions led to a rise in sCOD levels, indicating an improvement in solubility. Specifically, the experimental results revealed that sCOD increased by 750% in the feedstock pre-treated at a pH of 3, 555% in the feedstock pre-treated at a neutral pH of 7, and 625% in the feedstock pre-treated under alkaline conditions at a pH of 11. This enhancement suggests that the combined effect of acoustic cavitation with acid or alkali has a significant role in breaking down complex organic structures. In acidic conditions, the increase in sCOD can be attributed to the ability of acid to effectively cleave glycoside bonds present in starch molecules. This process facilitates the breakdown of complex starch polymers into simpler compounds with lower molecular weight, which exhibit significantly higher solubility in the solution [219]. The observed increase in sCOD under the alkaline condition can be attributed to the ability of the alkali to weaken the structural integrity of the waste material. This structural destabilization facilitates a more effective disintegration process, breaking down complex organic compounds into smaller, more soluble ones. As a result, the release of soluble organic matter is significantly enhanced, contributing to the overall rise in sCOD levels [220]. At alkaline conditions, the hydroxyl groups within starch molecules undergo ionization, which can loosen the starch structure, making the molecules more flexible and mobile. As a result, the cleavage of starch by acoustic cavitation might become easier, facilitating its release from the material matrix [221]. Additionally, the combination of US with acidic or alkaline pre-treatment can promote the breakdown of hemicellulose and lignin by creating localized microturbulence and shear forces. This improves the diffusion and interaction of chemical agents with the lignocellulosic matrix, which can intensify the disintegration of hemicellulose and lignin acting as barriers around cellulose. Therefore, it can enhance cellulose accessibility to enzymes, resulting in improved hydrolysis efficiency [110, 112].

As observed in **Figure 15**, the pre-treatment of feedstocks using the US at a neutral pH of 7, without the addition of acid or alkali, ultimately resulted in the highest concentration of L-lactic acid, reaching 10.0 g COD/L. In comparison, pre-treatment conducted at an acidic pH of 3 and an alkaline pH of 11 led to slightly lower peaks of L-lactic acid concentrations of 8.9 g COD/L and 8.4 g COD/L, respectively.

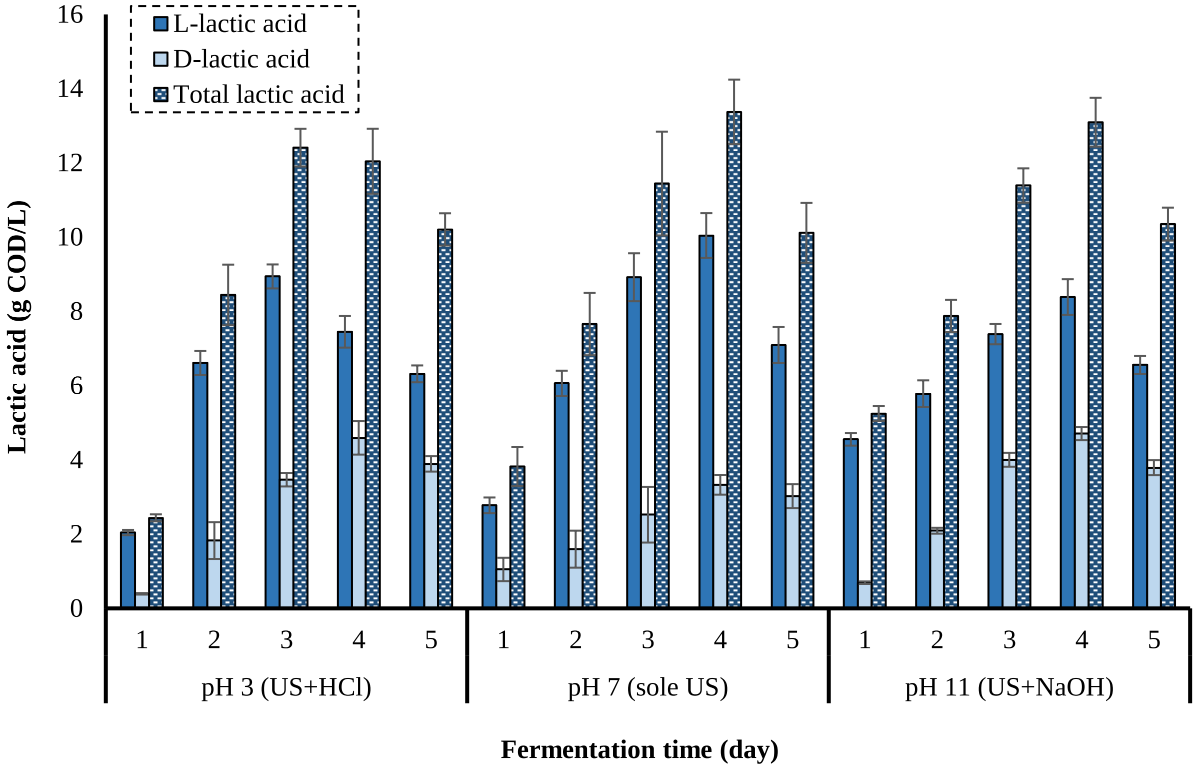


Figure 15. Effect of the combined application of the US with acid and alkali in the pre-treatment stage on the amount of lactic acid (*sono*-LF, power density: 400 W/L, rice concentration: 5% w/v, pre-treatment duration: 30 minutes) [204].

These variations in L-lactic acid production suggest that the chemical environment during the pre-treatment stage can have an effect on the properties of feedstocks, consequently, on the metabolic activity of microbial communities. The soluble compounds produced when the US was applied at acidic or alkaline conditions may have caused shifts in microbial composition, leading to a partial displacement of *Limosilactobacillus* species by other bacterial species. This shift could have impacted the efficiency of L-lactic acid production, highlighting the importance of pre-treatment pH. The higher L-lactic acid amount produced from the feedstock pre-treated by the US at a neutral pH of 7 is likely due to the dominant presence of *Limosilactobacillus* species in this particular environment. This dominance contributed to the higher L-lactic acid production [222]. Moreover, *Streptococcus* species and *Lactococcus* species, both of which are known to produce only L-lactic acid and not D-lactic acid, were found to be the most predominant microorganisms in this feedstock [223].

The feedstocks pre-treated by the US at both acidic and alkaline conditions resulted in the highest amount of D-lactic acid, reaching maximum values of 4.6 g COD/L and 4.7 g COD/L, respectively, occurring on the fourth day of the FPs. In comparison, the feedstock pre-treated at a neutral pH of 7 reached a peak of 3.3 g COD/L D-lactic acid. The observed increase in D-lactic acid concentration is likely associated with the higher presence of *Lactobacillus* species in the feedstock pre-treated by the US at pH 3 and pH 11. *Lactobacillus* species is known for its ability to produce D-lactic acid, which distinguishes it from other bacterial species such as *Lactococcus* species. These *Lactococcus* species are limited to the production of only L-lactic acid [223]. The maximum concentrations of total lactic acid obtained from the feedstocks pre-treated by the US at pH 7 and pH 11 were almost the same. In contrast, the peak of total lactic acid produced from the feedstock pre-treated at pH 3 was slightly lower.

It is hypothesized that there may have been a limit to the amount of nutrients that microorganisms could consume within a given time, meaning that supplying additional nutrients did not further enhance productivity. Another possibility is that the microorganisms reached a maximum production capacity for the desired products, and beyond a certain concentration, further production stopped. This could be due to the inhibitory effect of the products themselves, as high concentrations may become toxic to the bacteria. As a result, despite the higher sCOD values generated by the US under acidic and alkaline conditions, no further increase in total lactic acid yield was observed. **Figure 16** presents the results that demonstrate how feedstocks pre-treated by the US in combination with both acid and alkali influence the production of VFAs.

