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Recent advances in biotechnological valorization of agro-food wastes (AFW): Optimizing integrated approaches for sustainable biorefinery and circular bioeconomy

Timothy Prince Chidike Ezeorba^{a,b,c,*}, Emmanuel Sunday Okeke^{b,d,e,*}, Mida Habila Mayel^f, Charles Ogugua Nwuche^g, Tobechukwu Christian Ezike^{b,c,*}

^a Department of Environmental Health and Risk Management, College of Life and Environmental Sciences, University of Birmingham Edgbaston, Birmingham B15 2TT, United Kingdom

^b Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State 410001, Nigeria

^c Department of Genetics and Biotechnology, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State 410001, Nigeria

^d Natural Science Unit, School of General Studies, University of Nigeria, Nsukka, 410001, Enugu State, Nigeria

^e Institute of Environmental Health and Ecological Security, School of the Environment and Safety, Jiangsu University, 301 Xuefu Rd., 212013 Zhenjiang, Jiangsu, China

^f Enzymology and Protein Chemistry Research Unit, Department of Biochemistry, Faculty of Pure and Applied Sciences, Federal University Wukari, Taraba State, Nigeria

^g Department of Microbiology, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State 410001, Nigeria

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ABSTRACT

Achieving renewable clean energy to meet increasing global demand and counter overreliance on depleting unsustainable sources has recently drawn significant research interest. Similarly, to attain the sustainable development goals (SDGs) “zero hunger” agenda, massive agricultural/food productions are embarked on, leading to increased agro-food waste (AFW) generation with enormous handling costs to evade its contribution to environmental pollution. The advent of biorefineries has fostered a healthy balance for tackling challenges from AFW and unsustainable energy – thereby promoting circular bioeconomy (CBE). Integrating several emerging microbial/enzymatic bioconversion processes has facilitated the overall increase in process efficiency. This review, therefore, provides extensive information on the ecological and environmental impacts of AFW, as well as its biorefinery processes for a circular bioeconomy and environmental sustainability. We also critically reviewed advances in integrated bioconversion processes and microbial/enzymatic engineering for AFW valorization. Finally, limitations and prospects for real-life application of these recent approaches were suggested.

1. Introduction

The constant growing global population is driving increased demand for food and essential commodities, leading to a significant upsurge in the production of waste biomass. This rampant production not only poses environmental challenges but also escalates the costs associated with waste disposal. Agriculture, as one of the most widespread human activities, generates surplus crop residues year-round, a substantial portion of which often goes to waste (Kosre et al., 2021; Prasad et al., 2020). With the advent of modern civilization, agriculture has undergone rapid commercialization, with production and consumption processes becoming highly mechanized and technologically advanced. The globalization of agricultural markets has further intensified competition within the sector. Efficient management of Agro-Food Wastes (AFW) is

imperative to mitigate environmental and health concerns (Chen et al., 2020; Klai et al., 2021). In the pursuit of sustainable practices and resource optimization, the biotechnological valorization of AFW has emerged as a pivotal focus for both researchers and industries.

A significant portion of biomass waste in the agricultural sector originates from post-harvest and post-processing activities (Sadh et al., 2018a). To effectively navigate challenges and ensure connectivity to domestic and international markets, as well as to promote environmental conservation, resource management, and food security, modern agriculture must address various obstacles. Annually, approximately 998 million tons of agricultural wastes are generated globally (Awogbemi and Von Kallon, 2022). The management of agricultural residues poses a complex challenge that impacts both the economy and daily operations of the agricultural and agro-industrial sectors. These residues

* Corresponding authors at: Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State 410001, Nigeria.

E-mail addresses: timothy.ezeorba@unn.edu.ng (T.P.C. Ezeorba), emmanuel.okeke@unn.edu.ng (E.S. Okeke), tobechukwu.ezike@unn.edu.ng (T.C. Ezike).

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constitute one of the most abundant and renewable resources on the planet, encompassing materials such as straws, husks, bagasse, pulp, whey, hulls, pomace, feathers, among others, the accumulation of which contributes to global pollution and environmental degradation if left unmanaged (Klai et al., 2021).

Food waste, defined as the discarding or loss of edible food throughout the supply chain from production to consumption, represents a significant global challenge with far-reaching economic, social, and environmental implications. Economically, food waste results in the squandering of resources, including water, energy, and labor, leading to inefficiencies for businesses and households alike. Socially, it exacerbates issues of food insecurity, as edible food is discarded while millions suffer from hunger and malnutrition. Environmentally, food waste contributes to greenhouse gas emissions, as decomposing organic matter generates methane in landfills, a potent greenhouse gas. Furthermore, the resources invested in food production, such as land and water, are wasted when food is discarded. Strategies to combat food waste encompass awareness campaigns, supply chain enhancements, innovative packaging solutions, and initiatives to redistribute surplus food to those in need. Addressing food waste is crucial for promoting sustainability, mitigating environmental impact, and fostering equitable distribution of food resources globally. In the United States alone, food waste accounts for an estimated 30–40 % of the food supply, with over one-third of all available food going uneaten (USDA, 2022).

Both agricultural waste and food waste significantly impact food security, economic prosperity, and environmental sustainability (Pharino, 2021). Agricultural waste, in particular, poses a pressing issue, with farming activities generating 14.9 million tons of surplus produce in 2023. Shockingly, over 80 % of this surplus is abandoned in fields without harvesting, with only a meager 1.6 % being donated for hunger relief (USDA, 2022). Solutions to reduce food waste at the farm level include implementing practices to minimize the loss of 10 million tons of cosmetically imperfect or unharvested food annually (ReFED, 2022). Such solutions aim to reestablish the connection between consumers and food producers. Additionally, the wasted materials could otherwise be utilized to produce valuable products such as food, fuel, feed, and various chemicals and bioactive substances. Agricultural and agro-processing wastes represent promising feedstocks for environmentally sustainable products, characterized by their abundance, biodegradability, renewability, and affordability (Duque-Acevedo et al., 2020; Ibitoye et al., 2021).

The escalating global energy demand, propelled by urbanization and population growth, is projected to increase by 50 % over the next 15 years (UNDESA, 2018). Fossil fuels, including petroleum, natural gas, and coal, currently satisfy approximately 80 % of this demand (Mehta et al., 2019). However, their non-renewable nature and associated environmental drawbacks necessitate the exploration of alternative, eco-friendly fuel sources (Liu et al., 2020). Moreover, reliance on conventional fuels exacerbates economic challenges, particularly in developing countries, due to fluctuating energy prices stemming from resource scarcity (Sharma et al., 2020). Additionally, the combustion of fossil fuels releases harmful gases such as NO_x, CH₄, CO₂, SO_x, contributing to climate change, global warming, and acid rain (Srivastava et al., 2020a(Srivastava et al., 2020b)). Consequently, there is an urgent need to transition towards sustainable energy sources.

In response, various pretreatment techniques, including physical, chemical, physicochemical, and environmentally friendly methods, have been developed to optimize the production of value-added products from AFW and support a circular bioeconomy. The efficacy of these pretreatment procedures depends on the nature and composition of AFW and the desired target products (Sirohi et al., 2021). Lignocellulosic wastes, comprising hemicellulose, cellulose, lignin, proteins, sugars, resins, and pigments, represent crucial components for biofuel generation, biochemicals, and other value-added products in biorefineries (Rishikesh et al., 2021; Šelo et al., 2021). Effective management of AFW not only enhances agricultural output and resource

utilization efficiency but also contributes to environmental health by reducing pollutants.

Enzymes and microbes play pivotal roles in AFW biorefinery processes, ensuring environmental sustainability within a circular economy framework. This review discusses microbial and enzymatic valorization processes in AFW biorefineries, encompassing dark fermentation, photo-fermentation, anaerobic digestion, and other integrated approaches. Furthermore, it explores the diverse range of value-added products derived from AFW biorefineries, the circular economy aspects, and prospects for advancing AFW biorefinery through microbial and enzymatic engineering, bioaugmentation, biostimulation, among other strategies. By this review, we hope to emphasize and contribute to the scientific understanding of the application of microorganisms and enzymes in AFW biorefineries to ensure environmental sustainability.

2. Environmental and ecological impact of AFW

The advent of industrialization and modernization has led to the generation of enormous amounts of waste, resulting in the accumulation of wastes and negative environmental impacts to alarming levels. This poses a severe danger to humanity's ability to holistically manage and account for such wastes to improve the environment. The great majority of agro residues are annually generated by agro-based industries. Improper disposal and poor management of AFW could pollute the environment, harming human and animal health and leading to water pollution and loss of aesthetics (Sadh et al., 2018b). Most AFW, being untreated and underutilized, are disposed of through burning, dumping, or unintentional landfilling, contributing to increased greenhouse gas emissions and various climate change issues. Additionally, the use of fossil fuels further exacerbates greenhouse gas (GHG) emissions. Approximately 15 % of the total garbage produced by each country comprises agricultural waste, making it one of the most significant contributors to pollution from agriculture and agro-food processing industries. As global demand increases for environmentally sustainable industrial practices, pollution control, and financial considerations, waste management systems are evolving to view wastes as new sources of resources for creating value-added products (Kosre et al., 2021).

The improper disposal of agricultural solid wastes can lead to the obstruction of water channels and drainage systems, significantly contributing to floods. The indiscriminate disposal of agricultural solid wastes can block water channels, leading to potential loss of life and property due to floods. Increased fine sediment deposition from tilled fields in receiving streams can restrict crucial spawning sites for many fish species, altering the form and flow regime of channels. Suspended sediments can reduce light penetration, interfering with respiration and feeding mechanisms in fishes and other macroinvertebrates (Kosre et al., 2021; Prasad et al., 2020).

Efforts to achieve the crucial 17 Global Sustainable Goals, including ending hunger, ensuring food security, enhancing nutrition, and promoting sustainable agriculture by 2030, are facing challenges. Currently, there are 821 million hungry people globally, a decade ahead of the goal's deadline (WFP, 2020). It has been argued that agricultural solid wastes, particularly food wastes, contribute to food insecurity, especially in regions with the fastest-growing populations like Africa and Asia, where food insecurity and ineffective waste management are prevalent (Akegbejo-Samsons and Akegbejo-Samsons, 2022).

3. An overview of strategies for recovering bioactives/valuable compounds from agro-food waste

The approach of recovering valuable compounds and bioactives from agro-food waste (AFW) has garnered significant interest as an effective strategy to mitigate its environmental impact. Recent advancements in recovery systems have made this approach increasingly feasible and attractive (Abbasi-Parizad et al., 2022). Bioactives found in plant-based AFWs encompass a diverse array of compounds such as flavonoids,

uronic acids, quercetin, polyphenols, carotenoids, anthocyanins, Gallic acid, catechin, rutin, ferulic acid, vanillin, sugars, limonoids, among others. These compounds exhibit valuable applications, including nutraceuticals or functional foods, active pharmaceutical ingredients (APIs), cosmetics and personal care products, as well as in biodegradable materials, among others (Bala et al., 2022; Chukwuma et al., 2023; Ejike et al., 2023; Ezema et al., 2022; Ezeorba et al., 2022a; Ezeorba et al., 2022b). The recovery of these compounds not only leads to a significant reduction in waste but also ensures resource conservation, thus contributing to the promotion of a sustainable society and the establishment of a circular economy (Mármol et al., 2021).

