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Advancing Sustainable Lactic Acid Production: A Mini Review of Biomass Substrates, Fermentation Techniques, and Industrial Applications

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ABSTRACT

Lactic acid (LA) has garnered global recognition for its versatile industrial applications, especially as a precursor to polylactic acid (PLA), a biodegradable polymer. This mini review explores sustainable LA production using *Rhizopus sp.,* with emphasis on the utilization of lignocellulosic agricultural residues as low-cost carbon sources. Conventional substrates such as glucose offer high yields but are economically and environmentally unsustainable. Instead, agro-wastes like empty fruit bunches, cocoa pod husks, cassava peels, coconut husks, and banana peels are rich in cellulose and hemicellulose, making them promising feedstocks. However, their complex structures necessitate pretreatment methods, including ethylenediamine (EDA) delignification, to enhance fermentability. The morphology of *Rhizopus*—pellet versus filamentous form—further influences LA yields, with pelletized forms offering higher productivity. Morphological control through fermentation parameters and neutralizing agents (e.g., $CaCO_3$) has shown significant effects on LA synthesis. Despite its promise, very few explorations have been made in this topic in recent years due to technical complexity, methodological challenges, and limited applicability across industries. Resource constraints and the need for novel experimental techniques or substantial funding have further hindered comprehensive investigations. Nevertheless, this scarcity of research also underscores the value of this field in niche applications where sustainable bioprocessing is critically needed. This review emphasizes the need for integrated approaches combining biomass pretreatment, optimized fermentation conditions, and morphology control to improve LA yields. Ultimately, advancing sustainable fungal LA production supports waste valorization, circular economy goals, and green technology development in agriculture and industry.

Keywords: lactic acid; *Rhizopus*; fungal fermentation; lignocellulosic biomass; sustainable biotechnology; morphological control

1.0 LACTIC ACID (LA)

Carl Wilhelm Scheele, a Swedish scientist, made the initial discovery of LA in 1780, also known as milk acid, which is a hydroxycarboxylic acid [1]. Many industries, including the food, pharmaceutical, and cosmetic industries, use LA [2] as LA is resurfacing in the polymer market as a promising potential feedstock for biodegradable polymers owing to increased environmental concerns [3]. With an annual global manufacturing volume of 370,000 metric tons, lactic acid (2-hydroxy propionic acid) is one of the most abundant microbial chemical compounds. In the past, acidity and preservation were the primary uses of LA in foods.

In addition to many other industries, LA is used in cosmetics, pharmaceuticals, and leather tanning [4]. The increasing market demand for LA in sectors such as bioplastics, pharmaceuticals, and food preservation are driving the need for more sustainable production methods. Conventional LA production relies heavily on refined sugars and starches, which can compete with food resources and incur higher production costs. This has led to growing interest in alternative raw materials, particularly lignocellulosic substrates, which are abundant, renewable, and do not compete with food supplies. Utilizing lignocellulosic biomass, such as agricultural residues, not only provides low-cost feedstock but also aligns sustainable production goals by reducing waste and lowering environmental impact. This shift toward renewable substrates could significantly enhance the economic and environmental sustainability of LA production, supporting its expansion across various industries.

The global LA market is predicted to reach USD 5.02 billion by 2028. It is predicted to grow at a compound annual growth rate (CAGR) of 8.0 % from 2021 to 2028 [5]. As previously stated, commercial raw materials utilized in LA manufacturing are generated from feedstocks, such as agricultural by-products. Nonetheless, these sources are insufficient because they must compete with demand from other industries, particularly the food processing and everyday consumption industries.

LA has been made for generations from either pure sugars or edible plants. However, the price of raw materials is a significant issue in LA manufacturing. As a result, the price of LA has increased. Furthermore, the price of LA is affected by the quality of the feedstock used in its manufacturing. Therefore, to ensure the success of LA commercialization, it is necessary to investigate dependable and less expensive raw materials and optimize the bioconversion conditions. LA serves as a monomer for PLA, a green or biodegradable product that emphasizes low-carbon activities [6]. While looking for an alternative raw material that is cheap and environmentally friendly, it was discovered that natural resources, particularly biomass (plant fiber), can meet these criteria. Furthermore, Malaysia cheaply exports cassava starch to China and other large corporations to synthesize LA. However, Malaysia imports LA exorbitantly. Therefore, a solution to this problem is urgently required. In Malaysia, most industries are still in the low value-added category, with low technology adoption. Thus, with this development, the diversity of products and services will reach an optimum level, and reliance on poverty-based exports will be reduced, in accordance with the Key Economic Growth Activities (KEGA) 7 of Malaysian Vision 2030 [7]. KEGA 12 – This study is relevant to the government policy of KEGA 12 of the green economy, which includes operations centered on plastic alternative products.

The majority of LA is produced via microbial fermentation of carbohydrate-rich feedstocks. LA bacteria (LAB) are the primary microorganisms used for LA production due to their ability to efficiently convert sugars into LA. The LA production process typically involves the following steps: pretreatment of the feedstock to release fermentable sugars fermentation of the sugars by LAB to produce LA, and downstream processing to purify and concentrate the LA [8]. The key steps in this process are:

1. Pretreatment: The feedstock, such as carbohydrate-rich biomass, undergoes pretreatment to break down the complex structures and release the fermentable sugars. This can involve physical, chemical, or enzymatic methods [9].
2. Fermentation: LABs are used to ferment the released sugars and convert them into lactic acid. LABs are the primary microorganisms utilized for LA production due to their ability to efficiently carry out this conversion [10].
3. Downstream Processing: After fermentation, the LA-containing broth undergoes purification and concentration steps to isolate the lactic acid product. This may include processes like filtration, distillation, or ion exchange chromatography [11].