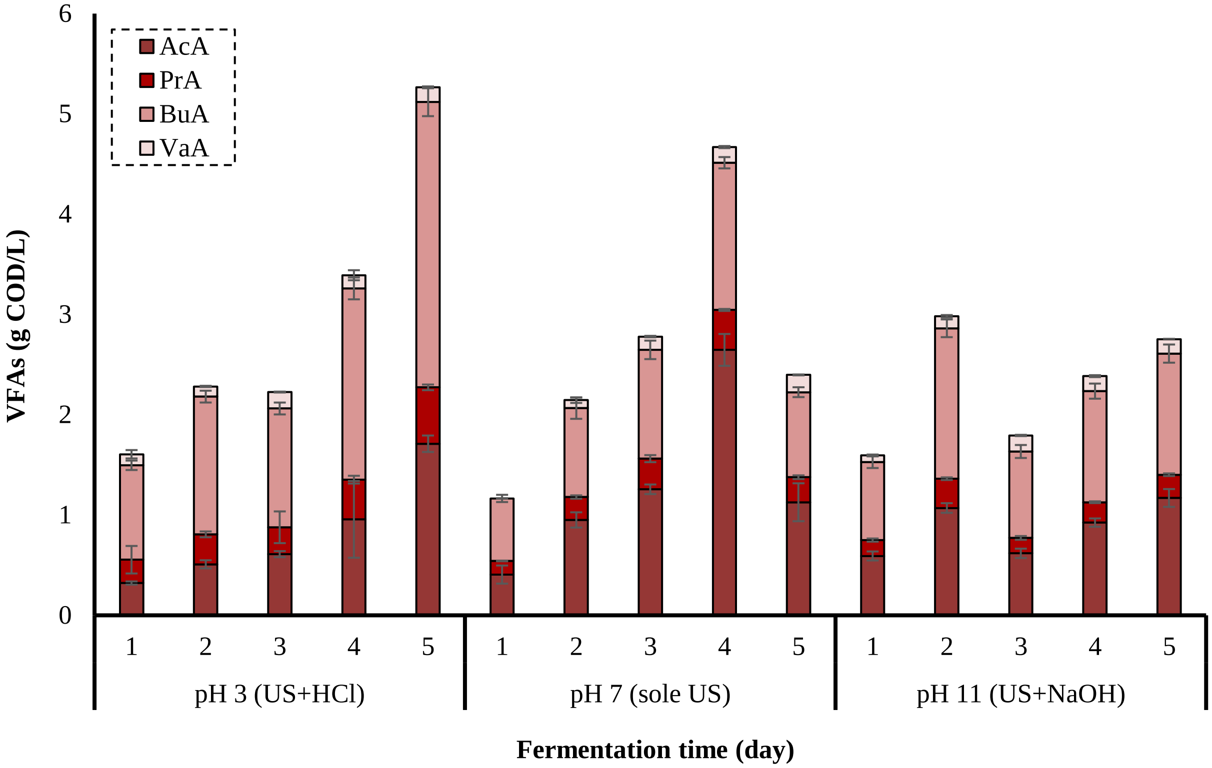


Figure 16. Effect of the combined application of the US with acid and alkali in the pre-treatment stage on the amount of VFAs (*sono*-LF, power density: 400 W/L, rice concentration: 5% w/v, pre-treatment duration: 30 minutes) [204].

The production of VFAs from the feedstocks that were pre-treated by the US under acidic, neutral, and alkaline conditions resulted in the peak concentrations of total VFAs occurring at different times during the FPs. Specifically, the highest concentration of total VFAs, 5.3 g COD/L, was observed on the fifth day of the FP of the feedstock pre-treated under the acidic condition. In the case of the pre-treatment under the neutral condition, the peak concentration of 4.7 g COD/L was reached on the fourth day of the FP, while for the feedstock pre-treated under the alkaline condition, the peak was 3.0 g COD/L, occurring on the second day of the FP. Among the VFAs produced, AcA and BuA were the dominant products. In particular, the feedstock pre-treated by the US at pH 3 yielded the highest amounts of BuA (2.8 g COD/L) and PrA (0.6 g COD/L) on the fifth day of the FP. *Romboutsia* species are known for their ability to produce BuA during FPs. Microbiological analysis conducted during the study revealed that this particular species was most abundant in the feedstock that was pre-treated by the US at an acidic pH of 3. On the other hand, the feedstock pre-treated by the US at an alkaline pH of 11 showed the lowest abundance of *Romboutsia* species [224]. These findings indicate that the growth of *Romboutsia* species, and consequently their BuA production, may have been influenced by the pH conditions during the pre-treatment process.

4.3.2. Ultrasounds combined with H_2O_2 in the pre-treatment stage

AOPs are effective methods that generate reactive radicals, which can break down target molecules. These radicals can oxidize complex compounds, transforming them into simpler forms [139]. In this study, a hybrid pre-treatment method was employed, where the low-frequency US was combined with H_2O_2 at a concentration of 500 ppm. This low concentration of H_2O_2 was selected for 5% w/v of rice in the pre-treatment stage to decrease any harmful effects of H_2O_2 residue on the microorganisms during the FP.

The process was conducted in *sono*-LF at a power density of 400 W/L and a pH of 7, with 5% w/v feedstock concentration for 30 minutes. The results of this combined approach were compared to the results obtained from the sole pre-treatment methods to evaluate if the combined treatment influenced the solubility of organic matter, as reflected by sCOD levels, as well as the production of lactic acid and VFAs.

The combination of cavitation and H_2O_2 results in the formation of $^{\bullet}OH$ according to **Eq. 12**.

$$H_2O_2 + \textit{cavitation} \rightarrow {}^{\bullet}OH \qquad (12)$$

By the oxidative breakage of molecular bonds through $^{\bullet}OH$, the larger, more complex structures are broken down into smaller, simpler components [113]. As a result, the organic compounds become more bioavailable, allowing microorganisms to more easily access and consume them.

Figure 17 presents the results that demonstrate how the combination of H_2O_2 with the US ($US+H_2O_2$) affected the solubility of the feedstock, quantified by sCOD.

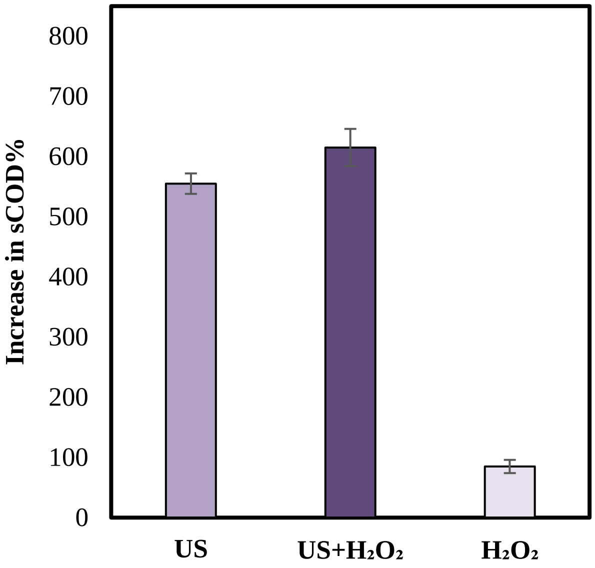


Figure 17. Effect of the combined application of the US with H_2O_2 in the pre-treatment stage on sCOD (*sono*-LF, power density: 400 W/L, rice concentration: 5% w/v, [H_2O_2]: 500 ppm, pre-treatment duration: 30 minutes) [204].

In the analysis of rice content breakdown, the combination of the US and H_2O_2 led to an increase in sCOD by 615%, while sole H_2O_2 increased sCOD just by 85%.

The impact of the combination of the US and H_2O_2 on lactic acid production was examined, with the findings presented in **Figure 18**.

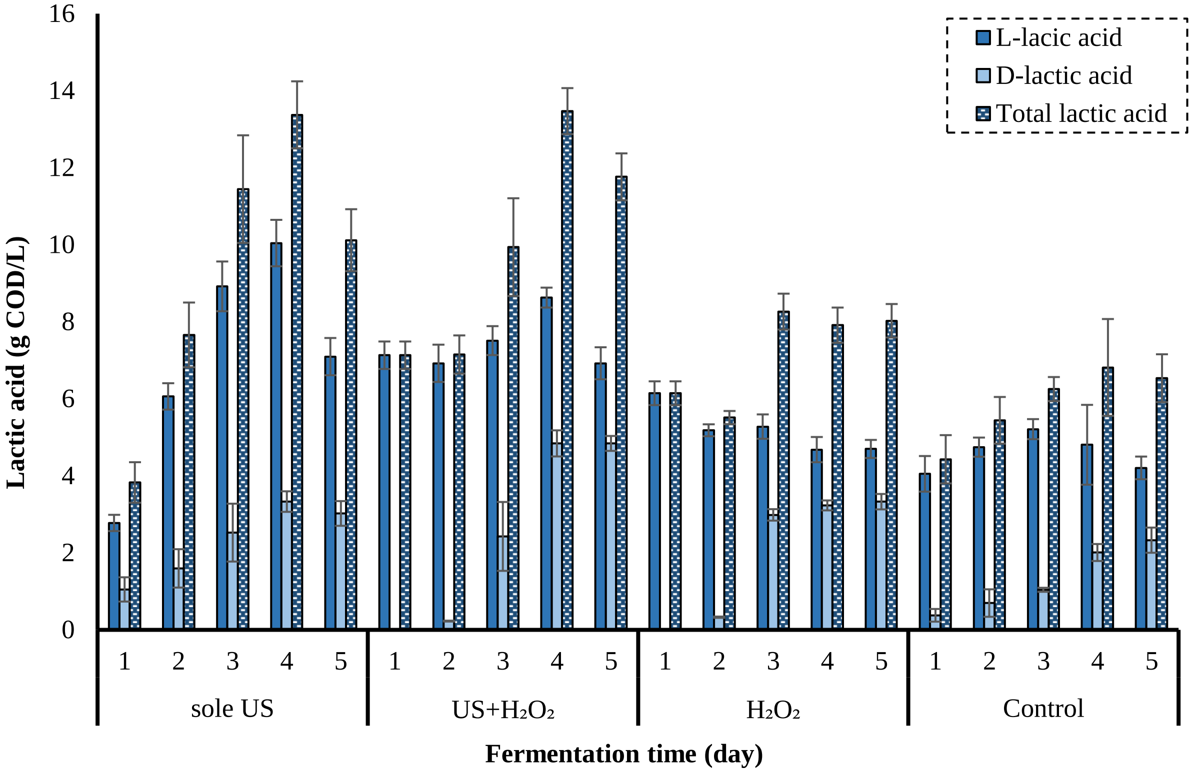


Figure 18. Effect of the combined application of the US with H_2O_2 in the pre-treatment stage on the amount of lactic acid (*sono*-LF, power density: 400 W/L, rice concentration: 5% w/v, [H_2O_2]: 500 ppm, pre-treatment duration: 30 minutes) [204].