The inherent diversity and varying composition of agro-food waste (AFW) stemming from different sources present challenges in devising a singular technological system for optimal bioactive recovery across multiple AFW sources. Hence, it is crucial to first identify the target valuable compounds through diverse assays and analytical approaches before embarking on the recovery process (Brennan, 2024). Several methodologies can be employed to comprehend AFW characteristics prior to recovery. These include comprehensive characterization of AFW through compositional and physicochemical analyses, leveraging existing knowledge through literature surveys, databases, or repositories, qualitative bioassays or biological activity screenings, and quantitative analyses using spectrometric and chromatographic techniques, among others. Evaluating the bioactive constituents beforehand facilitates the customization of recovery strategies, resulting in enhanced efficiency in both valorization and waste reduction/management endeavors (Withanage et al., 2021).

Recovering bioactives from agro-food waste (AFW) entails the utilization of diverse extraction techniques aimed at efficiently isolating and concentrating the target compounds. Various extraction methods are commonly employed for bioactive recovery from AFW, including solid-liquid extraction, ultrasonic-assisted extraction, subcritical water extraction, microwave-assisted extraction, supercritical fluid extraction, fermentation, and enzymatic processes (Lemes et al., 2022). Each of these techniques offers unique advantages and can be customized based on the specific properties of AFW and the target bioactives. It is crucial to optimize extraction parameters such as solvent type, extraction temperature, pressure, and duration to maximize yield and purity while minimizing energy consumption and environmental impact (Vilas-Boas et al., 2021).

While these recovery methods have their merits, they also present certain drawbacks. Conventional methods, particularly solid-liquid extractions, are often time-consuming and yield products of inferior quality despite their simplicity. The adoption of solid-liquid extraction for recovery is sometimes hindered by the cell wall barrier or the recalcitrant lignocellulose composition, especially in plant-based AFW (Ben-othman et al., 2020). Consequently, suitable pretreatment steps such as hydrolytic enzymatic treatment may be necessary to break down the cell walls and enhance yield (Gomes-araújo et al., 2021). On the other hand, incorporating enzymatic systems into the recovery process, despite their eco-friendliness, can significantly increase costs. Factors contributing to these costs include enzyme purification, immobilization, and the maintenance of optimal pH, temperature, and substrate concentration. Alternatively, ultrasonic-assisted extraction and microwave-assisted extraction are also eco-friendly options that offer shorter processing times and improved product quality. However, they require high energy demands (Gomes-araújo et al., 2021; Lemes et al., 2022). Therefore, critical considerations regarding the cost-effectiveness of the overall process are essential for ensuring the profitability of the extraction process.

Furthermore, downstream processing steps, including purification and concentration, are often conducted using various membrane technologies to obtain bioactives of the desired quality for diverse applications (Bala et al., 2023; Gomes-araújo et al., 2021). Membrane processes represent an innovative approach for bioactive recovery from agro-food waste (AFW), selectively separating target compounds from the complex

matrix of the waste material. These processes employ semi-permeable membranes to allow the passage of specific components based on their size, charge, or solubility, while retaining others (Tapia-Quirós et al., 2022). Pressure-driven membrane processes such as microfiltration, ultrafiltration, nanofiltration, and reverse osmosis are commonly utilized due to their scalability, operational simplicity, low energy consumption, and high separation efficiency in recovering bioactives such as sugars, proteins, antioxidants, and carbohydrates from AFWs (Papaioannou et al., 2022).

In recent years, pervaporation has emerged as a popular membrane recovery process, particularly for separating liquid mixtures containing volatile components. This technique relies on the selective permeation of one or more components through a specialized membrane, driven by a vapor pressure difference. Tailoring the operating conditions, such as temperature and pressure, along with utilizing specific selective membranes, allows pervaporation to be effectively employed for the recovery of bioethanol and other volatile bioactives produced from agro-food waste (AFW) (Peng et al., 2021). Recovered products and bioactives from AFW can be further valorized into a variety of value-added products, including biopharmaceuticals, food supplements/additives, bio-surfactants, antioxidants, fragrances, thickeners, emulsifiers, dyes, biofertilizers, and biofuels (Anaduaka et al., 2023; Ben-othman et al., 2020; Kumar et al., 2017; Okagu et al., 2023).

4. Concept of AFW biorefinery and circular bioeconomy

The escalation of global agricultural practices to meet the demands of a growing population has resulted in a substantial volume of waste generated during agricultural processing, which often goes unrecycled, contributing to environmental pollution. Agro-Food Wastes (AFW) encompass remnants obtained from cultivating, harvesting, and processing agricultural products such as fruits, vegetables, meat, poultry, dairy products, and crops (Sadh et al., 2018b). Despite being discarded, these wastes contain components with economic value and potential benefits for mankind. Their chemical compositions are contingent upon their sources and processing methods, existing in liquid, slurry, or solid forms (Obi et al., 2016). Within the framework of the circular bioeconomy, these agro wastes can serve as raw materials for producing value-added products like bioethanol and biogas (Yaashikaa and Kumar, 2022).

The imperative for green energy solutions has driven the quest for sustainable and renewable energy sources to mitigate carbon emissions and preserve ecosystems. Most agricultural waste materials harbor significant quantities of lignocellulose, comprising components like hemicellulose and cellulose, which, upon complete hydrolysis, yield simple sugars fermentable to generate bioenergy in a zero-waste approach (Okeke et al., 2022b). Developing bioprocesses to valorize these abundant agricultural wastes is pivotal for establishing a sustainable circular economy and advancing United Nations Sustainable Development Goals (SDGs) (Baiano, 2014). Therefore, leveraging biomass generated by the agricultural sector for AFW biorefinery is essential. Bio-based energy, typically derived from renewable sources, can be utilized to produce a myriad of value-added products such as bioethanol, biogas, and biodiesel (Awasthi et al., 2022).

Beyond mere energy generation from AFW, the concept of AFW biorefinery encompasses scientific innovations, sustainable bioconversion processes, and infrastructure for processing and converting biomass feedstock into a diverse array of valuable, marketable products (Takkellapati and Li, 2019). Analogous to a traditional refinery, wherein physical and chemical processes transform crude oil into various petrochemical products, biological processes in a biorefinery convert agricultural wastes into valuable commodities, including bioenergy. The overarching goal of a biorefinery is to harness the chemical energy stored in biomass while fostering sustainability (Palmeros Parada et al., 2017; Rajendran et al., 2021). The choice of an AFW biorefinery's type, concerning end products, may be dictated by the nature and availability

of AFW in a given locality. For instance, regions with a predominant focus on animal breeding may produce more animal-based agro wastes, conducive to biofertilizer production, while areas emphasizing crop production may yield more lignocellulosic biomass, ideal for bioenergy production (Gontard et al., 2018).

The linear economic model, characterized by the ‘take-make-dispose’ approach, no longer ensures sustainable development, as it depletes limited resources required by future generations and fosters the accumulation of harmful wastes. In contrast, a circular bioeconomy presents a superior economic model, ensuring the sustainable and efficient valorization of wastes by intersecting bioeconomy and circular economy principles (Marami et al., 2022; Ptak et al., 2021; Salvador et al., 2022). In a circular bioeconomy, biomass conversion into valuable products, such as foods, energy, and chemicals, is sustainably achieved through cascades of interlinked bioprocesses that loop backside streams of wastes into the technosphere, ensuring zero waste generation (Tan and Lamers, 2021; Venkatesh, 2021).

Agro waste biorefineries play a pivotal role in a circular bioeconomy. Driven by technological advancements, biorefineries address the challenges encountered in biotechnology, thereby enhancing the circular bioeconomy (Fig. 1) (Kardung et al., 2021). Various factors may limit the extent to which AFW biorefineries impact the circular bioeconomy, aligning with documented challenges facing circular bioeconomy initiatives. Inadequate technology poses a hindrance to successful bioresource interconversions in biorefineries. To circumvent such challenges, scientists may resort to genetically manipulating microorganisms or enzymes to confer novel catalytic abilities, thus achieving efficient valorization of AFW (Salvador et al., 2022).

5. Microbial and enzymatic valorization processes in AFW biorefinery

The conversion of agricultural wastes into valuable green chemical products necessitates a sophisticated chemical reaction involving delignification, hydrolysis of plant polysaccharides, and fermentation to produce biofuels such as bioethanol. These processes rely on microorganisms that secrete enzyme consortiums for biocatalysis and bioconversions. Initially, agricultural waste is gathered, dried, and pulverized to achieve the appropriate particle size. Subsequently, microorganisms are introduced, which release enzymes breaking down the lignocellulosic components into lignin, cellulose, and hemicellulose (Rajendran et al., 2021). Additionally, agro wastes rich in starch content undergo hydrolysis into fermentable sugars. Ultimately, yeasts aid in fermenting these sugars into bioethanol (Munasinghe and Khanal, 2010). This bioconversion process is pivotal for attaining a high final product yield (Table 1). The ease of valorization of AFW hinges on its source. For instance, plant-based agrowastes, abundant in lignocellulosic materials, are more recalcitrant than animal-based agrowastes. Nevertheless, a judicious combination of bioconversion processes can be applied to AFW for valorization. Common bioconversion strategies encompass anaerobic digestion, dark fermentation, electro-fermentation, photofermentation, and integrated approaches (Fig. 2).

5.1. Anaerobic digestion

Anaerobic digestion (AD) is a natural process of breaking down organic materials into smaller chemical components without oxygen, concomitantly generating biogas (Rajaonison et al., 2020). This biological process occurs naturally in mammals’ gastrointestinal tracts, swamps, and wetlands. The concept has been widely utilized, including in the digestion of primary and secondary sewage sludge, municipal solid wastes, up-flow anaerobic sludge blanket reactors, and activated sludge systems (Tsegaye et al., 2021).