Understanding the production mechanism of LA is crucial for optimizing its industrial applications, particularly in the fields of biodegradable plastics, pharmaceuticals, and food processing. Efficient LA production supports the development of sustainable bioprocesses, reducing dependency on petroleum-based resources and lowering environmental impact. As global demand for eco-friendly materials rises, enhancing LA yields from renewable biomass sources offers a promising pathway for sustainable and scalable production. Insight into the biochemical transformations during LA synthesis enables targeted optimizations, which can improve process efficiency and reduce waste, aligning with the principles of green chemistry and the circular economy.

1.1 Mechanism of LA Production

LA production typically involves fermentation of carbohydrates by LAB such as *Lactobacillus*, *Streptococcus*, and *Bifidobacterium*. The process starts with the selection of substrates, such as glucose, sucrose, lactose, or starch. Microorganisms convert these sugars to LA under anaerobic conditions. The general reaction is based on Equation (1):

$$\underset{\text{(Glucose)}}{C_6H_{12}O_6} \rightarrow 2\underset{\text{(LA)}}{C_3H_6O_3} \quad (1)$$

LA production involves several key steps, starting with pretreatment, where complex carbohydrates such as cellulose are broken down into fermentable sugars using physical, chemical, or enzymatic methods. This is followed by fermentation, where lactic acid bacteria (LAB) such as *Lactobacillus* (*L. plantarum*, *L. delbrueckii*), *Streptococcus* (*S. thermophilus*), and *Bifidobacterium* species convert sugars, such as glucose, sucrose, lactose, or starch, into LA. This fermentation typically occurs at a pH of 5.0-6.5 and temperatures between 30-45°C. Once fermentation is complete, LA is separated from the fermentation broth and purified for use [12]. Common substrates for the process include simple sugars, such as glucose and sucrose, as well as complex carbohydrates, such as starch and cellulose. The by-products of this process include acetic acid, ethanol, and carbon dioxide. However, this process has limitations, such as the high cost of pure substrates [12] the presence of inhibitory compounds in substrates that can affect the efficiency of LAB [13] and the need to maintain optimal conditions to maximize LA yield.

Overall, efficient LA production relies on the selection of suitable substrates, optimization of fermentation conditions, and effective management of by-products and inhibitors.

1.2 Lignocellulosic Substrates

Due to the growing awareness of environmental issues, several industries are making significant efforts to valorize lignocellulosic crop waste. Recognizing this trend, researchers are focusing on developing LA from natural fibers and eco–friendly products to replace synthetic and chemical-based products. The demand for LA has significantly increased due to its potential as a monomer in creating biopolymers, which are utilized as a substitute for synthetic polymers generated from petroleum resources [14].

Due to its abundant nature, cellulose is a possible and potential natural resource that can be used in utilizing LA since it contains glucose. Thus, using cellulose biomass as an alternative raw material in the manufacturing of LA is a solution for minimizing waste disposed of in the agricultural sector. However, due to the structural components' cellulose, lignin, and hemicellulose, agricultural biomass possesses a complex and intricate structure. This complexity poses challenges in directly utilizing such biomass to produce LA. Therefore, cellulose biomass cannot be directly employed to synthesize LAs. Biomass must first be processed to liberate glucose, eliminate lignin and hemicellulose, and decrease cellulose. Physical, chemical (acid and alkaline), and biological treatments are all accessible; however, chemical treatment is the most widely employed. There are many other types of treatment methods accessible, including physical, chemical (acid and alkaline), and biological treatment; nevertheless, chemical treatment is the most widely used by many researchers because it is less expensive than the other methods [12].

1.2.1 Palm oil- empty fruit bunch (EFB)

Malaysia's palm oil industries generate a substantial quantity of biomass, particularly after pruning or harvesting the fresh bunch, being the world's second-largest producer and exporter of palm oil behind Indonesia. The palm oil biomass comprises discarded EFB [15]. Approximately 20-23 tons of non-productive oil palm tree products are produced yearly in Malaysia. Some of their outer layers are removed and utilized to make plywood and pellets, but 99% of this resource is burned or dumped. Using agricultural by-products as a carbon feedstock for LA production provides an economical solution to create value-added products while preserving good environmental practices [16]. LA production from microwave-alkali pre-treated EFB using recombinant *Rhizopus oryzae* pellet has been studied by [17]. The findings demonstrated that by employing fungi pellets, microwave-assisted pre-treated EFB produces more lactate than clump *Rhizopus*. After 96 hours of culture, the lactate yield was 0.77 g/g; meanwhile, the productivity of the *Rhizopus oryzae NRRL* was 0.089 g/L in clump form and 0.099 g/L in pellet form. The most recent similar publication studied the same microwave-assisted method using EFB to upscale LA was done by [18] aimed to scale up LA production using microwave-alkali-pretreated EFB from palm oil biomass. The scaling-up process moved from a 16-liter to a 150-liter scale using *Rhizopus oryzae NRRL 395,* focusing on maintaining a constant volumetric oxygen mass transfer coefficient (k_La). The study found that optimal LA production at the larger scale required adjusting aeration rates to maintain k_La values like those on the smaller scale. At the 150-liter scale, an aeration rate of 0.5 vvm was optimal, resulting in a lactic acid yield of 6.8 g/L, matching the efficiency of the smaller 16-liter scale. The first study to explore the fermentation of EDA-pretreated EFB with *Rhizopus sp.* was done by [19]. The HPLC study found notable variations in EFB's sugar content both before and during EDA treatment. Glucose was present in low concentration of 0.02% before treatment; other sugars like fructose (0.70%) and maltotriose (0.33%) were also found. Glucose concentration dropped even more to a minimal level following EDA pretreatment (5.5% EDA at 30°C), but maltotriose rose significantly to 5.3%, and maltose, before undetectable, showed at 1.10%. The total removal of fructose and the conversion of simple sugars into oligosaccharides point to considerable structural change and reorganization of carbohydrate content resulting from the EDA treatment. These findings show that although glucose was first present, the pretreatment preferred the conversion of glucose and other monosaccharides into more complex sugar forms, or their fast consumption during the enzymatic hydrolysis phase, therefore restricting free glucose availability post-treatment [19].