The highest concentration of L-lactic acid, reaching 10 g COD/L, was obtained from the feedstock pre-treated by the sole US, while a peak of 8.6 g COD/L was achieved from the feedstock pre-treated by the US combined with H_2O_2. This decrease in L-lactic acid amount obtained from the feedstock pre-treated by the combined application of the US and H_2O_2 as pre-treatment could be attributed to the reduced presence of *Limosilactobacillus* species, a microorganism responsible for L-lactic acid production, in this feedstock. The properties of the feedstock pre-treated by this combined process might have altered the microbial composition, leading to a lower percentage of

Limosilactobacillus species, and consequently, a decrease in L-lactic acid production. On the other hand, when the US was combined with H_2O_2 in the pre-treatment stage, a noticeable increase in D-lactic acid produced through the FP was observed, with the highest concentration reached 4.8 g COD/L, compared to only 3.3 g COD/L D-lactic acid produced from the feedstock pre-treated by the sole US. This rise in D-lactic acid production could be attributed to a higher abundance of *Lactobacillus* species, a bacterial species that can produce D-lactic acid [223]. In other words, while the pre-treatment by the combined application of the US and H_2O_2 subsequently had a negative impact on L-lactic acid production, it enhanced the production of D-lactic acid, likely due to a shift in microbial dynamics favoring *Lactobacillus* species over *Limosilactobacillus* species. H_2O_2 applied alone resulted in significantly higher peaks of both L-lactic acid (6.1 g COD/L) and D-lactic acid (3.3 g COD/L) compared to the control process.

The total lactic acid produced from the feedstocks which were pre-treated by the sole US and the US combined with H_2O_2 were almost identical.

Additionally, it was observed that feedstocks pre-treated by H_2O_2 resulted in faster L-lactic acid production. Specifically, on the first day of the FPs, the amount of L-lactic acid produced from feedstocks pre-treated by either H_2O_2 alone or the combination of the US and H_2O_2 was higher compared to those pre-treated by the sole US or the control process. **Figure 19** provides a representation of the effect of H_2O_2 on the production of VFAs.

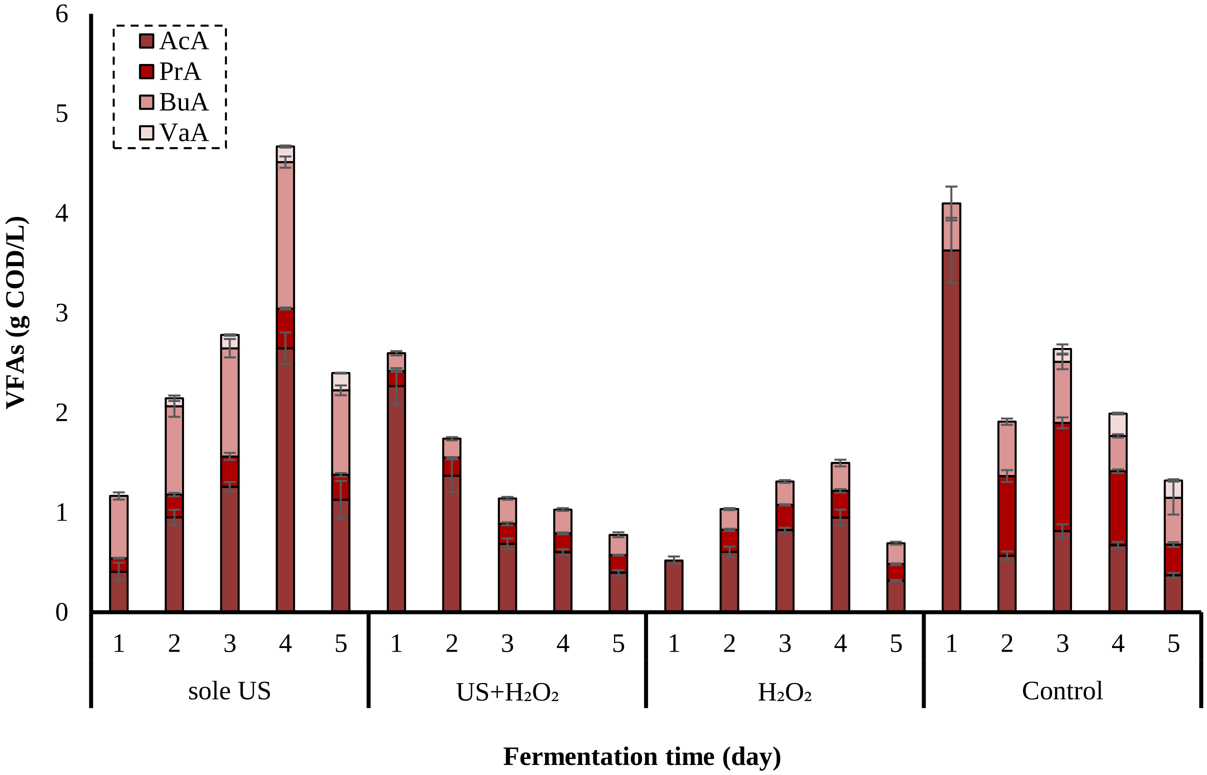


Figure 19. Effect of the combined application of the US with H_2O_2 in the pre-treatment stage on the amount of VFAs (*sono*-LF, power density: 400 W/L, rice concentration: 5% w/v, [H_2O_2]: 500 ppm, pre-treatment duration: 30 minutes) [204].

When the feedstock was pre-treated by the US combined with H_2O_2, there was a noticeable decrease in the production of VFAs, and the peak of VFAs concentration was 2.6 g COD/L, occurring on the first day of the FP. When only H_2O_2 was used, the peak of VFAs concentration reached 1.5 g COD/L, occurring on the fourth day. Again, AcA was identified as the predominant VFAs produced in the presence of H_2O_2.

The utilization of H_2O_2 in the pre-treatment stage appeared to have a detrimental effect on certain microorganisms, particularly those responsible for producing VaA. Furthermore, the pre-treatment of feedstocks by the combined application of the US and H_2O_2 might have produced compounds which hindered the microorganisms that typically generate BuA.

For further experiments, rice as a model food waste was substituted with actual food waste to better assess the impact of acoustic cavitation on the pre-treatment of actual food waste and to obtain more realistic and applicable results. Additionally, both anaerobic and aerobic sludge were employed to produce lactic acid and VFAs, allowing for a comparative analysis of their effectiveness. In these experiments, two key parameters were investigated: the duration of ultrasonic pre-treatment and the concentration of feedstocks in the ultrasonic reactor. The results related to these parameters are discussed in detail in the following paragraphs.

4.4. Effect of ultrasonic pre-treatment duration

The reduction in particle size generally occurs rapidly, with the most significant decrease taking place within the initial 15 minutes of ultrasonic treatment [200]. However, food waste with a high lignin content, such as that made of fruits and vegetables, requires a longer duration for effective solubilization of organic matter. This is due to the complex structure of lignocellulosic materials, which resists immediate breakdown and requires prolonged exposure to cavitation forces for optimal disintegration [201].

The US was employed in durations of 10 minutes, 20 minutes, and 30 minutes, and the effect on sCOD, lactic acid, and VFAs enhancement was investigated. The effect of the ultrasonic durations on sCOD enhancement is observed in **Table 1**.

Table 1. Effect of pre-treatment duration on sCOD (*sono*-LF, power density: 400 W/L, food waste concentration of 5% w/v).

Pre-treatment Process	***Pre-treatment duration (minutes)***	**Increase in sCOD%**	**Total nitrogen (g/L)**
US	***10***	275	0.690
US	***20***	441	0.722
US	***30***	492	0.795

A prolonged duration of the ultrasonic pre-treatment led to a steady rise in sCOD levels. Extending the ultrasonic duration during the pre-treatment stage from 10 to 20 minutes significantly increased sCOD enhancement from 275% to 441%. However, further increasing the duration to 30 minutes resulted in a smaller difference, with sCOD enhancement reaching 492%. This trend suggests that extending the pre-treatment duration can enhance the decomposition of organic matter, improving the solubilization of food waste components.

The observed increase in sCOD of food waste through cavitation can be attributed to the mechanical effects of cavitation, helping break down high-molecular-weight proteins (over 20 kDa) by disrupting their structure, leading to the formation of amino acids and peptides [178]. This process effectively weakens the hydrogen bonds that stabilize large protein polymers, resulting in the production of smaller, more soluble protein molecules [179].