Amid the current energy crisis, AD has been employed to produce biogases from waste materials such as agricultural wastes, poultry manure, sewage sludge, municipal wastes, and food and vegetable

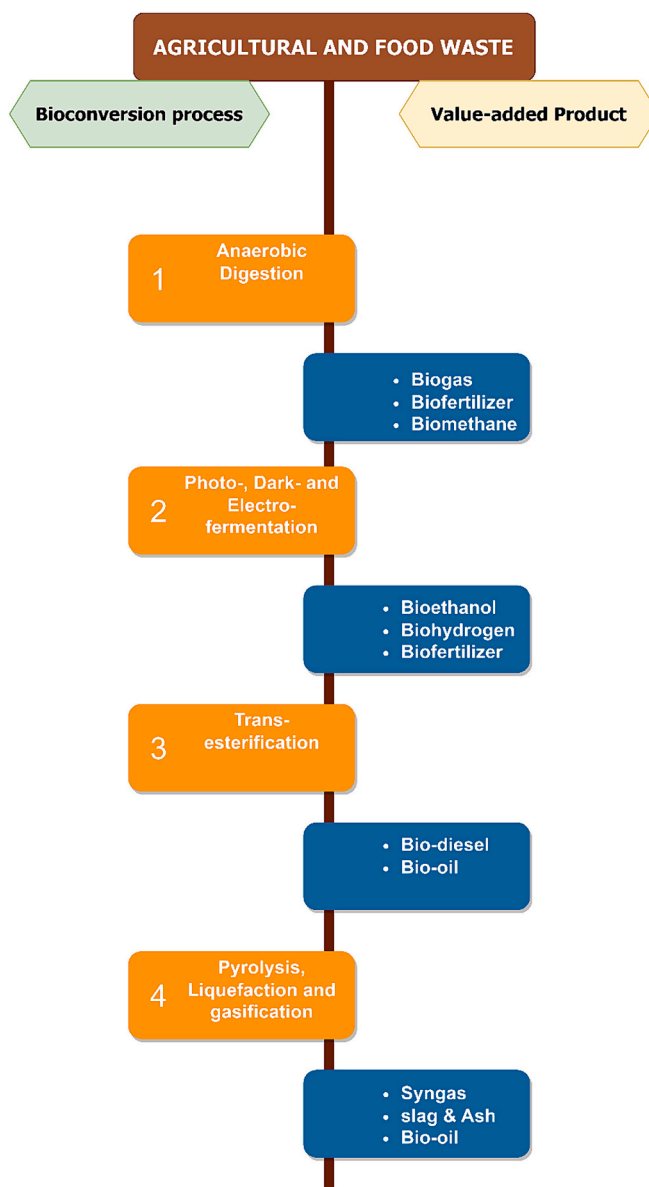


Fig. 1. Bioconversion process for valorization of agro wastes into biogas, bioethanol, biohydrogen and biodiesel. Anaerobic digestion often results in the generation of biogas and diesel whereas photofermentation, dark fermentation and electro-fermentation generate bioethanol and biohydrogen.

wastes, yielding methane, volatile fatty acids, and hydrogen as the primary products (Náthia-Neves et al., 2018; Tsegaye et al., 2021). These biogases can serve as heat, electricity, car fuel, and other fuel sources. Moreover, the digestate, rich in nutrients, can be utilized as a biofertilizer (Rajendran et al., 2021).

Anaerobic digestion is a dynamic system with numerous complex interactions involving microbial, biochemical, and physical-chemical processes concurrently in four steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (see Fig. 3) (Náthia-Neves et al., 2018). The overall outcome of the process is determined by hydrolysis, which is the reaction’s rate-limiting step. Microorganisms produce extracellular hydrolytic enzymes during the hydrolysis process, breaking down complex molecules like proteins, carbohydrates, and fats into more easily soluble substances like amino acids, sugars, and free fatty acids (Richard et al., 2019). Facultative and obligate anaerobes then transform these simple molecules to produce short-chain organic acids or volatile fatty acids and alcohols in the process known as acidogenesis

Table 1
Highlights of Biorefinery process for valorization of agrowastes from a few selected studies.

Agro wastes	Bioconversion process	Microorganism/ Enzymes	Products	Yields	Reference
Fruit and vegetable waste	Integrated approach (Dark fermentation and anaerobic digestion)	Mixed culture of digested sludge	Biohydrogen and Methane	115.2 L H ₂ /kg VS 334 L CH ₄ /kg COD	(Yeshanew et al., 2016)
waste activated sludge	Dark fermentation	–	Biohydrogen	1.3 to 14.2 mL/g VSS	(Li et al., 2022)
Date byproduct (Deglet-Nour)	Dark fermentation	–	Biohydrogen	292 mL H ₂ /g VS	(Ben Yahmed et al., 2021)
Sawdust	Enzymatic hydrolysis/fermentation	–	Bioethanol	351 L/ton	(Abdou Alio et al., 2021)
Food, vegetable and animal waste	Anaerobic digestion	–	Biogas (60 % methane content)	670 NL biogas/kg VS	(Kastner et al., 2012)
Spent coffee grounds	Fermentation	<i>Clostridium beijerinckii</i>	Biobutanol	7.1 g/L	(López-Linares et al., 2021)
Rice straw	Electro-fermentation (Cathodic)	Undefined mixed culture	Butyric acid	5.54 g/L	(Zhang et al., 2021b)
Kitchen waste	Enzymatic process	Immobilized oxidase and glucoamylase	Ethanol	30 g/L	(Ma et al., 2014)
Waste cooking oil	Enzymatic process	Immobilized lipase	Biodiesel	–	(Vescovi et al., 2016)
Chestnut shells	Fermentation	Saccharomyces cerevisiae	Bioethanol	14.6 g/L	(Morales et al., 2018)
Corn fiber	Enzymatic hydrolysis and biodetoxification	–	Bioethanol	70.2 g/L	(Zhang et al., 2021c)
Sweet sorghum bagasse	Fermentation	Saccharomyces cerevisiae, Clostridium acetobutylicum	Ethanol, Butanol	144.8 g/L, 17.3 g/L	(Su et al., 2020)
winery wastewater	Photofermentation	PNSB consortium	Biohydrogen, Poly-β-hydroxybutyrate	468 mL L ⁻¹ , 203 mg/L	(Policastro et al., 2020)
garden wastes	Dark fermentation	<i>Escherichia coli</i>	Biohydrogen	97 mL of H ₂ /g	(Ramprakash and Incharoensakdi, 2022)
Glucose	Electro-fermentation (Cathodic)	<i>Clostridium beijerinckii</i>	Butanol and hydrogen	0.30 ± 0.02 g/g and 206.53 ± 8.20 mL/g	(Zhang et al., 2021a)
Waste Soybean oil	Biocatalysis	<i>Pseudomonas cepacia</i> , Burkholderia sp	Biodiesel	881–885 kg/m ³	(Ağbulut et al., 2024)
Potato peel	Glucose-adapted fermentation	Thermococcus onnurineus NA1	Biohydrogen	3.3 to 6.7 g/L	(Lee et al., 2023)
Date palm waste (trunk, leaves, leaf sheath, pedicels, date cake, and seeds)	Liquid hot water, ethanol organosolv, and catalyzed ethanol organosolv (CEO) pretreatment and anaerobic fermentation	<i>S. cerevisiae</i>	Ethanol, methane, and lignin.	806.9 mL ethanol, 902.8 L methane, and 528.0 g lignin	(Shokrollahi et al., 2024)
Apricot seed	Non-catalytic transesterification	–	Biodiesel	43.06 wt%,	(Kim et al., 2024a)

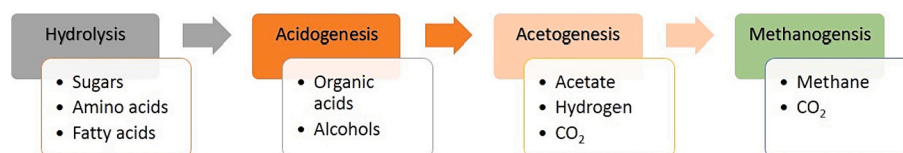


Fig. 2. The process flow from anaerobic digestion. The components of the agricultural waste are hydrolyzed into simpler compounds, which are then fermented through the processes of acidogenesis and acetogenesis to produce precursor compounds for the production of biogas.

(fermentation), which are further converted into acetate, hydrogen, and carbon dioxide by acetogenesis. Finally, methanogenic bacteria react with the organic acids generated in the preceding steps to release methane and carbon dioxide (Rajaonison et al., 2020; Rajendran et al., 2021).

Anaerobic digestion can be conducted in either a one-step or two-step bioreactor system. The one-step design is straightforward and less expensive since all the steps occur in one compartment of the bioreactor system. However, this method encounters various challenges, such as intermediate product inhibition, hindering subsequent reactions and reducing system efficiency and product yield. Conversely, the two-step system is partitioned so that acidogenesis and acetogenesis occur in different compartments of the bioreactor system. This approach has proven more effective in overcoming the drawbacks of single-step design, including process instability, acidifications, and the combined release of methane and hydrogen gas (Tsegaye et al., 2021).

In various studies, AD has shown promising results in generating biogas. For instance, AD yielded 8495 m³ of biogas per day with a

methane content of 60 % (v/v) from animal wastes, while a biogas output of 640 L/kg VS was generated using a mixture of food wastes, poultry litter, and sewage sludge in the ratio of 1:1:2 (Liu et al., 2016; Lohani et al., 2021). The yield of the desired product in AD is influenced by various physicochemical and nutrient parameters, including pH, temperature, the composition of the agro wastes, the C/N ratio, operational time, and inoculum. Optimization of these parameters is necessary to increase the yield of the final product.

Despite its advantages, anaerobic digestion also faces drawbacks such as process instabilities, inhibition, and the capital-intensive nature of setup. The intricate microbial interactions involved in anaerobic digestion necessitate meticulous monitoring and management to prevent disruptions and optimize biogas production. Moreover, the variability in biogas yields due to factors like waste composition and operational conditions underscores the importance of ongoing research and development to enhance process efficiency and reliability. Overcoming these challenges requires collaborative efforts among policy-makers, researchers, and industry stakeholders to promote the

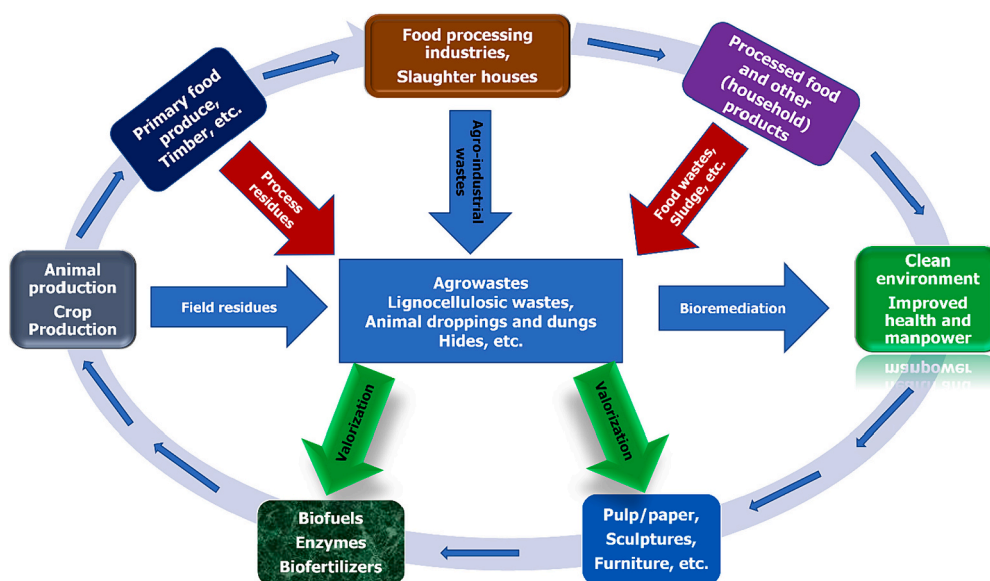


Fig. 3. Relationship between agrowastes biorefinery and circular bioeconomy.

widespread adoption of anaerobic digestion as a sustainable solution for organic waste management and renewable energy production.

5.2. Electro-fermentation

Electro-fermentation, as the name suggests, combines electrodes and fermentation (Schievano et al., 2016). Fermentation, with its historical significance in food production and human history, involves organic molecules serving as both electron donors and acceptors in the glycolysis metabolic process, sustaining ATP in the absence of oxidative phosphorylation (Schievano et al., 2016). Glycolysis is pivotal as the sole energy extraction process in fermentation, transforming pyruvate into various substances, including H₂, industrial enzymes, alcohols, polyols, short-chain fatty acids (SCFAs), amino acids, and polysaccharides (Civelek Yoruklu et al., 2019).