1.2.2 Cocoa pod husk (CPH)

Malaysia produced 537 tons of cocoa fruits in 2021 [20]. Malaysia, the sixth in the world, is one of the major hubs for grinding and processing cocoa beans [21]. The biomass waste from the cocoa business that results from the digesting process is called cocoa pod husk (CPH). It makes up roughly 70–75% of the fresh cocoa fruit. CPH contains active alkaloids that are considered to act as inhibitors of the microbes utilized in LA synthesis [13]. The study explores how alkaloids affect the fermentation process with *Lactobacillus plantarum* bacteria to produce

LA from CPH. It compared substrate consumption (glucose), cell dry weight, and LA generation during 48 hours at 50°C and 100 rpm agitation. Alkaloids alter product growth patterns, resulting in a consistent maximal growth rate (0.69 h^{-1}) and substrate inhibition constant (3.89 g/L) that remains unaffected by inhibitors. Despite alkaloids, the study underlines CPH's potential for LA generation. However, no research on CPH fermentation using fungi has been studied.

1.2.3 Coconut husk (CH)

With most of the plantations in Sabah and Sarawak, coconuts are Malaysia's fourth largest industrial crop after oil palm, rubber, and rice. The Malaysian Agricultural Research and Development Institute (MARDI) reported that Malaysia is one of the top 10 producers of coconuts in the world [22]. In Malaysia, coconut waste has grown to represent a significant volume of waste. Although CH is a biodegradable material, it takes a long time to degrade because it is sturdy and resilient. In addition, CH has been recycled into handicrafts, natural ropes, and mattress [23]. For the utilization of lignocellulosic residues from CHs in the synthesis of LA, the structural complexity of lignocellulosic biomass, typically called recalcitrance, hinders microbiological and enzymatic treatments. CH pretreatment using ethylenediamine (EDA)has not been studied yet in *Rhizopus* fermentation.

1.2.4 Cassava peels (CP)

In the Southeast Asia region, Malaysia produces a small amount of cassava. Cassava waste is produced in large quantities in Malaysia because there are many cassava plantations. Cassava is harvested in Malaysia from a 39 000-acre area per year for 400,000 tons of cassava production [24]. Johore accounts for about 83 % of the production. Nevertheless, it may be assumed that Malaysia will always consume some domestically because cassava appears to be a typical traditional food source for Malay groups [25]. Other than that, cassava is grown mainly in Malaysia for its starch. CP also contains high starch residues resulting from the peeling process. CP, which makes up roughly 10% of the raw root's weight is frequently discarded, thus contributing to soil acidification and waste issues [26]. Researchers looked at L-LA production by utilizing cultures of *Rhizopus oligosporus* to harness CPs. The best LA production was recorded at 50.2 g/100 g of hydrolyzed CP substrate [27]. Similar efforts using *Lactobacillus casei* ATCC334 were made to develop a purification and recovery method that would be less expensive than those that are now in use. Unhydrolyzed substrate produced 4.80% of the yield, alkali hydrolysate produced 4.75%, and acidic hydrolysate produced 10.53 %wt. The most excellent LA output was achieved at pH 6.0, 2.0% v/v inoculum size, 25% w/v substrate concentration, and 5% nitrogen supply concentration [26]. However, most research did not correlate with the morphology formation, LA production yields, and EDA pretreatment method simultaneously. Thus, our study covers the discovery of utilizing these agro-wastes to achieve our objectives.

1.2.5 Banana peels (BP)

BPs have been characterized and found to contain promising sugar that can be used to produce LA. In Malaysia, 26,210 ha of banana plantations and 313,811 tons of bananas were produced in 2021 [28]. Bananas from Malaysia are exported to Singapore, Brunei, Hong Kong, Japan, and other regional nations [29]. After harvest, about 60% of the biomass from bananas is wasted. Banana waste loss amounts to roughly 114.08 million metric tons worldwide, contributing to environmental issues such as the excessive emission of greenhouse gases [30]. With the help of various processes, including bacterial fermentation and anaerobic degradation, these wastes can be converted into bioplastics, organic fertilizers, and biofuels like ethanol, biogas, hydrogen, and biodiesel. They also contain a high concentration of materials of utmost industrial importance, such as cellulose, hemicellulose, and natural fibers [31]. Recently a study by [32] recorded the conditions of pH 4.5, temperature of 7 °C, and inoculum size of $1x10^4$ spores/g resulted in the greatest LA production of $8.13x10^{-2}$g/L. Consequently, producing LA by fermentation of banana peels using *R. oryzae* could be a potential strategy, especially with the lack of EDA integration in the pretreatment process.

As shown in Figure 1 and Table 1, the primary components of these agricultural wastes are rich in cellulose and hemicellulose, making them the preferred raw materials for *Rhizopus* productions of LA since they include many fermentable sugars.