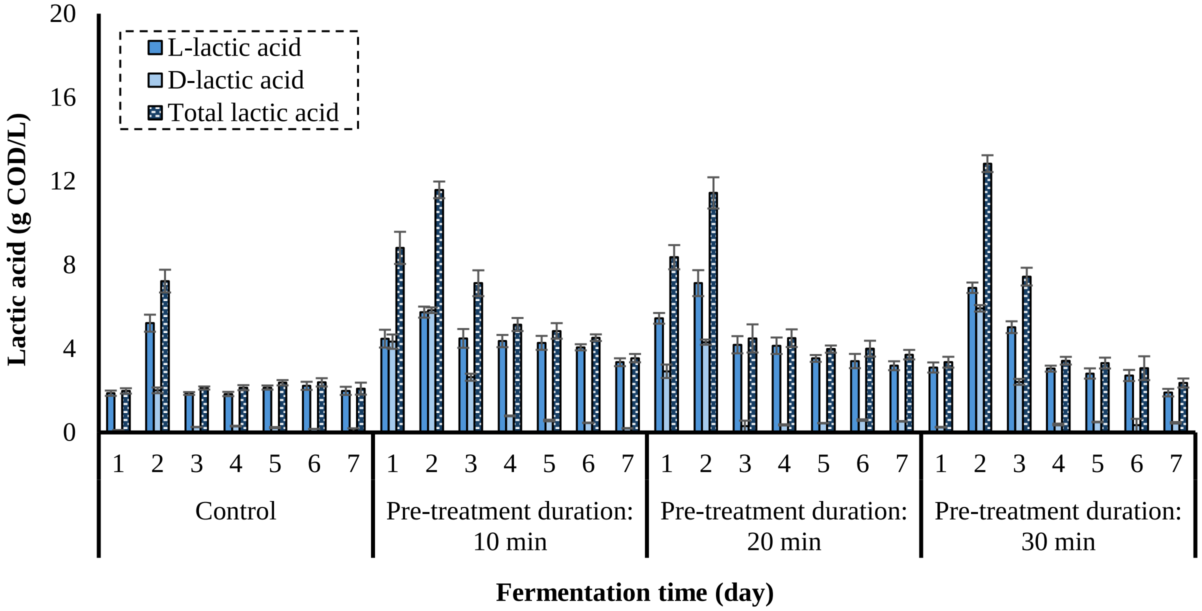
Additionally, in the case of the lignocellulosic components of food waste, such as fruits and vegetables, cavitation pre-treatment induces structural modifications. The intense mechanical action creates surface disarray, reducing particle size and lowering the degree of polymerization [175]. By breaking down the cell wall structure, this technique facilitates the release of cellulose molecules into the solution, improving the solubility of organic matter [5]. Beyond these mechanical effects, the formation of $^{\bullet}$OH within high-energy cavitation hot spots contributes to the breakdown of intermolecular hydrogen bonds and facilitates the oxidative degradation of lignin components, further enhancing the process efficiency [176].

The determination of the optimal pre-treatment duration is important, as excessive pre-treatment can lead to re-flocculation, causing an increase in particle size rather than further reduction. This phenomenon is attributed to the release of intracellular polymers due to extensive cell lysis, which can act as binding agents, effectively reversing the initial particle size reduction [225]. However, in this study, such re-flocculation was not observed.

In addition to process efficiency, the determination of the suitable pre-treatment duration is also essential from an economic and energy consumption perspective. Extended pre-treatment duration may lead to unnecessary energy consumption without substantial additional benefits, making optimization an important factor in achieving both effective and cost-efficient cavitation pre-treatment.

The effect of ultrasonic pre-treatment duration on the production of lactic acid through the subsequent FPs by anaerobic sludge and aerobic sludge is demonstrated in **Figures 20 A** and **B**, respectively.

(A) Anaerobic sludge



(B) Aerobic sludge

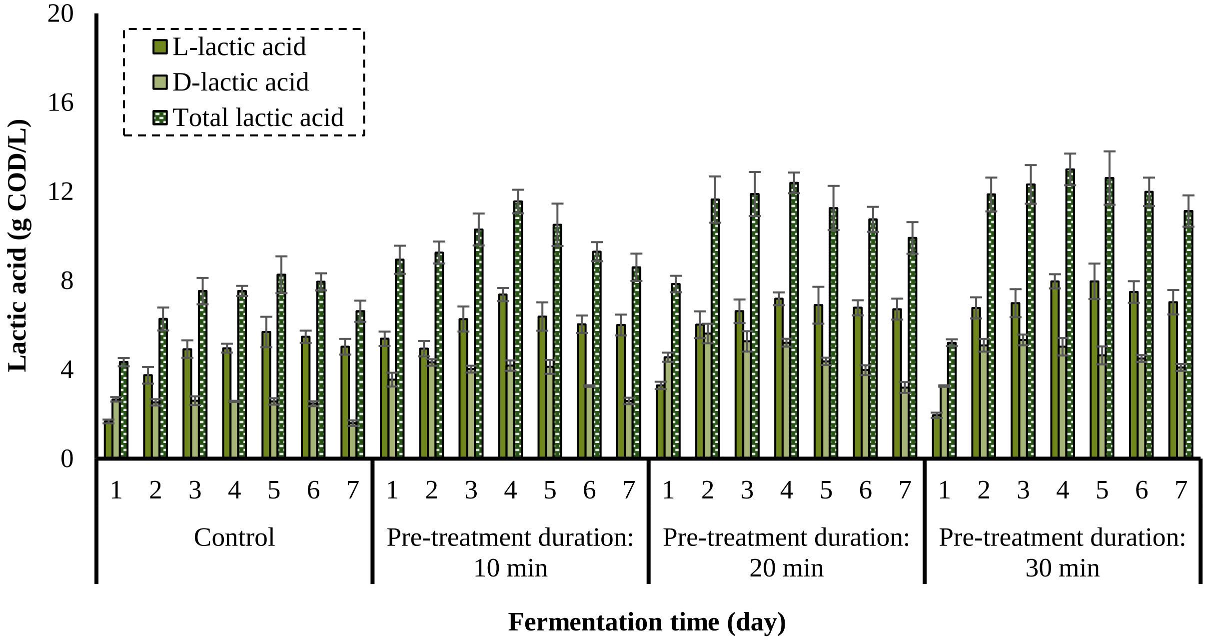


Figure 20. Effect of pre-treatment duration on the amount of lactic acid produced through the FPs by A) anaerobic sludge, B) aerobic sludge (*sono*-LF, power density: 400 W/L, food waste concentration: 5% w/v).

An increase in the pre-treatment duration was found to slightly enhance the total lactic acid production.

In the case of the FPs by anaerobic sludge, using the US (*sono*-LF, power density: 400 W/L, food waste concentration: 5% w/v), an increase in pre-treatment duration from 10 minutes to 20 minutes enhanced the peaks of L-lactic acid from 5.7 g COD/L to 7.1 g COD/L, both obtained on the second day of the FPs. In contrast, the peak concentration of D-lactic acid from feedstock pre-treated for 10 minutes was 5.8 g COD/L, and extending the ultrasonic duration to 30 minutes resulted in only a negligible increase. In the control process, the peaks of L-lactic acid and D-lactic acid achieved through the FPs by the anaerobic sludge were 5.2 g COD/L and 2.0 g COD/ L, respectively, both observed on the second day of the FPs.

In the case of the FPs by aerobic sludge, under the same operating conditions of the US (*sono*-LF, 400 W/L, 5% w/v), an increase in pre-treatment duration from 10 minutes to 30 minutes, increased the peak of L-lactic acid from 7.4 g COD/L to 8.0 g COD/L (both observed on the fourth day of the FPs). An increase in ultrasonic duration from 10 minutes to 20 minutes enhanced the peak of D-lactic acid from 4.3 g COD/L to 5.6 g COD/L (both observed on the second day of the FPs). In the control process, the peaks of L-lactic acid and D-lactic acid were 5.7 g COD/L (occurring on the fifth day) and 2.7 g COD/L (occurring on the first day), respectively.

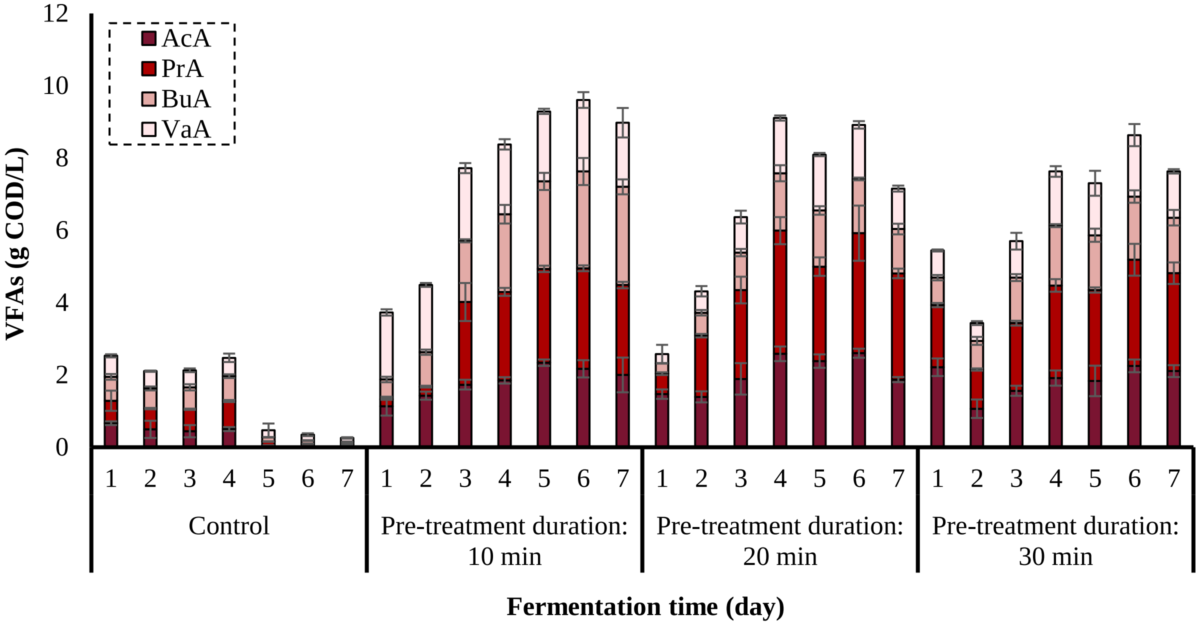
In general, aerobic sludge consistently resulted in greater lactic acid accumulation compared to anaerobic sludge. In the case of anaerobic sludge, lactic acid concentrations declined after reaching the maximum values, likely due to microbial interactions that utilize lactic acid as an intermediate metabolite. Within anaerobic microbial communities, particularly those dominated by obligate anaerobes and methanogens, lactic acid is rapidly converted into other metabolic byproducts, preventing its prolonged accumulation in the system. The interplay between these microbial populations leads to dynamic shifts in lactic acid levels, impacting the overall efficiency of its production.