The economic potential of industrial fermentation faces challenges such as time consumption, capital intensity, low yield, suboptimal product purity, high selectivity by culture media, environmental pollution, and the need for highly optimized strains of microorganisms (Bhagchandani et al., 2020). Electro-fermentation leverages electroactive microorganisms, utilizing electrodes as electron mediators (acceptors or donors), to control the redox potential of the fermentation broth, leading to a more economically viable biotechnological innovation (Bhagchandani et al., 2020). This innovative approach facilitates the conversion of CO₂ into value-added products and biofuels from lignocellulosic biomass, thereby reducing environmental pollution and greenhouse gas emissions (Dessi et al., 2021).

A Bioelectrochemical system (BES) is a promising technology comprising an anode, cathode, and generally, a membrane separating both (Civelek Yoruklu et al., 2019). BES utilizes biocatalysts in the cathode, anode, or both, acknowledging their crucial role. Both microorganisms and enzymes can power BES, with microbial fuel chains (MFCs) and microbial electrolysis cells (MECs) being the two main forms of BES powered by microorganisms (Hernández-Correa et al., 2017). MECs require a steady electricity supply, while MFCs can produce power from organic substrates by oxidizing organic compounds (Hernández-Correa et al., 2017).

In BES, electrolyte solutions, often aqueous or wastewater containing reactants and/or products, surround the electrodes. The membrane-containing electrolyte solutions assist ions in moving into the cells through a membrane, creating a parallel electrical circuit between the electrodes. Consequently, the anode undergoes oxidation, while the

cathode undergoes reduction (Civelek Yoruklu et al., 2019; Rabaey and Rozendal, 2010).

Incorporating bioelectrochemical systems (BESs) into the fermentation process is a novel idea that enhances product recovery and process efficiency. The use of electrodes improves the fermentation environment, optimizing conditions to produce higher-purity products, promoting microbial cell proliferation and density, and achieving chain elongation (Schievano et al., 2016). Rabaey and Rozendal (2010) and Rabaey and Ragauskas (2014) reviewed and referred to this method as electro-fermentation (EF).

The basic tenet of electro-fermentation (EF) technology lies in the electrochemical regulation of microbial fermentative metabolism. In the EF method, electrodes can change the medium, influencing the fermentation's redox balance. Electrodes function as electron acceptors (anodic EF) or donors (cathodic EF), circumventing metabolic constraints during fermentation (Schievano et al., 2016). The EF method offers several benefits, including the ability to drive carboxylates' carbon chain elongation, provide pH control without the use of salt by transporting ionic products from the broth, and extract and/or convert target products using a selective membrane (Civelek Yoruklu et al., 2019).

5.3. Photofermentation

Photofermentation, also known as light fermentation, is a biological process wherein light serves as an additional energy source to drive microbial fermentation of agro residues for energy production. Unlike dark fermentation, which occurs without light, photofermentation relies on light energy for the fermentation process to proceed. Photosynthetic microorganisms harness light energy to produce biohydrogen from available organic wastes in a nitrogen and oxygen-deficient medium (Sağır and Hallenbeck, 2019). Purple non-sulfur bacteria (PNSB), such as *Rhodospseudomonas palustris*, *Rhodospseudomonas spheroides* O.U001, and *Rhodospirillum rubrum*, are commonly used in photofermentation due to their ability to yield high amounts of biohydrogen and other essential substances (Keskin et al., 2011).

Photosynthetic bacteria secrete ATP-dependent nitrogenases, which are activated by light energy through reverse electron flow (Melitos et al., 2021; Mishra et al., 2019; Sağır and Hallenbeck, 2019). These nitrogenases then utilize ATP to reduce metabolically derived protons to biohydrogen by reducing nitrogen to ammonia. This process continues until nitrogen is completely reduced. In the absence of nitrogen,

nitrogenase, along with ATP, produces biohydrogen, the primary goal of photofermentation (Mishra et al., 2019). Since this reaction occurs under oxygen-deficient conditions, organic compounds like acetate, butyrate, and lactate are also broken down to form H₂ and a small amount of CO₂ (Singh and Sarma, 2022).

The significance of photofermentation lies in its ability to generate biohydrogen, which is an ideal biofuel (Keskin et al., 2011). Hydrogen production is rapid, and it has several advantages over other biofuels due to its lack of harmful elements like carbon found in other fossil fuels and biofuels. Moreover, hydrogen finds wide-ranging applications in energy production, heat and electricity generation, as well as in the production of methanol and ammonia, valuable products in the chemical industry.

Hydrogen serves as the primary fuel in many internal combustion engines powering cars, trains, airplanes, and ships. Importantly, the only byproduct of hydrogen combustion is water, making it an environmentally friendly biofuel with no harmful emissions that could affect human health or the environment negatively (Melitos et al., 2021). Overall, photofermentation offers a promising approach to generate biohydrogen from agricultural residues, contributing to sustainable energy production and reducing environmental impact compared to traditional fossil fuels.

5.4. Dark fermentation

Dark fermentation, unlike photofermentation, occurs in the absence of light and oxygen to produce hydrogen. Facultative and obligate anaerobes act on organic materials in this process, generating hydrogen, carbon dioxide, and organic acids through acidogenesis, which can subsequently be transformed to produce methane (Ferreira and Gouveia, 2020; Kamran, 2021). Organic materials for dark fermentation can originate from various sources such as lignocellulosic wastes, food products, industrial wastewater, municipal solid wastes, and sugar-rich crop residues. Prior to dark fermentation, pretreatment is crucial to enhance process efficiency. Selecting sugar-rich agro-wastes and appropriate microorganisms for fermentation is key to achieving maximum yield. Despite the production of significant volatile free fatty acids and a relatively small amount of hydrogen (approximately 4 H₂ per glucose molecule), dark fermentation remains attractive due to its straightforward reactor design and high turnover rate (Ding et al., 2016).

Various factors influence dark fermentation for the production of biohydrogen and other valuable organic acids from agro-food wastes (AFWs), including pH and temperature of the medium, nutrient availability, achieving optimal hydraulic retention time or substrate loading rate, the presence or absence of inhibitors/toxic compounds, potential feedback inhibition due to hydrogen partial pressures, and the adapted fermentation mode and reactor configuration (Mohanakrishna et al., 2023).

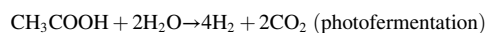
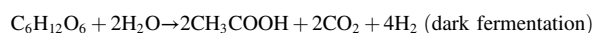
Temperature is a critical factor affecting the efficiency and hydrogen yield of dark fermentation. The activity and growth rates of fermentative microorganisms are temperature-dependent. Optimal temperatures typically range from 30 °C to 40 °C, although specific microorganisms may have different temperature optima. For example, food wastes fermented at 34 °C produced 53.5 mL H₂/g VS, while the yield decreased to 37.6 mL H₂/g VS at a higher temperature of 55 °C (Ghimire et al., 2022). Byproducts of the system, including organic acids and the buildup of hydrogen (measured as hydrogen partial pressure), also affect production yield. Feedback inhibition varies depending on the pathway the dark reaction follows; studies have shown that acetate and butyrate pathways often yield more biohydrogen than alcohol and lactate pathways (Liu et al., 2013; Saady, 2013). Inhibitors inherent in waste materials, such as furan derivatives, heavy metals, and bacteriocins, can affect AFW fermentation. Managing inhibitor concentrations through strategies like dilution, operational parameter adjustment, and removal or inactivation is crucial (Chen et al., 2021).

Different fermentation modes (e.g., batch, continuous, fed-batch) and reactor configurations (e.g., stirred-tank, fixed-bed, fluidized-bed) can impact process performance, hydrogen yield, and operational stability. Selecting an appropriate fermentation mode and reactor design is essential for achieving desired outcomes. Recent studies have shown that solid-state fermentation (SSF) yields better hydrogen compared to submerged fermentation (Mohanakrishna et al., 2023), though outcomes may vary depending on substrate and consortium. Substrate acidification is another approach to improve DF efficiency; for instance, strong acidification inhibits non-hydrogen producers while favoring acidophilic hydrogen-producing bacteria (Xue et al., 2023).

Optimizing these conditions can significantly increase biohydrogen and volatile organic acid yields. Various approaches have been successful in overcoming limiting or inhibiting factors (Mohanakrishna and Pengodeth, 2024). For example, codigestion of AFWs by *Bacillus subtilis* CDBB 555 and *Clostridium acetobutylicum* ATCC 824 in a syntrophic association has been successful in bio-hydrogen production (Ríos-González et al., 2024). Other approaches include adding biochar to the fermentation system (Lou et al., 2024), optimizing pretreatment for AFW hydrolysis (Fasheun et al., 2024), metabolic or genetic engineering of microbial candidates (Krishnan et al., 2023), combining short-circuited electrodes with DF (Truong et al., 2024), and starch extrusion and enzymatic hydrolysis (Fasheun et al., 2024). Finally, for practicality and economic feasibility, lactate-driven DF, bioaugmentation, construction of synthetic microbiomes, and metabolic engineering are recommended (Villanueva-Galindo et al., 2023).

5.5. Integrated Approach

The integrated approach entails combining one or more systems to valorize AFW residues for biofuel generation. This approach has proven to enhance the economic viability of food waste treatment, reduce production costs, and maximize product yield and energy recovery (Mabalane et al., 2021; Morero et al., 2020; Tsegaye et al., 2021). Integrating approaches such as dark fermentation and microbial fuel cells (MFC) or other methods for producing biohydrogen and other valuable products in waste-based biorefineries is gaining traction (Poggi-Valardo et al., 2014). The coupling of dark fermentation and photofermentation notably improves biohydrogen production, enhancing biohydrogen yield under dark fermentation conditions by photosynthetic bacteria (PNSB) during photofermentation (Mishra et al., 2019; Ventura et al., 2021). This integrated approach can be executed in a single or two-stage system, with the latter often preferred due to the specific optimum conditions and treatment required for the metabolic byproducts of dark fermentation (Rai and Singh, 2016). The general reactions of dark and photofermentation are outlined below:



In addition to combining biological treatments, hybrid systems can incorporate various chemical and thermal methods. For example, thermal pretreatment of starch wastewater enriched with groundnut de-oiled cake significantly increased biohydrogen production. Similar results were observed when nano-metal oxides were introduced to rice mill wastewater during dark fermentation by *Clostridium beijerinckii* DSM 791. The addition of NiO and CoO nanoparticles increased biohydrogen yields by 109 % and 90 %, respectively. Overall, the integrated approach tends to yield more promising results for commercial hydrogen production compared to other methods. Table 1 provides a summary of different strategies for waste valorization and their product yields.

6. Value-added products of AFW biorefinery

The AFW biorefinery represents a contemporary and optimal

approach to managing wastes and byproducts from agricultural activities. These technologies offer a novel avenue for valorizing wastes into environmentally friendly and value-added products, including biofuel, biofertilizers, platform chemicals, biopolymers, and more (Chavan et al., 2022). Biofuels, derived from biological systems, are a significant output of AFW biorefinery processes, resulting from the degradation of complex biopolymers. Biofuels encompass bioethanol, biomethane, biohydrogen, biohythane, and biodiesel (Isah and Ozbay, 2020).

Of note, the products of AFW biorefineries hold the potential to reduce reliance on fossil fuels and promote the utilization of renewable and bio-sourced energy. Moreover, these systems are highly sustainable, as AFW serves as a potential food source without competing interests. Ultimately, AFW biorefinery initiatives contribute to the advancement of a circular economy (Leong et al., 2021; Philippini et al., 2020). This section provides a brief overview of some of the value-added products of AFW biorefinery processes (see Fig. 4).