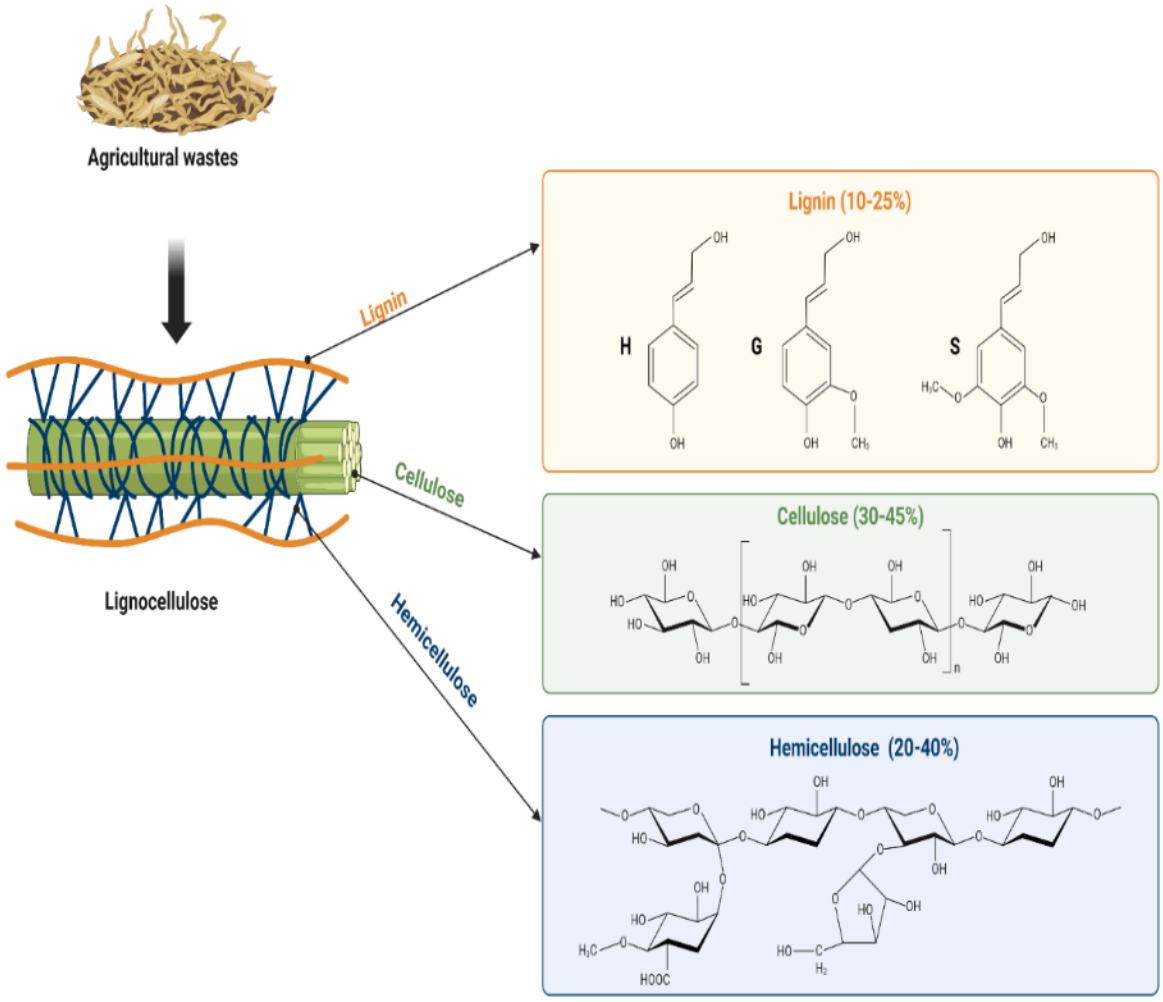

Figure: 1 Main components in agricultural waste (Illustrated using Biorender app).

Table 1 Proximate composition of EFB, CPH, CP, CH, and BP.

Substrates	Content						Ref.
	Protein (%)	Hemi-cellulose (%)	Cellulose (%)	Lignin (%)	Crude Fiber (%)	Sugar (%)	
EFB	4.87	36.60	28.30	35.00	67.00	6.00	[33], [34]
CPH	6.80	26.10	12.80	28.00	46.6	10.0	[35], [36]
CP	3.00	29.81	5.04	4.81	15.8	7.5	[37] [38]
CH	1.12	31.10	42.56	44.68	32.39	0.26	[39]
BP	0.90	41.38	9.90	8.90	31.70	14.0	[40]

2.0 Lignocellulose Substrates Pretreatment

Lignocellulosic biomass, obtained from plant materials such as wood, agricultural leftovers, and grasses, is an abundant and renewable raw resource to produce biofuels, biochemicals, and other important goods [41]. However, the complex and resistant structure of lignocellulose, which is predominantly composed of cellulose, hemicellulose, and lignin, presents substantial hurdles to efficient conversion procedures. Pretreatment is an important step in the bioconversion of lignocellulosic biomass, since it disrupts the intricate matrix of these components, increasing the accessibility and digestibility of cellulose and hemicellulose.

2.1 Physical Pretreatments

Mechanical comminution, which involves advanced milling and grinding techniques, has seen significant improvements aimed at enhancing the efficiency of biomass size reduction while reducing energy consumption [42]. This pretreatment employs mechanical force to disrupt the main cell wall, hence enhancing cellulose accessibility for subsequent treatments. Consequently, the specific surface area increases while crystallinity diminishes (assessed by the crystallinity index: CrI), thereby enhancing the overall efficiency of the final process. The significance of pretreatment is paramount, as enhancements in subsequent processes will result in decreased equipment size, less residues, and shorter residence time [42]. These advancements have allowed for more effective processing of materials, leading to better preparation for subsequent treatments like biochemical or thermochemical conversions. However, despite these improvements, one major disadvantage remains: the process still demands high energy consumption. This drawback limits the overall sustainability and cost-effectiveness of mechanical comminution, especially when scaling up for industrial applications [43]. The integration of ultrasonic and microwave pretreatments has also proven to be highly effective in enhancing the disruption of lignocellulosic structures, which improves enzyme accessibility and boosts sugar yields [44]. These methods offer a significant advantage in terms of accelerating the breakdown of complex biomass components, making the process more efficient. However, despite these benefits, a notable disadvantage is the high energy consumption associated with both ultrasound and microwave technologies. This limitation poses challenges for their widespread adoption, particularly in large-scale industrial settings where energy efficiency is a critical factor.