The rise in lactic acid production with the extended pre-treatment duration can be attributed to the improved solubility of organic matter. A longer duration of US facilitates the breakdown of complex cellulose structures into simpler, more readily available components [226]. Additionally, the increased surface area of degraded organic matter allows for better enzymatic hydrolysis, further accelerating FPs.

The effect of ultrasonic duration on the production of VFAs by anaerobic sludge and aerobic sludge is illustrated in **Figures 21 A** and **B**, respectively.

Pre-treatment of 5% w/v food waste in 10 minutes resulted in the highest VFAs production through the FPs. The maximum total VFAs values were 9.9 g COD/L, happening on the fifth day of the FP by the aerobic sludge and 9.6 g COD/L, happening on the sixth day of the FP by the anaerobic sludge, indicating that a 10-minute pre-treatment duration is optimal for maximizing VFAs yields. It can be hypothesized that extending the pre-treatment duration to 20 and 30 minutes may have led to the formation of species acting as inhibitors, such as furfurals and phenolic compounds. These byproducts can negatively impact acidogenic bacterial activity, initially promoting lactic acid accumulation while suppressing the subsequent production of VFAs. The presence of these inhibitory substances can create unfavorable conditions for microbial communities, slowing down metabolic pathways that contribute to VFAs formation. In contrast, a milder pre-treatment allows for a more controlled breakdown of organic matter. In the control process, the peaks of VFAs achieved by the aerobic sludge and anaerobic sludge were 5.8 g COD/L (observed on the third day) and 2.5 g COD/L (observed on the first day), respectively.

(A) Anaerobic sludge



(B) Aerobic sludge

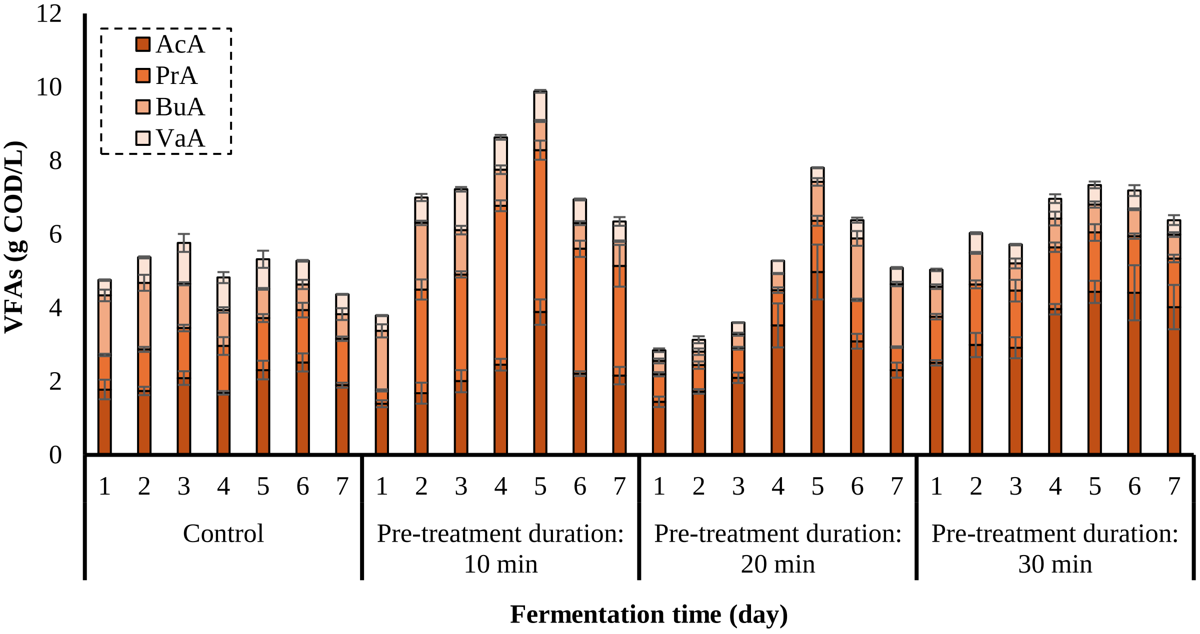


Figure 21. Effect of pre-treatment duration on the amount of VFAs produced through the FPs by A) anaerobic sludge, B) aerobic sludge (*sono*-LF, power density: 400 W/L, food waste concentration: 5% w/v).

4.5. Effect of feedstock concentration in the ultrasonic reactor

Feedstock concentration in cavitation reactors in the pre-treatment stage is typically selected below 10% w/v [5]. Hence, food waste concentrations of 3%, 5%, and 7% w/v were pre-treated in *sono*-LF at a power density of 400 W/L for 30 minutes to investigate the effect of this parameter on sCOD, and the subsequent production of lactic acid, and VFAs. The results reported in **Table 2** indicate that increasing food waste concentration improved sCOD, reflecting a greater release of soluble organic compounds. An increase in the food waste concentration during the pre-treatment stage from 3% to 5% w/v led to a significant rise in sCOD, increasing by 213% and 492%, respectively. However, when the food waste concentration was further increased to 7% w/v, the difference in enhanced sCOD was smaller.

Table 2. Effect of food waste concentration in the ultrasonic reactor on sCOD (*sono*-LF, power density: 400 W/L, pre-treatment duration: 30 minutes).

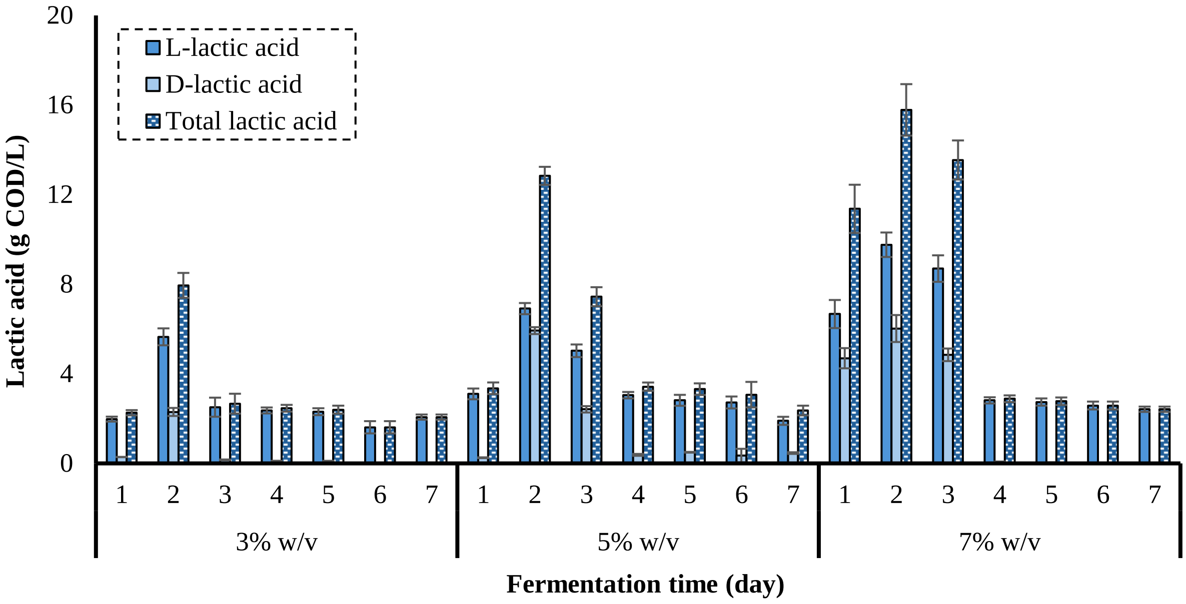
Pre-treatment Process	***Food waste Concentration (% w/v)***	**Increase in sCOD%**	**Total nitrogen (g/L)**
US	***3***	213	0.632
US	***5***	492	0.795
US	***7***	582	0.881

The observed trend can be attributed to the role of food waste particles in promoting cavitation. These particles act as initiation nuclei for bubble formation, reducing the energy required for cavitation to begin. Once cavitation is initiated, the bubbles rapidly expand and collapse, intensifying the overall cavitation effect. This suggests that the presence of food waste particles plays a role in enhancing cavitation efficiency by facilitating bubble generation and amplifying its impact [202].