6.1. Bioethanol

Bioethanol is a quality and high-octane profile fuel produced from the fermentation of carbohydrate feedstock. The bioethanol from AFW is commonly referred to as second-generation bioethanol. Agro wastes bioconversion to ethanol is a means towards a bioeconomy and is preferred over food crops (First-generation ethanol) (Ramesh et al., 2021). Agro wastes are rich in lignocellulosytic substances, which enzymes or other physicochemical pretreatments should degrade to simple

sugars and further fermentation to bioethanol. Several concerted research efforts have been made to optimize the bioethanol production systems, which have been discussed in detail in several focused reviews (Bisht et al., 2022; Khaire et al., 2021). In a recent study by Tiwari et al. (2022), rice husk was valorized for bioethanol production when incubated with *Klebsiella oxytoca* ATCC 13182. It was reported that a pH of 7.36 and a temperature of 36 °C for 48–72 h was optimal for a biorefinery system (giving a yield of 32.61 ± 0.45 g/L). Interestingly, the optimal conditions for this biosystem are easily achievable at a minimized cost.

Moreover, other supplementation, such as adding different nitrogen sources (ammonium chloride, peptone, and beef extract) and trace metal (Zn²⁺ and Mg²⁺), improved the bioethanol yield to about 35.13–44.60 g/L. Finally, the impact of AFW pretreatment before bio-fermentation cannot be overemphasized. Pretreatment enhances the accessibility of usable carbohydrates from the lignocellulosytic AFW. In the study on Rice husk and *K. oxytoca* biorefinery, acid and biological pretreatment (with *Aspergillus niger*) was optimal, giving up to 1.47 fold increase in yield (Tiwari et al., 2022). Other studies, as summarized in Table 2, have reported optimal production of bioethanol from other AFW sources such as wheat bran, sago wastes, rice bran, sugarcane tops, rice straws, banana wastes, piggery excreta, and others (Doreswamy et al., 2021; Khaire et al., 2021; Le et al., 2022; Rajesh and Gummadi, 2022; Sawarkar et al., 2022).

Pervaporation, a combination of permeation and evaporation, stands out as a promising method for purifying and recovering bioethanol from

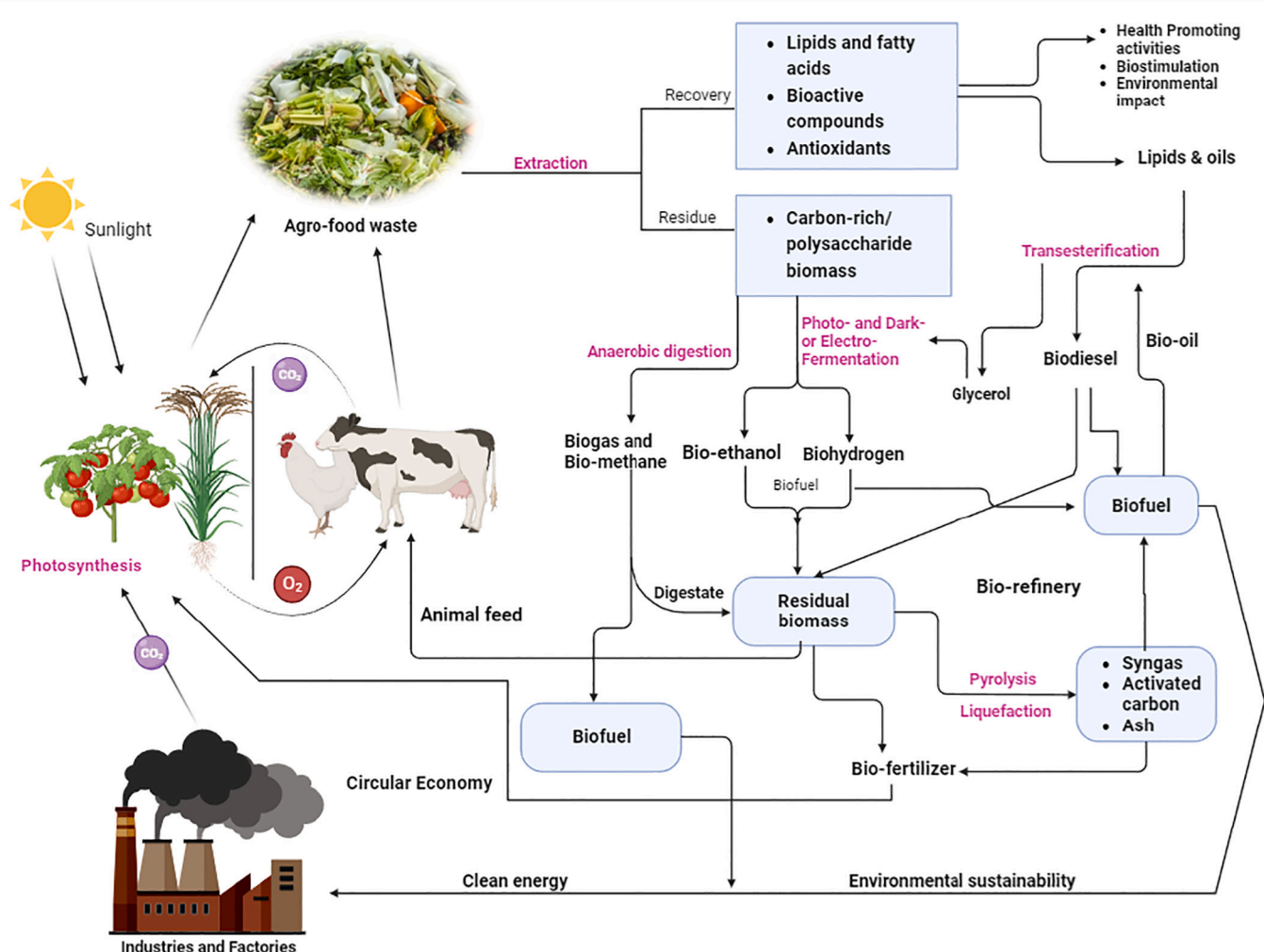


Fig. 4. Valorization of agro-food waste processes into value-added product through several concerted biorefinery, ultimately achieving a circular bioeconomy.

Table 2
Summary of selected studies on the bioethanol production from agricultural waste.

Agro wastes	Microorganisms	Pretreatment	Optimal conditions	Bioethanol Yield	Ref
Rice Husks	<i>Klebsiella oxytoca</i> ATCC 13182	Acid and biological pretreatment (<i>A. niger</i>)	pH - 7.0 Temp - 36 °C Fermentation time - 48–72 h	32.61–47.98 g/L	(Tiwari et al., 2022)
Wheat bran	<i>Bacillus</i> sp. PM06	In situ enzyme pretreatment (cellulase and amylase)	pH - 7 Temp - 40 °C Fermentation time – 60 h	1.83 g/L from (3 % w/v) wheat bran	(Rajesh and Gummadi, 2022)
Sugarcane Tops	<i>Saccharomyces cerevisiae</i>	In situ enzyme pretreatment (cellulase and xylanase from <i>Trichoderma reesei</i> Rut C30)	pH - 5 Temp - 50 °C Fermentation time – 7 h	27.2 g/ L	(Sherpa et al., 2019)
Banana leaves	<i>Saccharomyces cerevisiae</i>	Acid and alkali pretreatment (0.1 N NaOH and H ₂ SO ₄ in 1:10 (w/v) –autoclave at 121 °C for 1 h)	pH – 5.5 Temp - 30 °C, Fermentation time – 30 h Agitation – 150 rpm	15.43 g/L	(Suhag et al., 2020)
Banana leaves	<i>Saccharomyces cerevisiae</i>	Acid pretreatment (2.5 % H ₂ SO ₄) autoclaved at 121 °C for 30 min	Temp - 30 °C, Agitation – 150 rpm Fermentation time - 96 h Microbial Inoculum rate – 10 %	8.1 g/L	(Shankar et al., 2020)
Banana Pseudostem	<i>Kluyveromyces marxianus</i>	Acid pretreatment 0.5 % v/v H ₂ SO ₄	Temp-35 °C Fermentation time - 24 h Agitation – 150 rpm	5.35 g/L	(Gatdula et al., 2021)
Banana Pseudo stem	<i>Saccharomyces cerevisiae</i>	Alkali pretreatment (3 % NaOH)	Temp-30 °C Fermentation time - 48 h Agitation – 150 rpm	17.6 g/L	(Legodi et al., 2021)
Banana Peels	<i>Saccharomyces cerevisiae</i>	Autoclaving at 121 °C, 15 min	pH – 4.8 shaker Speed – 150 rpm Temp - 35 °C, Fermentation time - 64 h	32.6 g/L	(Palacios et al., 2021)
Piggery (<i>Sus scrofa</i>) excreta	<i>Saccharomyces cerevisiae</i>	Acid pretreatment with 4 % H ₂ SO ₄	pH – 6-7 Fermentation time - 3–5 days Temperature – Room temperature	0.765–1.02 g/200 mL of 89.59 % pure bioethanol	(Doreswamy et al., 2021)

fermentation broths. This purification step is crucial in bioethanol production as it directly impacts the volume and, consequently, the cost of the recovered ethanol. Various strategies have been explored to enhance bioethanol recovery using pervaporation, leveraging membrane-based separation techniques (Peng et al., 2021).

Recent advancements have led to the development of membranes with remarkable separation efficiency, low energy consumption, and reliance on external power sources. Notably, Mansy et al. (2024) reported the successful fabrication of a membrane using sulphonated polyvinyl chloride and poly(2-acrylamido-2-methyl-1-propanesulfonic acid), demonstrating promising results for bioethanol recovery.

Additionally, researchers have investigated alternative membrane materials to improve pervaporation performance. Zhang et al. (2024) demonstrated the superior separation capabilities of zwitterionic polyamide membranes, offering stability and high permeation flux. He et al. (2024) explored the use of carbon nanotube arrangements within polydimethylsiloxane (PDMS) membranes, leveraging electric-field effects to enhance mass transfer and bioethanol recovery efficiency. Furthermore, Kalahal et al. (2022) introduced gelatinated membranes, highlighting their high selectivity in recovering azeotropic bioethanol.

The selection of membrane type significantly influences the efficiency of ethanol purification by pervaporation, as the interaction between membranes and permeating components is multifaceted. For instance, properties such as membrane thickness, affinity for permeate components, volatility, and diffusion coefficient dictate permeation flux (Peng et al., 2021).

Although pure PDMS membranes are commonly used for bioethanol recovery, they suffer from limitations such as low water/ethanol separation factor and broad permeability flux range. Efforts to overcome these challenges include reducing effective membrane thickness, utilizing composite PDMS membranes, hybridization, and nano-engineering. Several PDMS-derived membranes have been critically reviewed, shedding light on their potential for bioethanol recovery (Cheng et al., 2024; Peng et al., 2021; Shahzad et al., 2022).