2.2 Chemical Pretreatments

Refinements in acid (e.g., sulfuric acid) and alkali (e.g., sodium hydroxide) pretreatment methods have significantly improved their efficiency by reducing the degradation of sugars and minimizing the formation of inhibitory by-products [45]. These improvements have made these methods more effective for biomass processing, particularly in increasing the yield of fermentable sugars for biofuel production. However, several disadvantages persist. The corrosive nature of acids and alkalis can lead to equipment degradation over time, increasing maintenance costs [46]. Additionally, the handling and disposal of these chemicals raise environmental and safety concerns. Furthermore, if not properly controlled, there is still potential for the formation of inhibitory by-products, which can negatively impact downstream processes such as fermentation [47]

Ionic liquids and deep eutectic solvents have also attracted significant attention for their ability to dissolve lignin and hemicellulose, thereby increasing the accessibility of cellulose for enzymatic hydrolysis [48]. This capability enhances the overall efficiency of biomass pretreatment, offering a promising alternative to traditional methods. However, these solvents come with several disadvantages. The high cost of ionic liquids and deep eutectic solvents makes large-scale implementation economically challenging. Additionally, the recycling and recovery processes for these solvents are often complex and energy-intensive, which can further limit their industrial viability. Furthermore, some ionic liquids pose potential toxicity and environmental risks, raising concerns about their long-term sustainability [49].

Finally, organosolv pretreatment, which employs organic solvents like ethanol and acetone to solubilize lignin, has been optimized to enhance lignin recovery and improve downstream processing efficiency [50]. This method offers the advantage of producing high-quality lignin while making cellulose more accessible for enzymatic conversion. However, the use of organic solvents presents several challenges. These solvents can be costly, and their flammability and potential toxicity require stringent safety measures during handling and processing. Additionally, the recovery and recycling of the solvents add complexity and increase operational costs. Moreover, any residual solvent left in the biomass can interfere with downstream processes, potentially hindering fermentation or enzyme activity [51].

2.3 Biological Pretreatments

Microbial and enzymatic methods, which utilize fungi, bacteria, and their enzymes (e.g., laccases, cellulases), have significantly advanced in terms of enzyme production, stability, and activity for lignin degradation and cellulose hydrolysis. These biological approaches offer a more environmentally friendly alternative to chemical and physical pretreatments, with the added benefit of selective breakdown of biomass components [52]. However, there are notable disadvantages. The reaction rates of microbial and enzymatic methods are relatively slow compared to physical and chemical processes, which can limit their efficiency. Additionally, these methods are highly sensitive to environmental conditions such as temperature and pH, requiring precise control to maintain optimal [52] Furthermore, the costs associated with enzyme production and purification remain high, posing economic challenges for large-scale applications [53]. A study done by [54] used laccase and cellulase to enhance saccharification and bioethanol production from rice straw. The laccase-mediated pretreatment resulted in a 6.7% delignification of rice straw. The process also enhanced accessibility of cellulose and hemicelluloses. The single-step simultaneous delignification, saccharification, and fermentation method yielded 26.7 ± 0.2 g/L of sugar and 6.47 ± 0.16 g/L of ethanol, making it an appropriate single-step method.

Genetically engineered microorganisms have been also developed with enhanced lignocellulose-degrading capabilities, thanks to advances in genetic engineering. These engineered strains can break down biomass more efficiently, offering a promising approach to improving the overall process of biofuel production and biomass utilization. However, there are significant disadvantages associated with this technology [55]. Regulatory and public acceptance issues surrounding genetically modified organisms (GMOs) can create barriers to widespread adoption [56]. Additionally, the development and maintenance of genetically engineered strains involve high costs and complexity, making the technology less accessible for industrial use ([57]. There are also concerns about potential risks such as genetic drift or unintended environmental impacts, which must be carefully managed to avoid negative consequences on ecosystems [58].

2.4 Combined Pretreatments

Hybrid approaches, which combine different pretreatment methods (e.g., chemical and physical), have demonstrated synergistic effects that improve overall efficiency and reduce the severity required for each individual method [59]. These combined approaches offer the potential to maximize biomass breakdown while minimizing the drawbacks associated with single-method pretreatments. However, hybrid methods also come with notable disadvantages. The integration of multiple techniques increases the complexity of the process, requiring more sophisticated equipment and expertise [60]. This added complexity can lead to higher capital and operational costs, making the approach less economically feasible for some applications. Moreover, careful optimization is essential to balance the synergistic effects while minimizing negative interactions between the different methods, which can be challenging and time-consuming [61].

Meanwhile, the sequential pretreatments, where methods such as alkaline treatment are followed by acid pretreatment, have been optimized to maximize sugar yields while minimizing

the formation of inhibitory by-products. This approach allows for a more thorough breakdown of biomass components, improving overall efficiency in processes like biofuel production. However, sequential pretreatments also come with certain disadvantages. The process time and complexity increase due to the need for multiple stages, which can slow down production. Additionally, cumulative energy consumption and associated costs may rise, reducing the economic viability of the method. Careful control and optimization are essential to prevent the formation of inhibitors, adding further challenges to process management.

Table 2 summarizes the existing strategies for lignocellulosic biomass pretreatment, highlighting its underlying key findings. Understanding these methodologies is critical for optimizing pretreatment procedures and moving forward with the development of sustainable pretreatment.