However, excessive food waste concentration can negatively impact cavitation efficiency due to reduced water availability. Since free water molecules are necessary for bubble formation and growth, an increase in solid content limits their presence, weakening cavitation intensity [7]. Moreover, a denser slurry with a higher proportion of solids resists bubble expansion and collapse, leading to a reduction in the effectiveness of cavitation. The increased viscosity further can dampen pressure fluctuations, suppressing the violent bubble collapses needed to generate strong cavitation forces [203]. Despite these potential limitations, our study did not observe a reduction in cavitation effects under the tested conditions, nevertheless, the difference in sCOD enhancement got smaller.

The effect of food waste concentration on lactic acid production through the FPs by the anaerobic sludge and aerobic sludge is displayed in **Figures 22 A** and **B**, respectively.

(A) Anaerobic sludge



(B) Aerobic sludge

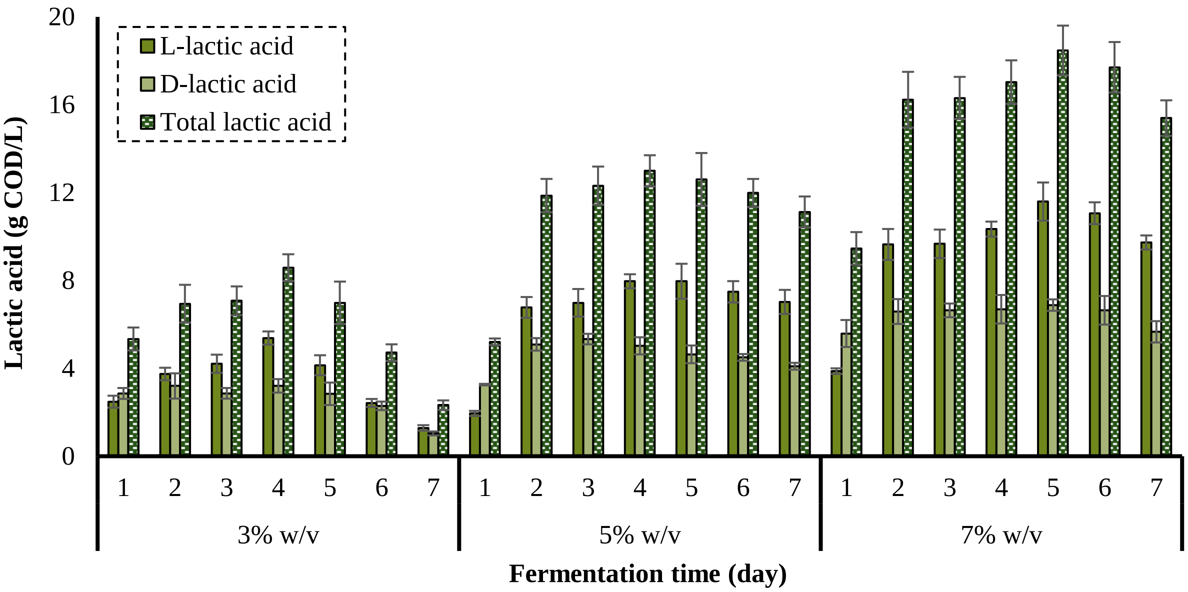


Figure 22. Effect of food waste concentration in the ultrasonic reactor on the amount of lactic acid produced through the FPs by A) anaerobic sludge, B) aerobic sludge (*sono*-LF, power density: 400 W/L, pre-treatment duration: 30 minutes).

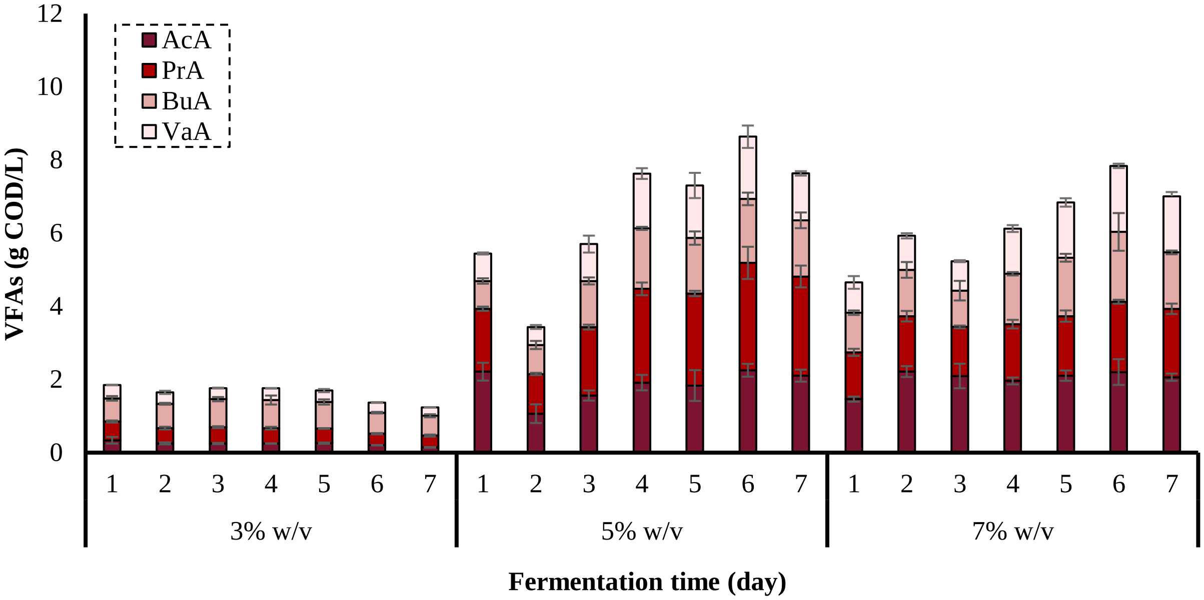
Increasing the food waste concentration from 3% w/v to 7% w/v during the ultrasonic pre-treatment stage resulted in a significant rise in lactic acid concentrations produced through the FPs by both aerobic sludge and anaerobic sludge.

In the case of anaerobic sludge, an increase in food waste concentration in the ultrasonic reactor from 3% w/v to 7% w/v led to an enhancement in L-lactic acid peak from 5.6 g COD/L to 9.8 g COD/L, and D-lactic acid peak from 2.3 g COD/L to 6.0 g COD/L, all observed on the second day of the FPs.

Similarly, through aerobic sludge, an increase in the food waste concentration in the ultrasonic reactor from 3% w/v to 7% w/v increased the peak of L-lactic acid from 5.4 g COD/L (occurring on the fourth day) to 11.6 g COD/L (occurring on the fifth day), while D-lactic acid peak rose from 3.2 g COD/L (occurring on the second day) to 6.9 g COD/L (occurring on the fifth day).

The effect of food waste concentration in the ultrasonic reactor on the production of VFAs by the anaerobic sludge and aerobic sludge is demonstrated in **Figures 23 A** and **B**, respectively.

(A) Anaerobic sludge



(B) Aerobic sludge

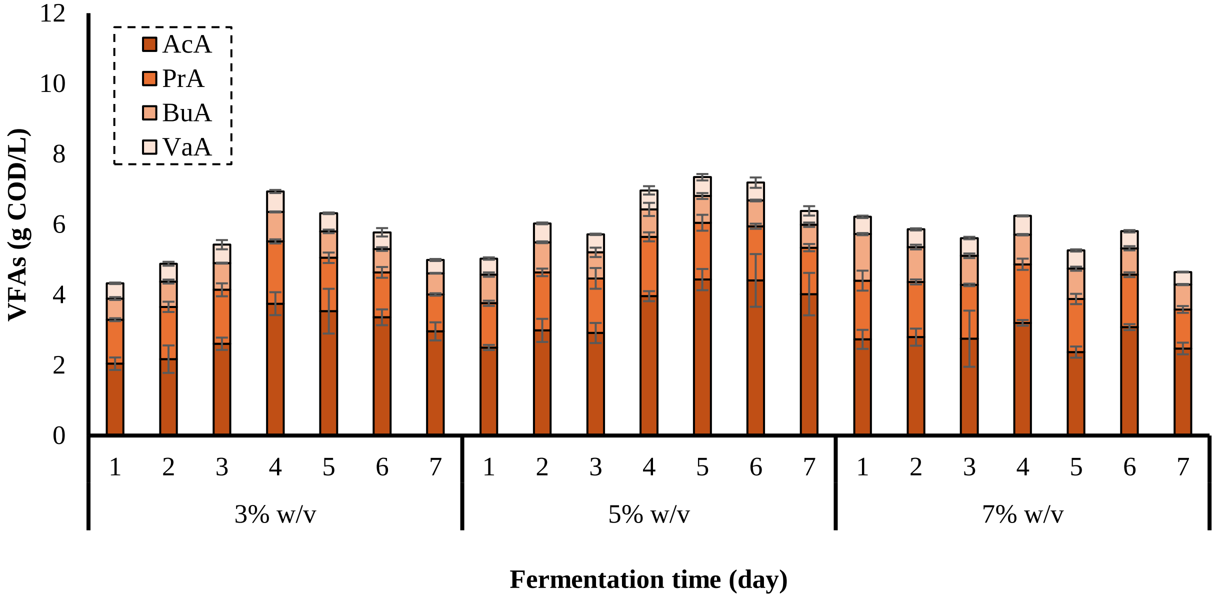


Figure 23. Effect of food waste concentration in the ultrasonic reactor on the amount of VFAs produced through the FPs by A) anaerobic sludge, B) aerobic sludge (*sono*-LF, power density: 400 W/L, pre-treatment duration: 30 minutes).