Hydrophobic inorganic membranes, such as zeolite membranes, offer exceptional chemical resistance, separation factors, mechanical properties, permeation flux, thermostability, long-term durability, and anti-fouling properties compared to polymeric membranes (Khalid et al., 2019; Peng et al., 2021). While mixed matrix membranes (MMMs) may exhibit lower separation performance than inorganic membranes, they present advantages in terms of cost-effectiveness, suitability for large-scale production, and anti-swelling properties (Khalid et al., 2019; Peng et al., 2021). In conclusion, while pervaporation holds great promise for ethanol purification and recovery from fermentation broths, the choice of membrane material significantly influences its efficacy. Continued research into membrane development and optimization is crucial for advancing pervaporation as a viable solution in bioethanol production processes.

6.2. Biodiesel

Biodiesel, a promising alternative fuel, can be produced from various

agro-food wastes rich in fats and oils. Typically, biodiesel is derived from the methanolic transesterification of fats or oils in the presence of a catalyst such as KOH, resulting in fatty acid methyl esters (FAMES) (Maheshwari et al., 2022). Agro-food processing wastes, particularly oil effluents, serve as excellent feedstock for biodiesel production, offering a sustainable solution for waste management. The byproducts of biodiesel production, including glycerol and de-fatted residues, hold additional value. Bio-glycerol can be purified for use in organic synthesis, while de-fatted residues can serve as feedstock for anaerobic biogas production (Philippini et al., 2020).

One of the main challenging issues for adapting AFW for biodiesel production is determining the most suitable technology for harvesting the biomass to perform the bioconversion. Several factors must be considered when choosing a harvesting technology, including feedstock type, quality, contamination potential, cost-effectiveness, environmental impact, scalability, and compatibility with the bioconversion process. Common harvesting technologies for AFW biomass include manual collection, mechanical harvesting, solvent extraction, and automated sorting systems, each with its advantages and limitations (Maheshwari et al., 2022).

Moreover, some oil-producing microorganisms have found several AFWs as suitable feedstock, and their oil is applied in biodiesel production (Odude et al., 2019). In a recent study by (Kanakdande and Khobragade, 2020), corn stover after acid hydrolysis (with 0.25 % HCl) was reported as an ideal AFW feedstock for oil-producing bacteria, *Bacillus amyloliquefaciens* (MF510169). By optimizing several culture conditions such as inoculum concentration, density, C/N ratio, temperature, pH, agitation speed, and fermentation time, the bacteria produced about 3.8 % oil, which was catalytically trans-esterified into different FAME compounds such as hexadecanoic acid methyl ester, 9–1, 2 octadecanoic acid methyl ester, octadecanoic acid methyl ester, and methyl stearate (Kanakdande and Khobragade, 2020). Additionally, research has demonstrated that several non-edible seeds perceived as waste possess high oil contents suitable for biodiesel production. Some of these seeds, including Mango kernel (*Mangifera indica*), Rambutan (*Nephelium lappaceum*), Pumpkin (*Cucurbita ps*), and Papaya (*Carica Papaya*, L), were studied for their oil content, which was extracted using Soxhlet extraction with n-hexane solvent (Abbas et al., 2019).

Upon completion of feedstock collection and preparation, as well as the extraction of the oily component from the waste, the subsequent step involves the transesterification process. Transesterification, recognized as the key bioconversion process in biodiesel production, involves the reaction of triglycerides present in agro-food waste with either methanol or ethanol in the presence of a catalyst, typically sodium hydroxide or potassium hydroxide. This chemical reaction facilitates the conversion of triglycerides into fatty acid methyl or ethyl esters (FAMES or FAEEs), constituting biodiesel, alongside glycerol, a valuable byproduct. Notably, certain agrowaste materials have showcased potential as biocatalysts to augment the transesterification of oil from AFW sources into biodiesel. Recently reported agro wastes exhibiting biocatalytic activities for biodiesel production includes banana peel, cocoa pod husk, oil palm frond, coconut shell, and groundnut shell (Abdullah et al., 2019; Odude et al., 2019). In scenarios where the agro-food waste contains elevated levels of free fatty acids (FFAs), acid esterification may be employed as a pretreatment step before transesterification. Acid esterification transforms FFAs into biodiesel-compatible esters using an acid catalyst.

The harvesting of biomass or the separation of biodiesel and its byproducts is a critical step in the biodiesel production workflow. Finding a suitable harvesting technology significantly influences the economic sustainability of the process, as energy costs can constitute up to 30 % of the overall production cost (Zheng et al., 2023). Efforts have been put forth to develop cost-effective, eco-friendly, rapid, versatile, and efficient biomass harvesting techniques (Kumar et al., 2023; Zheng et al., 2023). Common methods such as flocculation, sedimentation, centrifugation, filtration, and flotation are employed to harvest

microalgal biomass, each with its pros and cons. Flocculation has been used with gravity sedimentation of microalgal biomass so as to increase the sedimentation rate. Moreover, stopping the aeration and increasing the culture pH have been shown to usually enhance cell aggregation and autoflocculation (ref). Integrated systems have been developed to enhance the efficiency and the overall economical feasibility of the biodiesel production process. Techniques such as electrocoagulation, which utilizes electrical currents to induce the coagulation and precipitation of microalgae cells from the culture medium as well as bio-flocculation which harnesses the natural flocculation properties of certain microalgae strains or bacteria to facilitate biomass separation, have been common in recent years (Kumar et al., 2023). Co-cultivation of microalgae with flocculating bacteria, induction of autoflocculation by adjustment of pH, coprecipitation with Mg^{2+} and/or Ca^{2+} , and the use of chitosan-based flocculation have yielded appreciable results (Ananthi et al., 2021; Cheirsilp et al., 2020; Kumar et al., 2023).

Efforts to develop cost-effective biomass harvesting techniques, achieved through both modification of existing methods and introduction of novel approaches, have resulted in a diverse array of techniques tailored to specific parameters such as harvesting efficiency, speed, cost-effectiveness, and environmental impact (Ananthi et al., 2021; Muhammad et al., 2021). The choice of harvesting technique is contingent upon the source and characteristics of the biomass. For instance, sedimentation rates during gravity sedimentation vary depending on factors such as cell size, density, and water turbulence, which can differ between species or cultures. Conversely, while centrifugation offers rapid harvesting, it may lead to cell disruption due to strong shear forces (Ananthi et al., 2021; Cheirsilp et al., 2020).

Flocculation, along with its derivatives, is widely regarded as the superior harvesting technique for algal biomass due to its numerous advantages over other methods (Muhammad et al., 2021; Yin et al., 2021; Zheng et al., 2023). Recent advancements in harvesting technologies have been substantial, opening up new avenues for improving the efficiency and sustainability of biodiesel production. Therefore, it is imperative that future research efforts continue to explore the potential of agro-food wastes for biodiesel production, driving innovation and progress in the field.

6.3. Biogas (Biomethane and Bio-hythane)

Biogas is a mixture of gases (methane, CO_2 , H_2 , and others) generated from the syntrophic activities of anaerobic microbes. Biogas is useful for combustion and generating other forms of energy. Complex agro wastes rich in organics can be used as feedstock for biogas generation, in which pretreatment hydrolytic steps catalyzed by microbial hydrolytic enzymes break down complex polymers into simple organic moieties for methanogenesis (Calbry-Muzyka et al., 2022). Biomethane and bio-hythane can be obtained from biogas through a series of purification or gas separation processes. While biomethane contains 100 % methane, bio-hythane contains a mixture of methane and 5–25 % hydrogen gas (Liu et al., 2018). Several recent studies have reported adopting AFW from plants and animals as feedstock for biogas production. Mohapatra et al. (2023) reported the optimum co-digestion of the plant (arrowroot, cauliflower, and jackfruit leaves) and animal (cow dung) agricultural wastes in the ratio of 60:40 for biogas production. The optimum yield was achieved with sewage water (1:10 (w/v)) as an inoculum to prime the process, and a constant thermophilic temperature (55 °C) was maintained for a 17-day hydraulic retention time (HRT). In another study, the biogas yield was much higher when co-digestion of waste-activated sludge (WAS) and agricultural waste straw was performed rather than mono-digestion of WAS (Potdukhe et al., 2021). Three straws, namely wheat straw, rice straw, and soybean straw, caused a 2.57, 2.52, and 2.27 times increase in biogas yield on co-digestion with WAS, respectively. In summary, this study and others confirm the potencies of AFW to be valorized for biogas production (Arekemase and Aweda, 2021; Calbry-Muzyka et al., 2022; Kokieva

et al., 2020; Moustakas et al., 2021; Potdukhe et al., 2021; Sumardiono et al., 2022).

Biogas recovery is a crucial aspect to consider for the overall feasibility of the process. Among various methods for biogas upgrading, membrane-based gas separation stands out as a preferred option for removing CO₂ and other minor impurities compared to alternatives like chemical adsorption, absorption (including physical and chemical methods like water scrubbing and amine scrubbing), cryogenic separation, and hydrogenation (Koukovinos et al., 2024; Sulewski and Ignaciuk, 2023). However, a notable drawback of membrane systems is the need for frequent membrane replacement due to their relatively short lifespan caused by regular wear and tear (Gkotsis et al., 2023; Sulewski and Ignaciuk, 2023).

Various membranes have been developed to enhance the separation of CH₄ from CO₂, including the use of multistage membrane-based purification configurations. Pilot studies have demonstrated significant CH₄ recovery rates, with up to 95.7 % achieved using a two-stage membrane-based upgrading strategy with polyimide membranes (Koukovinos et al., 2024), and over 97 % CH₄ recovery with a cost-effective three-stage membrane separation configuration (Abejón et al., 2024). Despite recent attention on methanation as an alternative approach, its suitability for in situ applications is limited due to factors such as the poor solubility of H₂, the need for external H₂ supply, and elevated partial pressure of H₂ (Pierro et al., 2023; Rao et al., 2024).

The economic feasibility of membrane-based biomethane upgrading is a critical consideration. Factors such as membrane lifetime, system configuration, and energy consumption influence economic parameters and require careful analysis (Araújo et al., 2024; Haider and Lindbr, 2016; Soto et al., 2022). Inorganic membranes generally offer better durability compared to organic membranes over their lifetime. Membrane upgrading systems typically consume less energy than conventional methods, and to mitigate maintenance costs, multi-stage approaches and the use of stronger and more durable membranes, such as zeolite-based mixed-matrix membranes, are employed (Zito et al., 2022).

Membrane separation is recognized as a cost-effective approach with lower CH₄ loss, making it suitable for large-scale production. Its efficiency, compactness, lightness, reduced need for skilled labor and maintenance, and consistent performance regardless of concentration make it superior to other methods (Ahmed et al., 2021; Brunetti and Barbieri, 2021; Yusuf and Almomani, 2023).

6.4. Biofertilizers

The byproducts of biogas and other biofuel generation processes are great sources of clean and odorless organic fertilizers and are useful to boost agriculture processes and promote crop yields. Digestate is one of the most common biofertilizers obtained from the end products of different waste fermentation processes and biogas generation (Dar et al., 2021). The nutrient composition of biofertilizers may vary depending on the wastes' proximate composition. Moreover, their application improves soil properties such as texture, porousness, and retaining capacities (Kumar et al., 2022). Other biofertilizers potentially derived from agro wastes are compost and dehydrated manures (dung and droppings). Composting improves the organic nutrient contents of recalcitrant agricultural wastes by the activities of fermentative and degradative microbes. These systems have an odor as their major challenge. However, advancements in research and technologies are being developed to manage the odor generated from the composting system (Lin et al., 2022). A recent study by Mekki et al. (2017) reported the improvement of several soil properties such as pH, conductivity, water retention, and organic matter contents by adding compost, dehydrated manure, and digestate. Moreover, a significant improvement in the microbial and respirometric soil activities was achieved with those biofertilizers as well as the germination index and growth evolution of crops such as Tomato (*Lycopersicon esculentum*), Alfalfa (*Medicago*

sativa), Wheat (*Triticum durum*), and Sorghum (*Sorghum bicolor*) (Mekki et al., 2017). Several other studies have reported the potencies of AFW biofertilizers in boosting agricultural processes and crop yield (Al-Suhaibani et al., 2020; Al-suhaibani et al., 2021).