Table 2 Current Techniques in Lignocellulosic Biomass Pretreatment

Pretreatment Methods	Plant source	Method	Key Findings	Ref.
Physical Treatments				
Torrefaction	ash-wood (*Fraxinus ssp.*), beech (*Fagus ssp.*), miscanthus (*Miscanthus ssp.*), pine (*Pinus pinaster*) and wheat straw (*Triticum ssp.*)	Heated storage loop system to sample volatile species released at 11 different temperatures.	• The extraction of different biomass samples led to a higher detection of 23 species than previous studies with commercial compounds. • The optimized extraction procedure preserved the properties of the extracted fractions, resulting in lower volatile species release. • The study assessed volatile species production profiles based on literature formation mechanisms and correlated polysaccharide-based fractions with their sugar composition.	[62]
Mechanical Comminution	Corn stover, sycamore branch, and moso bamboo	Entrained-flow gasification and multi-staged comminution	• Threebiomasses (corn stover, sycamore branch, and moso bamboo) revealed that two-stage milling decreased energy consumption by 27.98% and 16.84%, respectively, in comparison to single-stage milling. • The energy of Moso bamboo diminished by 8.59% as a result of the energy required to separate and fracture its robust fibres. •	[63]
Chemical Treatments				
Acid and Alkali Pretreatments	Corn stover	Pretreatment with dilute acid (DA) and alkaline sodium sulfite (ASS)	• Resulted in 83.8% lignin removal • Cellulose conversion and ethanol concentration reaching 86.6% and 50 g/L, respectively, after 72 hours of	[64]

			enzymatic hydrolysis and fed-batch semi simultaneous-saccharification and fermentation	
Ionic Liquid (IL) and Deep Eutectic Solvents (DES)	Sawmill chips, grass, walnut wood, walnut leaves, walnut shells, walnut shells, and rye straw	DES: 1-methylimidazole IL: Choline chloride	• Transformed ~78% of the cellulose present in the pretreated biomass	[65]
Hybrid Approaches				
Physical + Chemical Pretreatment	Wheat straw	Ultrasound pretreatment combined with alkaline NaOH	• Effectively delignified wheat straw, enhancing enzymatic hydrolysis and sugar yield • Achieved 59.2% lignin removal and a 68 ± 5.7% sugar yield	[66]
Chemical + Biological Pretreatment	Rice straw	Utilizing urine-prepared NaOH solution (NUr)	• Significantly improved hydrolysis rates and hydrogen production. • The peak biohydrogen yield was 184.46 mL/g substrate, with 86.98% holocellulose retention and 59.84% lignin removal. NUr pretreated rice straw also increased cellulose and hemicellulose exposure, and its energy conversion efficiency was 10.38%, surpassing raw rice straw by 62.19%.	[67]
Other Techniques				
Organic acid	Bamboo	Mandelic acid (MA), a natural and eco-friendly organic acid	• Under optimized conditions, a substantial yield of 65.9% xylooligosaccharides (XOS) was achieved, with a cumulative yield of 43.5% for xylobiose and xylotriose. • The recovered lignin showed a purity of 95.2% and improved crystallinity and accessibility. • The combined strategy provided an effective method for co-producing XOS, lignin, and glucose from bamboo.	[68]
Hydrothermal Pretreatment	Wheat straw	Combination of a multi-effect evaporator and	• The hydrothermal concept can be executed energy-efficiently without supplementary	[69]

		pressure-driven ultrafiltration	auxiliaries, with specific energy needs for pretreatment and hydrolysate processing being 0.28 kWh/kg and 0.13 kWh/kg dry wheat straw mass respectively. • The integration of a multi-effect evaporator with pressure-driven ultrafiltration can decrease heating and cooling energy consumption by 29% and 44%, respectively.	

3.0 Key Challenges and Future Directions of Lignocellulose Pretreatment

The pretreatment of lignocellulosic biomass is essential in converting biomass into biofuels and chemicals. Despite breakthroughs in various pretreatment technologies, some obstacles still need to be overcome, impeding these processes' economic and technological viability. This section discusses the critical obstacles in lignocellulose pretreatment and future directions for addressing these issues to improve efficiency, lower costs, and minimize environmental impacts.

- **Inhibitor Formation**: Reducing the formation of inhibitory compounds during pretreatment remains a significant challenge. For instance, acid processes produce many inhibitory byproducts and require corrosion-resistant equipment. While the alkali method boasts gentler conditions and effectively breaks down the lignocellulose structure to yield more cellulose and hemicellulose [70], it requires the removal of excess salts from the biomass, resulting in heightened costs.
- **Cost and Scalability**: Developing cost-effective and scalable pretreatment technologies is crucial for commercial viability.
- **Sustainability**: Ensuring the environmental sustainability of pretreatment processes by minimizing energy consumption and chemical use is essential.
- **Integration with Downstream Processes**: Seamless integration of pretreatment with downstream processes (e.g., enzymatic hydrolysis, fermentation) to optimize overall biomass conversion efficiency [71].

Among other pretreatment techniques, alkaline pretreatment is one of the most powerful methods that can improve the enzymatic hydrolysis of fibers by disrupting lignin and breaking the bond of lignin carbohydrates [72]. This method also showed the advantages of effectively reducing the crystallization rate by swelling, excluding the acetyl group and uronic acid replacements from hemicelluloses, and breaking down the ester bonds. It is based on the meta-review analysis by [73]. Table 3 compares the alkaline treatment and acid methods. This table compares various parameters, such as cellulose content increase, hemicellulose content reduction, lignin content reduction, fermentable sugar yield, ethanol yield, formation of inhibitory compounds, cost, and reaction time, between acidic and alkaline pretreatment methods.

Thus, alkaline pretreatment is more effective in removing lignin and increasing fermentable sugar yield, leading to higher product yields. In contrast, acidic pretreatment is less effective and often results in the formation of undesirable inhibitory compounds at higher temperatures. Despite being costlier and requiring longer reaction times, the efficiency of alkaline pretreatment in producing higher sugar yields makes it more favorable for commercial applications. Additionally, alkaline pretreatment operates under conditions more favorable for preserving and converting biomass components into ethanol. In contrast, acidic pretreatment

requires careful management to avoid inhibitory compounds, which can negatively impact the overall efficiency of the production process [73].

Table 3 Comparative Analysis of Acidic and Alkaline Pretreatment Techniques [73].