As observed in **Figure 23 A**, as the feedstock concentration in the ultrasonic reactor increased from 3% w/v to 5% w/v, the peak of total VFAs concentration produced later through the FPs by the anaerobic sludge remarkably rose from 1.8 g COD/L (observed on the first day) to 8.6 g COD/L (observed on the sixth day). Further increase in food waste concentration to 7% w/v led to a decrease in total VFAs to 7.8 g COD/L (observed on the sixth day).

In the case of the FPs by the aerobic sludge, an increase in the food waste concentration in the ultrasonic reactor from 3% w/v to 5% w/v slightly increased the total VFAs peak from 6.9 g COD/L (observed on the fourth day) to 7.3 g COD/L (observed on the fifth day) and an increase in food waste concentration to 7% w/v reduced total VFAs to 6.2 g COD/L (observed on the first day).

The observed decrease in the concentration of total VFAs can be attributed to the excessive lactic acid produced from the fermentation of feedstock pre-treated at 7% w/v concentration in the ultrasonic reactor, which resulted in a significant drop in pH and might have suppressed microbial activity and reduced overall VFAs yields.

4.6. Economic evaluation

The effectiveness of a pre-treatment method is mainly assessed by its ability to maximize the production of valuable products while minimizing waste generation. Achieving an optimal balance between these factors allows for more efficient resource employment and better waste management, making the process both economically feasible and environmentally sustainable [227]. Pre-treatment techniques have an important role in improving feedstock availability and boosting overall process efficiency. However, these benefits often come with the downside of higher operational costs. Specifically, using US as a pre-treatment step in traditional fermentation systems increases energy consumption. Therefore, an economic analysis is important to determine if the advantages of this approach justify the added costs and assess its viability for large-scale use.

In Poland, the electricity cost for industrial operations, including manufacturing, in 2025 is set at 83.74 EUR per MWh [228]. In Europe, H_2O_2 costs about 0.48 EUR per kilogram for industrial use [229]. The average market price for lactic acid is about 4 EUR per kilogram [230]. However, fluctuations in supply chains and production processes can influence lactic acid pricing trends across Europe.

Table 3 presents a summary of the economic analysis conducted for lactic acid production, which is the primary product of the FPs investigated in this study. Firstly, this evaluation was performed for rice as a model food waste. It examined the net savings achieved by the application of the US combined with H_2O_2, as well as the sole US at power densities of 400 W/L and 300 W/L, as pre-treatment techniques followed by the FPs, in comparison to the control processes (conventional FPs). The highest total lactic acid concentration achieved through the control process was 6.4 g/L observed on the fourth day of the FP. Applying the low-frequency US at power densities of 400 W/L and 300 W/L for 30 minutes (using 0.2 kWh and 0.15 kWh of energy, respectively) increased total lactic acid production to 12.5 g/L (an improvement of 6.1 g/L) and 9.5 g/L (an improvement of 3.1 g/L), respectively. Moreover, combining 500 ppm H_2O_2 with the low-frequency US at 400 W/L for 30 minutes for the pre-treatment of the rice resulted in 12.6 g/L total lactic acid, marking a 6.2 g/L increase compared to the control process.

Additionally, the assessment was done for the actual food waste pre-treated in different ultrasonic durations. In the case of the FPs by the anaerobic sludge, the peak of total lactic acid obtained from the control process was 6.7 g/L, recorded on the second day. The application of the low-frequency

US at power densities of 400 W/L for 10 minutes, 20 minutes, and 30 minutes led to the lactic acid production peaks of 10.8 g/L (4.1 g/L increase), 10.6 g/L (3.9 g/L increase), and 12.0 g/L (5.3 g/L increase), respectively, all observed on the second day of the FPs.

In the case of the FPs by the aerobic sludge, the total lactic acid peak achieved from the control process was 7.8 g/L, observed on the fourth day of the FP. Applying the low-frequency US at a power density of 400 W/L for 10 minutes, 20 minutes, and 30 minutes led to the lactic acid peaks of 10.7 g/L (2.9 g/L increase), 11.6 g/L (3.8 g/L increase), and 12.1 g/L (4.3 g/L increase), respectively, all observed on the fourth day of the FP.

Table 3. Economic assessment of ultrasonic pre-treatment compared to the control process per ton of pre-treated feedstock (*sono*-LF, feedstock concentration: 5% w/v [H_2O_2]: 500 ppm).

Feedstock	Power density (W/L)	Sludge type	Time (min)	H_2O_2 (€)	Energy input (kWh)	Energy input (€)	Δ lactic acid (kg)	Δ lactic acid (€)	Net saving (€)
Rice	300	Anaerobic	30		3000	251	62	248	-3
Rice	400	Anaerobic	30		4000	335	122	488	153
Rice	400	Anaerobic	30	4.8	4000	335	124	496	156
Food waste	400	Anaerobic	10		1333	112	82	328	***216***
Food waste	400	Anaerobic	20		2667	223	78	312	89
Food waste	400	Anaerobic	30		4000	335	106	424	89
Food waste	400	Aerobic	10		1333	112	58	232	120
Food waste	400	Aerobic	20		2667	223	76	304	81
Food waste	400	Aerobic	30		4000	335	86	344	9

Net saving compared to control process = Increase in total lactic acid (€) – Energy input (€) – additives (€).

The data in the table were derived from calculations based on one ton of pre-treated feedstock.

In the case of rice as a model food waste, this evaluation suggests that the application of the US (*sono*-LF, 400 W/L) combined with H_2O_2 at a concentration of 500 ppm is an economically viable method, yielding a net saving of €156 per ton of pre-treated feedstock. Using the sole US, under the same condition, generated a net saving of €153 per ton of the pre-treated feedstock, however, the US at a power density of 300 led to a negative financial outcome (€-3).

Actual food waste led to higher profits. The results of this analysis show that all examined ultrasonic treatments were economically viable. Among the different pre-treatment methods, the most cost-effective was the 10-minute ultrasonic treatment, followed by the FP via anaerobic sludge, yielding net savings of €216 per ton of pre-treated feedstock, respectively. The processes could also increase profit through the production of valuable byproducts like VFAs, which have potential applications in various industries. However, these additional benefits were not included in the current economic analysis.

While the addition of a pre-treatment system results in higher initial investment costs, its effectiveness in reducing solid content can lead to notable savings in subsequent processes such as dewatering, transportation, and landfill disposal. However, these factors have not been considered in the present analysis.

5. Conclusions

The global rise in food waste has become a major problem in recent years. This growing amount of food waste can lead to significant economic losses and serious environmental impacts, including increased greenhouse gas emissions and wasted natural resources. Converting waste into valuable products is an effective way to reduce its amount and support the principles of a circular economy. One efficient method for recycling food waste involves its use as a feedstock in biotechnological processes like anaerobic digestion and FPs. In these processes, hydrolysis is a key initial step, often acting as the rate-limiting stage due to the structural complexity of the organic material. To overcome this challenge, pre-treatment methods are applied to modify the structure of feedstocks, enhancing hydrolysis and improving overall processes.

Acoustic cavitation has a significant potential to be employed as a pre-treatment technique in FPs. Cavitation is a dynamic process involving the rapid formation, growth, and collapse of microbubbles in a liquid, which can happen by localized pressure changes through US. The collapse of these bubbles produces intense mechanical effects, including high-speed microjets, shock waves, and shear stress. These extreme conditions result in rapid energy transfer, breaking molecular bonds and decomposing organic materials. The compression of gas and vapor inside the bubbles creates local high temperatures and pressures, leading to the pyrolysis of water molecules and the generation of reactive species like $^{\bullet}OH$, $HOO^{\bullet}$, and H_2O_2, regarded as the chemical effect of cavitation. These reactive species can initiate chain reactions that break down complex molecules into simpler compounds. By disrupting cell structures and promoting the release of intracellular materials, cavitation enhances the solubilization of organic matters. As a result, these more accessible compounds become more easily metabolized by the microbial communities responsible for FPs, facilitating a more efficient and accelerated bioconversion process.