6.5. Platform chemicals

Platform chemicals are valuable and multipurpose chemicals that serve as substrates or starting ingredients for other higher-value-added products. The chemical composition of agricultural wastes varies greatly, making them potential sources of used platform chemicals. Several recent studies (Table 3) have reported producing and purifying useful platform chemicals such as furfural, 4-hydroxyvaleric acid, levulinic acid, succinic acids, and lactic acids from different AFW (Fatima et al., 2022; Kover et al., 2021). A recent study reported furfural production (a major precursor of furanic chemicals) from corncob by hydrolysis with an aqueous low transition temperature and autocatalysis. The aqueous low-temperature approach selectively converts xylan from the complex lignocellulose to xylose and fosters the auto-dehydration to furfural (Naga Sai et al., 2021). A thermochemical process produced another impressive platform chemical (Levulinic acid) and formate from rice straw and corncob. Co-produced formate was reused as a hydrogen source for the enzymatic hydrogenation of the generated levulinic acid (25.1 to 65.4 mM) to a valuable derivative - 4-hydroxyvaleric acid (11.32 mM). The reaction was catalyzed by an engineered 3-hydroxybutyrate dehydrogenase from *Alcaligenes faecalis*, fostering a significant conversion rate of about 48.2 % (Moon et al., 2021). Some other selected studies on the production of various platform chemicals from different agricultural wastes are summarized in Table 3.

6.6. Biopolymer

Biopolymers are broad polymers produced by plants, animals, or living organisms from sustainable sources such as agricultural waste. These polymers are useful in food and pharmaceutical industries and environmental management and are sustainable alternatives to synthetic counterparts (Ponce et al., 2022). Several biopolymers such as cellulose, hemicellulose, and other lignocellulosic-based polymers exist naturally in many AFW, whereas other biopolymers are produced either by physical treatment, chemical conversion, or biological activities of microorganisms on precursor polymers. Several recent studies have reported the production of biopolymers from various AFW (Bahçegül et al., 2020). An interesting study by Sayyed et al. (2021) reported on the production of Poly-β-hydroxybutyrate (PHB) from corn wastes (541.46 μg of PHB/mg of cell mass) and rice straws (379.98 μg of PHB per mg of cell mass) by *Alcaligenes faecalis* RZS4 and *Pseudomonas* sp. RZS1 respectively. The culture conditions were optimized at 30 °C for 48 h at 120 rpm, and 20 g/L of AFW was used. This study proposed an alternative route for sourcing PHB at a lowered production cost and a more sustainable and eco-friendly approach (Sayyed et al., 2021). Another study reported that wastes of tomato, pepper, and eggplants were viable substrates for producing Lignocellulose Nanofiber through mechanical and chemical pretreatment 2,2,6,6-tetramethylpiperidine-1-oxyl radical. The nanofiber produced was also shown to stabilize the mechanical properties, thermal resistance, chemical structure, antioxidant activity, water barrier, and optical properties of the synthetic polyvinyl alcohol (PVA) films. Several other similar studies have recently been reported (Nagarajan et al., 2022; Ponce et al., 2022; Santos et al., 2017).

AFW biorefineries can obtain several other integrated products with viable and sustainable advantages. Some products are useful enzymes/biocatalysts, biochar, nanocomposites, bioactive/medicinal chemicals, nano adsorbents, and many more (Cisse et al., 2022; Ezeorba et al., 2023; Okagu et al., 2021; Paul et al., 2020; Su et al., 2022; Yrjälä et al., 2022).

Table 3

A few selected recent studies on the valorization of agro-wastes to platform chemicals or their precursors.

Agrowaste	Platform chemicals	Biochemical processing or conversion	Yield	References
Corn cobs	Furfurals	Hydrolysis using an aqueous low-transition temperature mixture followed by autocatalysis and purification using hydrophobic deep eutectic solvents (HDES)	13.8 %	(Naga Sai et al., 2021)
rice straw and corncob	levulinic acid and formate	Thermochemical process	25.1 to 65.4 mM	(Moon et al., 2021)
rice straw and corncob	4-hydroxyvaleric acid (4-HV)	Enzymatic hydrogenation of levulinic acid, by 3-hydroxybutyrate dehydrogenase from <i>Alcaligenes faecalis</i>	11.32 mM and conversion rate of 48.2 %	(Moon et al., 2021)
Sweet sorghum bagasse	Succinic acid	*Conc Phosphoric acid pretreatment at a temperature of 40-85°C, *cellulase enzymatic hydrolysis to glucose *Fermentation of glucose by <i>Actinobacillus succinogenes</i> 130Z to succinic acid	130 g/L of biomass concentration yielded 29.2 g/L glucose and 17.8 g/L of succinic acid	(Lo et al., 2020)
Miscanthus straw	Succinic acid	Pretreatment by organosolv method, hydrolysis with cellulolytic enzyme cocktails and fermentation by <i>Actinobacillus succinogenes</i> 130Z	93.1 % glucose and 69.2 % xylose 75–82 % succinic acid	(Dąbkowska et al., 2019; Fatima et al., 2022)
cocoa pod husks	Bio-oils (9, 12-octadecadienoic acid and hexadecanoic acid)	Pyrolysis ≥ 500 °C	58%wt. of bio-oil, 30%wt. of bio-char	(Adjin-Tetteh et al., 2018)
cottonseed cake (CC), wheat straw (WS) and sugarcane bagasse (SB)	Lactic acids	Simultaneous saccharification by immobilize cellulase and co-fermentation by <i>Lactobacillus brevis</i>	0.22 g/g (CC), 0.49 g/g (WS), 0.52 g/g (SB) respectively	(Grewal and Khare, 2018)
coffee waste	Lactic acids	Pretreatment with H ₂ O ₂ and acetic acid, and fermentation with <i>Lactiplantibacillus plantarum</i>	22.8 g/L	(Kim et al., 2024b)
Cashew apple bagasse	Lactic acid	<i>Lactobacillus plantarum</i> FJ05311 and <i>L. plantarum</i> FJ05315	25 g/L	(Junior et al., 2024)
29.27 % dairy sludge; 24.77 % molasses; 10.49 % soybean meal	γ -Aminobutyric acid (GABA)	<i>Lactobacillus brevis</i> PML1, <i>Lactobacillus fermentum</i> 4–17, and <i>Lactobacillus plantarum</i> 1058	359.45 ppm	(Falah et al., 2022)
Citrus waste	Lactic acid	<i>Weizmannia coagulans</i>	44.8 g/L	Aulitto et al., 2024

7. Prospects for advancing AFW biorefinery

There is hope for advancing agro-biorefineries' efficiency through the use of microorganisms and enzymes, and technological advances potentiate these possibilities. Technological advances foretell prospects for agro-biorefineries (Fig. 5). Microorganisms have a wealth of molecular machines that can help valorize, with reasonable efficiency, the vast array of agro-industrial biowastes. *Saccharomyces cerevisiae* has been used to produce bioethanol from AFW like corn stover, grape pomace, and chestnut shells (Yaashikaa and Kumar, 2022). Microorganisms like *Saccharomyces cerevisiae* have enzyme toolboxes consisting of cellulases, amylases, ligninases, and many others, which help deconstruct the lignocellulosic structures in the biomass, hydrolyze complex carbohydrates to smaller units and then ferment them into a

vast array of bioproducts like ethanol, lactate, and acetate (Singh et al., 2022). Microorganisms could be manipulated generically in cases where the wild-type microbial candidates cannot metabolize key components of the biomass. Notwithstanding, we turn to the use of free enzymes due to some drawbacks of using whole cells. For instance, delay in adaptability and acclimatization, low bioavailability of substrates, susceptibility of microbial consortia to competitors and abiotic factors, etc.

7.1. Engineering the microbial consortium

Microbial consortia are used in bioconversions in biorefineries. Naturally, however, they do have some limitations. The microbial consortia used in a biorefinery may be genetically engineered to attain several pre-determined traits that make industrial operations efficient

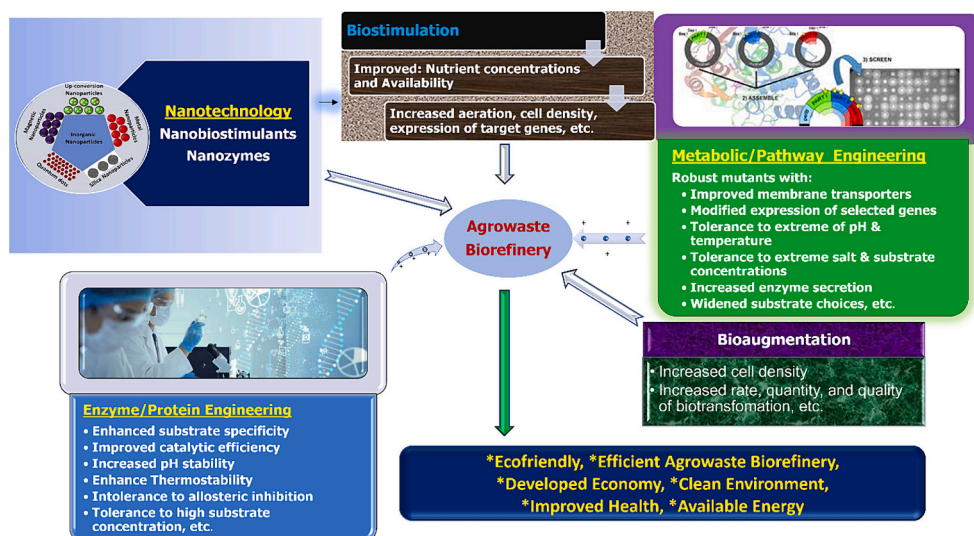


Fig. 5. Concerted effect of different technological approaches to enhancing successful eco-friendly agro-biorefinery.