Parameter/Attributes	Acidic Pretreatment	Alkaline Pretreatment
Cellulose Content Increase	Moderate	High
Hemicellulose Content Reduction	High	Moderate
Lignin Content Reduction	Moderate	High
Fermentable Sugar Yield	Moderate	High
Ethanol Yield	Moderate	High
Formation of Inhibitory Compounds	High	Low
Cost	Lower	Higher
Reaction Time	Shorter	Longer

4.0 Ethylenediamine (EDA)retreatment

The main hurdle of inorganic alkaline pretreatment is the release of large amounts of salt from black liquor. Black liquor is a byproduct of pulp mills that produce tree-based products like paper [39]. To overcome this problem, biomass was pretreated by the organic amine ethylenediamine (EDA) [74]. Additionally, EDA has been generally used to convert cellulose allomorph. The ecological information indicates that EDA does not contain components that have endocrine-disrupting properties at levels of 0.1% or higher. Additionally, the substance/mixture does not contain components considered to be persistent, bio-accumulative, and toxic or very persistent and bio-accumulative at levels of 0.1% [40]. Table 4 is the basic information on the physical and chemical properties of EDA:

Table 4: Physical and Chemical Properties of EDA

Property	**Value**
Physical state	Liquid
Color	Colorless
Odor	Amine-like
Melting point/freezing point	Melting point/range: 8.5 °C
Initial boiling point and range	118 °C
Upper flammability/explosive limit	17 %(V)
Lower flammability/explosive limit	2 %(V)
Flash point	38 °C - closed cup - DIN 51755 Part 1
Autoignition temperature	405 °C - DIN 51794
Decomposition temperature	> 120 °C
pH	12.2 at 100 g/l at 20 °C
Viscosity	Viscosity, kinematic: Viscosity, dynamic: 1.265 - 1.725 mPa.s at 25 °C
Water solubility	1,000 g/l - miscible
Partition coefficient: n-octanol/water	log Pow: -2.04 (Bioaccumulation is not expected, Lit.)

Property	Value
Vapor pressure	12 hPa at 20 °C
Density	0.899 g/mL at 25 °C
Relative density	0.897 at 20 °C
Relative vapor density	2.07 (Air = 1.0)

EDA can enter the natural crystalline cellulose as shown in Figure 2; it cracks the hydrogen bonds between adjacent cellulose chains, makes new hydrogen bonds between cellulose and EDA, and transforms cellulose I to EDA–cellulose I complex. The hydrogen bonds rebuild between cellulose units after eliminating EDA by drying under a vacuum or washing with polar/non-aqueous solutions (e.g., ethanol). The crystal configuration of cellulose I turn into cellulose III. Cellulose III can also return to cellulose I by heating above 200°C (Qin et al., 2018). In a study, after EDA pretreatment, the enzymatic transformation of cellulose was higher than 80%, which may refer to the fact that the enzymatic saccharification ratio of cellulose III is much higher than that of cellulose I. After EDA pretreatment, most hemicellulose and cellulose stay in a water-insoluble solid form. Lignin is translocated with ether and ester bonds in lignin–hemicellulose complex fraction to the surface of biomass, and incomplete crystal cellulose is converted to amorphous cellulose (Qin et al., 2018). Meanwhile, the EDA pretreatment method increased the nitrogen content of hemicellulose (Jia et al., 2024). This enhancement in nitrogen content improved fluorescence intensity, effectively realizing selective detection and information encryption. This indicates that the pretreatment method efficiently separated hemicellulose from cellulose and enhanced its functional properties for high-value applications (Jia et al., 2024).

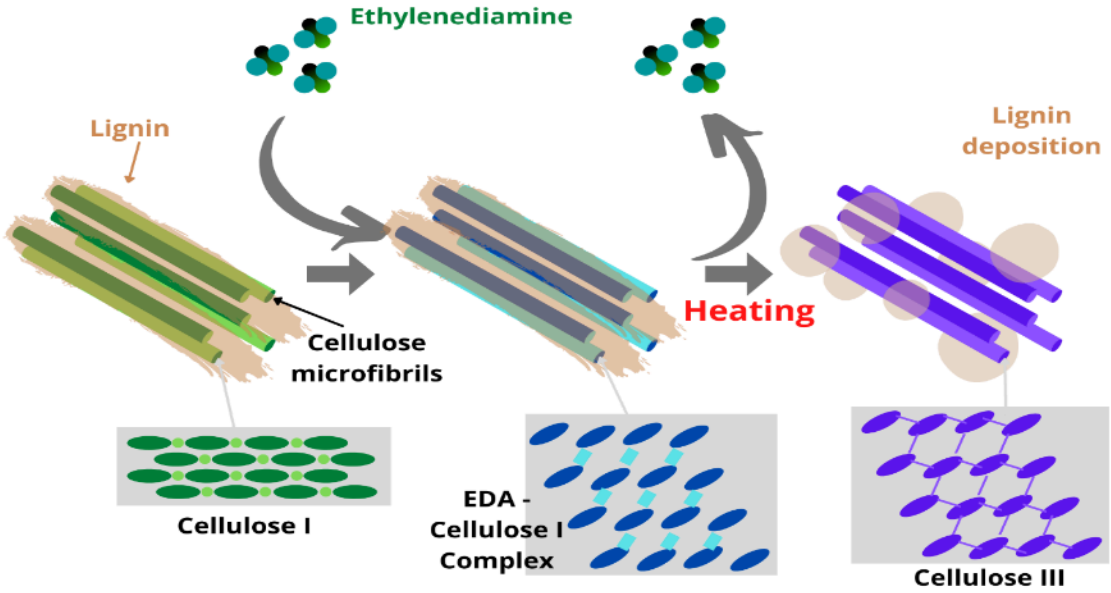

Figure 2 Approximate schematic for lignocellulosic biomass pretreated by EDA [75].