Lactic acid is a valuable compound with a wide range of applications. A mix of lactic acid isomers, primarily L-lactic acid and D-lactic acid can be produced through FPs. During FPs, lactic acid bacteria utilize sugars in anaerobic conditions to generate lactic acid, but slow hydrolysis as the first step of FPs can restrict the production of this compound. The application of US, inducing acoustic cavitation, for intensifying the production of lactic acid from the fermentation of food waste has been rarely reported in the literature. Therefore, this study examines the utilization of US to enhance the production of this compound as well as VFAs (as a by-product). Using rice (model food waste) as well as actual food waste as feedstocks, the research evaluates the effects of factors such as ultrasonic frequency, power density, pre-treatment duration, feedstock concentration in the ultrasonic reactors, and the combination of US with HCl, NaOH, or H_2O_2 on sCOD and the produced amounts of lactic acid and VFAs.

At the same power density of 300 W/L, *sono*-LF (operated at a frequency of 24 kHz) exhibited significantly better performance in increasing the sCOD of model food waste (rice) compared to *sono*-HF (operated at a frequency of 120 kHz). The generation of $^{\bullet}OH$ in both systems was verified using *p*CBA as a selective probe and potassium iodide as a supporting indicator. Among the studied reactors, the *sono*-LF generated a higher concentration of $^{\bullet}OH$. Low frequencies can also lead to stronger mechanical effects of cavitation. Thus, the *sono*-LF was used in the rest of the experiments.

Further research focused on the effect of power density on sCOD and the subsequent production of lactic acid and VFAs through the FPs of rice by anaerobic sludge. Increasing the power density from 100 W/L to 400 W/L resulted in a continuous rise in sCOD and L-lactic acid produced from the fermentation of rice. Pre-treatment in *sono*-LF at a power density of 400 W/L and a rice concentration 5% w/v for 30 minutes led to 555% increase in sCOD, and later during the FP, 92%

increase in L-lactic acid, 43% increase in D-lactic acid, and 15% increase in total VFAs compared to the control process (the FP without pre-treatment).

The combination of the US (*sono*-LF, 400 W/L, 5% w/v) with acid (HCl) and alkali (NaOH) during the pre-treatment led to significant increases in the sCOD of feedstock (rice), 750% and 625%, respectively, after 30 minutes of pre-treatment. Rice pre-treated by the US and acid resulted in 100% increase in D-lactic acid and 29% increase in total VFAs. The combination of the US and alkali in the pre-treatment stage also resulted in 104% rise in D-lactic production. Additionally, the US combined with H_2O_2 raised sCOD by 615% and D-lactic acid produced through the FP by 109%.

For actual food waste, using the US (*sono*-LF, 400 W/L, 5% w/v), extending the ultrasonic pre-treatment duration from 10 to 30 minutes led to an increase in enhanced sCOD from 275% to 492%. The FPs of actual food waste were performed by aerobic sludge as well as anaerobic sludge. The maximum amount of L-lactic acid was achieved in the FPs by the aerobic sludge, and pre-treatment for 30 minutes made 40% increase in the L-lactic acid amount produced through the FPs by this type of sludge. The highest enhancement in the production of D-lactic acid and VFAs made by the US was observed in the fermentation by anaerobic sludge. A prolonged pre-treatment duration increased L-lactic acid, but the increase in D-lactic acid was negligible. The feedstock pre-treated for 10 minutes resulted in 190% increase in D-lactic acid and 284% increase in total VFAs, obtained through the FP by the anaerobic sludge.

Furthermore, increasing the food waste concentration in the ultrasonic reactor (*sono*-LF, 400 W/L) from 3% to 7% w/v significantly raised the enhanced sCOD from 213% to 582% after the pre-treatment for 30 minutes. It also led to a continuous increase in lactic acid production, and the maximum amounts of lactic acid were obtained from the feedstocks pre-treated at a concentration of 7% w/v, leading to 88% increase in L-lactic acid and 200% increase in D-lactic acid produced through the FPs by the anaerobic sludge, as well as 103% increase in L-lactic acid and 156% increase in D-lactic acid produced through the FPs by the aerobic sludge.

For rice, as a model food waste, combining the US (*sono*-LF, 400 W/L) with 500 ppm H_2O_2 was found to be economically viable, yielding a net saving of €156 per ton of pre-treated feedstock. The sole US at the same operating condition resulted in slightly lower savings of €153 per ton of the feedstock. For actual food waste, the most cost-effective approach was a 10-minute ultrasonic pre-treatment (*sono*-LF, 400 W/L) followed by the FP with the anaerobic sludge, generating savings of €216 per ton of the pre-treated feedstock, respectively.

This research shows a significant potential to improve waste management by offering a practical pre-treatment method, which enhances the conversion of food waste into high-value products in FPs. This approach supports environmental objectives by reducing waste and lowering emissions, while also enhancing economic viability through an efficient conversion of waste. Future work could focus on optimizing the use of additives, including acid, alkali, and oxidants, further enhancing the process. Moreover, the application of cavitation-based pre-treatment for the modification of different types of food waste could reveal its wider applicability and promote sustainable bioresource utilization. Additionally, efforts should be directed towards scaling up the process, including the design and implementation of continuous-flow cavitation systems for industrial use. Overall, this study provides a strong foundation for adopting advanced pre-treatment techniques in bioengineering, with the potential to impact future research on renewable energy production and bioproduct synthesis.

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List of scientific achievements

Publications

- ***Z. Askarniya***, S. Baradaran, S.H. Sonawane, G. Boczkaj, A comparative study on the decolorization of Tartrazine, Ponceau 4R, and Coomassie Brilliant Blue using persulfate and hydrogen peroxide based Advanced Oxidation Processes combined with Hydrodynamic Cavitation, Chemical Engineering and Processing - Process Intensification 181 (2022) 109160, IF: 3.9,

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- ***Z. Askarniya***, M.P. Rayaroth, X. Sun, Z. Wang, G. Boczkaj, Degradation of bisphenol S - a contaminant of emerging concern - by synergistic ozone and percarbonate based AOP, Water Resources and Industry 29 (2023) 100208, IF: 7.5, DOI: 10.1016/j.wri.2023.100208.

- ***Z. Askarniya***, X. Sun, Z. Wang, G. Boczkaj, Cavitation-based technologies for pretreatment and processing of food wastes: Major applications and mechanisms - A review, Chemical Engineering Journal 454 (2023) 140388, IF: 13.2, DOI: 10.1016/j.cej.2022.140388.

- ***Z. Askarniya***, L. Kong, C. Wang, S.H. Sonawane, J. Mąkinia, G. Boczkaj, "Tuning" of food wastes bioavailability as feedstock for bio-conversion processes by acoustic cavitation and SPC, SPS, or H_2O_2 as external oxidants, Chemical Engineering and Processing - Process Intensification 195 (2024) 109626, IF: 3.9,

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- ***Z. Askarniya***, A.K. Khanzada, S. Ciesielski, Z. Wei, J. Mąkinia, G. Boczkaj, Influence of Acoustic Cavitation and Its Combination with H_2O_2, Acid, and Alkali as a Pre-treatment Technique on Lactic Acid Production from Rice as a Model Food Waste, Ultrasonics Sonochemistry 120 (2025) 107422, IF: 9.7,

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- ***Z. Askarniya***, Ł. Cichocki, S. Makowiec, C. Wang, G. Boczkaj, Degradation of dicamba - A persistent herbicide - By combined application of formic acid and UV as an advanced reduction process, Journal of Hazardous Materials 492 (2025) 137984, IF: 11.3, DOI: 10.1016/j.jhazmat.2025.137984.

- E.D. Dominguez, A.K. Khanzada, ***Z. Askarniya,*** S. Ciesielski, X. Xu, G. Boczkaj, X. Li, F.J. Fernández, J. Mąkinia, J.L. García-Morales, Enhancing lactic acid production from food waste through cavitation pretreatment and biocatalyst amendment – a comparative study (Submitted in Journal of Environmental Chemical Engineering).

Book chapter

- ***Z. Askarniya***, X. Sun, C. Wang, G. Boczkaj, Sustainable Management of Food Wastes Through Cavitation-Assisted Conversion into Value-Added Products, In book: Solid Waste Management, Publisher: Springer,

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Oral presentations

- **Z. Askarniya**, G. Boczkaj, Cavitation-assisted technologies for the bioconversion of food wastes into value-added products, 11th IWA International Symposium on Waste

Management Problems in Agro-Industry, Gdansk University of Technology, Gdansk, Poland, October 26-28, 2022.

- **Z. Askarniya**, G. Boczkaj, Advanced treatment processes for the removal of persistent compound, International Conference on Wider-Uptake of Water Resource Recovery from Wastewater Treatment (ICWRR2024), University of Palermo, Palermo, Italy, June 18-21, 2024.

Poster presentation

- **Z. Askarniya**, G. Boczkaj, The application of advanced reduction processes for the removal of halogenated compounds, Water-Excellent Science and Industrial Development Conference, Poznan University of Technology, Poznan, Poland, 15 - 17 November 2023.
- **Z. Askarniya**, G. Boczkaj, Cavitation-assisted technologies for the pretreatment of food wastes as a substrate of anaerobic digestion, 7th International Congress on Water, Waste, and Energy Management (WWEM-24), Lisbon, Portugal, 24-26 July 2024.

Internship

- 3 months (from 1.08.2024 to 31.10.2024) NAWA internships, Support for international mobility for the best doctoral students, Denmark, Aarhus University of Technology, Department of Biological and Chemical Engineering - Environmental Engineering, Host professor: Dr. Zongsu Wei.