(Kim et al., 2022). Sometimes, the goal of genetic engineering is to increase the rate of product formation. Wang et al. (2022) metabolically engineered *Yarrowia lipolytica* to improve the yield of scutellarin production from 15.11 mg/L to 94.79 mg/L. Increased production of enzymes like α -Galactosidase and endoxylanase by recombinant strains growing on corn cob and tofu liquid wastes have also been reported (Singh et al., 2022). Transporter engineering has been adopted to improve substrate influx in microbial cells and prevent efficiency loss in substrate uptake, especially at high concentrations. For instance, the rate of xylose transport into bacteria has been increased by modifying pre-existing hexose transporters like Hxt7, Hxt11, and Gal2 or by overexpressing heterologous transporters like Xyle from *Zymomonas mobilis* in the mutants (Kim et al., 2022). Genetic modifications may be metabolic engineering of mutants to integrate novel biochemical pathways aimed at degrading certain components of the AFW, generating mutants with rapid growth (shortened lag phase and lengthened log phase) or adaptability to extremities of pH, temperature, substrate concentration, etc. (Baptista et al., 2021; Gao et al., 2022; Yang et al., 2022b). Genetic engineering of *Escherichia coli* to hydrolyze lignin has been successfully done, whereas some microbial consortia have genetically been designed to convert CO into 3-hydroxy propionic acid (3-HP) and itaconic acid (ITA) (Cha et al., 2021). A wide range of gene-editing techniques are employed to manipulate the genomes of microorganisms. Examples are CRISPR/Cas9 technology, TALENS, gene guns, electroporation, and other recombinant DNA technology approaches (Fayyaz et al., 2020; Wang et al., 2022). The challenges facing genetic manipulation of microbial consortia for improved biotransformation include ethical concerns, limited knowledge of metabolic pathways, high cost of operation of techniques, and limited knowledge of factors controlling microbial growth and metabolism (Fayyaz et al., 2020; Srivastava and Bandhu, 2022; Xu et al., 2022). The generic heterogeneity of microbial consortia makes it challenging to control community resilience, stability, and robustness as each differs in physiology. However, integrative multi-omics and systems could be vital in curbing such challenges (Sasaki and Yoshikuni, 2022).

7.2. Bioaugmentation

Bioaugmentation may be defined as introducing autochthonous, allochthonous wild type, or genetically modified microorganisms to biomass or polluted environment to improve desired biotransformation rate, quantity, and quality (Goswami et al., 2018). Bioaugmentation is widely used in environmental bioremediation and valorizing several wastes in biorefineries. With bioaugmentation, we alter the genetic diversity by altering the microbial diversity. There are different approaches to bioaugmentation. Bioaugmentation is primarily used in concert with biostimulation (Goswami et al., 2018). Bioaugmentation may be cascaded such that a first inoculum is added, followed by successions of different other inoculant consortia, which will clear up the molecules that the preceding consortia left behind. This approach has produced biobutanol from lignocellulosic biomass (González-tenorio et al., 2020). An innovative integrated approach combining alkali pretreatment, temperature-phased aerobic digestion, and bioaugmentation techniques has been applied to produce biogas from lignocellulosic materials, up to a 47 % increase in yield (Donkor et al., 2022). Bioaugmentation has been successfully applied for the valorization of different agro wastes. *Methanoculleus bourgensis*, *Neocallimastix frontalis*, *Anaeromyces* sp., *Piromyces* sp., and *Orpinomyces* sp) have been used as inoculants in bioaugmentation to valorize cow and pig manure into biogas (Kumar et al., 2019). Production of propionic acid and other volatile fatty acids essential to several industries has been achieved by bioaugmentation, which increased the gene copy of *Propionibacterium acidipropionici* in cheese wastewater up to 20 times (Zhang et al., 2021d). Bioaugmentation is advantageous because it increases the digestibility of lignocellulosic biomass, increasing the overall efficiency of aerobic digestion (Donkor et al., 2022). Bioaugmentation has successfully been

applied in different other areas, such as valorization of household wastes into lactic acid (Zhang et al., 2022), enhanced maturation of compost from swine manure and rice straw (Wang and Liang, 2021); production of volatile fatty acids from corn stover (Murali et al., 2021); production of hydrogen from food wastes (Ortigueira et al., 2019), and in the valorization of many other agrowastes (Yaashikaa and Kumar, 2022). There are different limitations associated with bioaugmentation. Some of the factors influencing the success of bioaugmentation include the ability of the new inoculant to adapt to the new environment. This factor is compounded by the presence of competing indigenous consortia, predators, and abiotic factors, which, notably, include pH, temperature, moisture, substrate concentration, presence of toxic heavy metals, the bioavailability of metabolites, organic matter content, aeration, and nutrient content (Wang and Liang, 2021). Microorganisms are versatile enough to employ diverse adaptation strategies. However, this usually consumes time as they may have to synthesize many proteins to restructure their cell walls to control the influx and efflux of metabolites, secrete biosurfactants, and absorb heat shocks (Goswami et al., 2018). The fermentation medium's pH must be controlled, or it may affect the integrity of the products of interest (Zhang et al., 2022). Some limitations of bioaugmentation can be overcome by changing inoculum dosage, lengthened acclimatization periods, and biostimulation (Lebiocka et al., 2018).

7.3. Biostimulation

Biostimulation, the art of modifying some environmental factors of a microbial consortium to stimulate existing microbial candidates capable of biotransformation, is vital to biorefineries as it holds tremendous potential for advancing the eco-friendly operation of agro-biorefineries (Aamir et al., 2021). Biostimulation is usually done by adding various rate-limiting nutrients and different electron acceptors like P, N, O, or C. By this, the growth curve of the consortium can be altered to suit the biotransformation we want to achieve. Biostimulation can induce key enzymes to break down biomass components, shorten the lag phase, or even lengthen the exponential phase (Hiie et al., 2021). Biostimulation could be suitable for the bioconversion of oil-rich agro wastes like palm oil mill effluents. Also, biosurfactants or dispersants could be applied to solubilize the oil droplets, hence making them available to the microbiota for biotransformation into value-added products (Hiie et al., 2021). Biostimulation has been used for bioremediate pollutants such as petroleum hydrocarbons, pesticides, herbicides, and explosives. Biostimulation has been used to enhance biogas production from agro wastes. The biostimulants may be encapsulated in nanomaterials to enhance their uptake by the microbial cells (Abdelsalam and Samer, 2019). Aamir et al. (2021) reported a 1.9–2.4 % increase in hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) degradation rate through biostimulation. In some instances, lignocellulosic materials like sugar cane filter cake have been used as biostimulants to enhance the removal of pesticides from soils (Bhatt et al., 2021). Microbial biostimulation has been used to improve the treatment of pulp and paper industry wastewater (Ram et al., 2020). Biostimulation has been successfully increased, breaking down petroleum hydrocarbons by up to 57 % (Feng et al., 2021). Nitrate amendment has been used in aerobic and anaerobic consortia to degrade petroleum hydrocarbons (Sarkar et al., 2020). One setback of biostimulation, as practiced in biorefineries, is that it may be costly as there may be a need for continuous addition of stimulants like lactate and the rapidly depleted major inorganic nutrients (Ławniczak et al., 2020). Secondly, biostimulation may not be specific. There may be challenges involving substrate competition, strain compatibility, exchange of metabolites among various pathways, and reproducibility (Bhatt et al., 2021). The rapid growth of some species may lead to the elimination of others due to competition and the release of defense chemicals, limiting the diversity of enzymes required for total bioconversions of the feedstock. The limited knowledge about the ratios of the biostimulants and their diffusion mechanisms may hamper

biostimulation. Moreover, in ex-situ practices, the microbial consortia, not now in their native environment, may be difficult to control; i.e., there may be a need to control some environmental factors that call for higher expenses. There are some proposed approaches to advance biostimulation for application in agro-based biorefineries. Biostimulation could be applied in agro-biorefineries in feedstock pretreatment to remove toxic components like petroleum hydrocarbons and pesticides (Aamir et al., 2021). Moreover, it could be used to clean up environmental pollution caused by the biorefineries, or the potential it holds could be imported into in-situ or ex-situ valorization of AFW. If biostimulation is combined with techniques like bioaugmentation, nanotechnology, and genetic engineering, there might be better results (Okeke et al., 2022a, 2023).

7.4. Others

Nanotechnology may be employed to enhance bioconversions in agro-based biorefineries. Cu nanoparticles with peroxidase mimetic properties have been used for the aqueous phase conversion of fructose to levulinic acid (Thiyam et al., 2018). Nanozymes may help circumvent the demerits of enzyme immobilization in the future. Laccase-mimicking enzymes with high chemo selectivity for lignin have been used to cleave lignin into oligomers (Yang et al., 2022a). Nanotechnology has been used in the valorization of industrial wastewater (Ali et al., 2021), the immobilization of enzymes (Khoshnevisan et al., 2019), and the conversion of lignin to UV-protective nanomaterials (Kaur et al., 2021). Some demerits of nanotechnology include nanotoxicity and low yield of nanozymes.

Protein engineering and enzyme immobilization may also be exploited to achieve eco-friendly circular bioeconomic perspectives in agro-based biorefineries. Enzyme immobilization may be covalent or entrapment, which confers reusability to the enzymes. In contrast, enzyme engineering may be by directed evolution, rational design, or combining the two. It improves pH profile, substrate specificity, thermostability, solvent tolerance, ability to use novel cofactors, and the introduction of novel catalytic abilities or tolerance to extreme salt concentrations (Bernala et al., 2018). Computational modeling may have also been significant in generating highly efficient carbohydrate-active enzymes (Mendoza and Masgrau, 2021). Random mutagenesis, targeted mutagenesis, rDNA technology, and computational strategies for protein design have been used to design novel biocatalysts (Madhavan et al., 2021).

8. Conclusion

In conclusion, the valorization of agro-food waste (AFW) presents a promising avenue for sustainable fuel and chemical production through circular biorefineries. Across various sections discussed, it's evident that advancements in microbial engineering, biotechnology, and process optimization are driving significant progress in maximizing the efficiency and viability of AFW valorization. Firstly, the production of bioethanol and biodiesel from agro-food wastes (AFW) presents a sustainable solution for waste management while also contributing to the renewable energy sector. Various AFW sources, including rice husk, corn stover, and non-edible seeds, have been investigated as feedstocks for biofuel production, with optimized fermentation processes and pretreatment methods leading to increased yields. Pervaporation techniques have also been employed for the purification and recovery of bioethanol from fermentation broths, enhancing the overall efficiency of the process. Secondly, biodiesel production from AFW is being enhanced through enzymatic and microbial processes. By leveraging oil-rich AFW sources and employing microorganisms like *Bacillus amyloliquefaciens*, researchers are achieving substantial yields of fatty acid methyl esters (FAMES), offering a sustainable alternative to traditional biodiesel feedstocks.

Furthermore, the engineering of microbial consortia, coupled with

bioaugmentation and biostimulation techniques, is enabling the efficient conversion of AFW into valuable bioproducts. Genetic modifications and environmental manipulations are enhancing microbial performance, leading to improved biotransformation rates and product yields. Moreover, advancements in nanotechnology and enzyme immobilization are further enhancing the efficiency of AFW valorization processes. Nanozymes and engineered enzymes are enabling precise catalysis and waste conversion, contributing to the development of eco-friendly and economically viable biorefinery systems.

Looking ahead, future studies in AFW-biorefineries should focus on addressing key challenges such as environmental sustainability, cost-effectiveness, and scalability. Integrating multi-omics and systems biology approaches could provide insights into microbial community dynamics and metabolic pathways, enabling more efficient biotransformation processes. Additionally, exploring innovative biostimulation techniques, nanomaterial design, and enzyme engineering strategies will further advance the field towards sustainable and economically viable agro-biorefinery systems. Ultimately, continued research efforts in this area will contribute to the development of a circular bioeconomy, utilizing AFW as valuable resources for renewable energy and bio-based product production.

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Timothy Prince Chidike Ezeorba: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Emmanuel Sunday Okeke:** Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Mida Habila Mayel:** Writing – original draft, Visualization, Investigation, Formal analysis. **Charles Ogugua Nwuche:** Supervision, Project administration, Conceptualization. **Tobechukwu Christian Ezike:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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