EDA pretreatment has numerous benefits compared to other methods like dilute acid: it can be performed at ambient pressure, which decreases process cost; it can be carried out without adding water (a dry-to-dry process), which bypasses solid-liquid separation after pretreatment and overcomes energy consumption throughout pretreatment; it protects hemicellulose and

does not release fermentation inhibitors like furans. On the other hand, during the pretreatment process, EDA evaporates so it can be reused. Moreover, to lessen refinery costs, EDA should be eliminated from biomass. An earlier study also proposed that EDA loading and pretreatment temperature influenced EDA removal and enzymatic hydrolysis of biomass [76].

5.0 Morphological Control

Morphological control, widely used in many fields, including materials science, biology, chemistry, and engineering, is the intentional manipulation and regulation of the shape, structure, and form of materials, organisms, or systems at various scales, ranging from microscopic to macroscopic levels. In this context, morphological control refers to regulating the shape, structure, and growth form of the *Rhizopus sp.* fungus to optimize the production of LA under various conditions. Morphological control of *Rhizopus sp.* in an EDA-delignified cellulose medium involves optimizing the growth conditions to manipulate the fungal morphology for enhanced LA production. This process focuses on adjusting parameters such as pH, temperature, nutrient concentration, and agitation to influence the fungal mycelium's shape, size, and structure. The surface area for enzyme-substrate interactions can be maximized by achieving a desired morphology, leading to more efficient cellulose degradation and LA fermentation. This approach aims to enhance the metabolic activity of *Rhizopus sp.,* thereby increasing the yield and productivity of LA, a valuable bioproduct used in various industrial applications.

The development and productivity of filamentous fungi are closely linked to their specific morphological phenotypes. The macro morphology influences mixing, mass transfer, and broth concentration, thereby shaping the microenvironment of the hyphae and affecting product formation. For instance, the density of a filament network can impact mass transfer within a pellet, which is crucial for the viability of the filaments and potential substrate limitations. Enhancing the yield of desired products by controlling the morphology of filamentous organisms remains a challenging task [77].

Most of the LA production studies did not extensively investigate the Rhizopus's morphology to correlate with the LA yield of the fungi. Table 2.5 compares various *Rhizopus* species' LA production capabilities using different carbon sources and growth morphologies. *Rhizopus oryzae* is a common species studied across multiple substrates. For instance, *R. oryzae* using glucose, xylose, ribose, and yam peel hydrolysate shows high LA production of 80 to 100 g/L, influenced by its filamentous versus kinetic growth morphology [5]. Morphology studies of LA production by *Rhizopus oryzae* have also been reported [5] by using yam peel, which had shown the highest yield of all time in different tank setups. For the surface and submerged fermentation, the yam peel produced an LA yield of 80.03% and 75.63%, respectively. Synthetic glucose syrup (SGS) yielded values for surface and submerged fermentation of 77.36% and 38.96%, respectively, which were lower than these. Another study with *R. oryzae* utilizing *Sophora flavescens* residues reported an LA production of 46.78 g/L with a productivity rate of 0.97 g/L/h [78]

R. oryzae LA-UN-1 demonstrates different LA yields based on its morphological state; pellets produced 63.5 g/L, while the filamentous form yielded 41.5 g/L, indicating that morphology significantly impacts productivity [79]Similarly, [80] explores the use of grape stalk, a wine industry residue, as a solid substrate for *R. oryzae NCIM 1299* fermentation to obtain lactic acid Values obtained from equation fitting for yield growth on agar were 0.570 ± 0.018 g LA/g. This study advocates for the use of grape stalk, a byproduct of the wine industry, as a solid substrate for the fermentation of *R. oryzae NCIM 1299* to produce lactic acid. The application of filamentous fungus on grape stalks as a fermentative solid substrate has not been previously investigated, rendering this study a novel scientific endeavour. The chemical characterisation of Grape Stalk revealed its features, indicating it as a novel material with significant promise as a fermentation substrate. The assessment of R. oryzae growth kinetics requires measuring the biomass dry weight of the fungus over time; however, the penetration of filamentous fungi

into the solid substrate renders the quantitative removal of biomass from the substrate virtually impossible. A fungal growth experiment using membranes was conducted, enabling gravimetric determination of fungal biomass. Two media were comparatively utilised: potato dextrose agar and a medium formulated with agar and grape stalk, incubated for 5 days at 30 °C and 50% relative humidity. The Logistic, First Order, and First Order Plus Dead Time models were employed to model biomass increase, with the latter two models having not been previously utilised in microbial kinetics research. Growth kinetic parameters (X^{max}, μ^{max}, T^{p}, and T^{0}) were determined for all models, with the First Order model exhibiting the optimal fit, evidenced by an R2 value of 91.84%. The Luedeking-Piret model was employed to simulate lactic acid generation, yielding the values Yp/x and mp, with a R^2 of 90.05%. The kinetic parameters identified herein are highly relevant for subsequent bioreactor designs [80].

Using cassava pulp hydrolysate with immobilized R. *oryzae NRRL395* resulted in a high LA yield of 75.28 g/L, suggesting immobilization enhances productivity [81]. *Rhizopus oryzae* NRRL395 was utilised for LA synthesis from cassava pulp hydrolysate and glucose, adopting a method that included steam pretreatment, NaOH explosion, HCl treatment, and enzymatic hydrolysis. Immobilised fungal cells in a static bed fermenter exhibited enhanced lactic acid yield, with batch fermentation producing 75.28 g/L over 96 hours (productivity: 1.05 g/L/h) and continuous fermentation attaining 0.72 g/L/h over 215 hours, underscoring the viability and efficiency of immobilised cell systems for continuous operation [82]. In a separate investigation, *R. oryzae NBRC 5384* was utilised on paper sludge that had been progressively treated with NaOH and HCl under aerobic fermentation at an ideal temperature of 40 °C, resulting in a production of 80 g/L of lactic acid with a productivity of 1.11 g/L/h over 72 hours, so exhibiting effective bioconversion of mixed sugars [83]. Furthermore, *R. microsporus*, extracted from a commercial Ragi Tempeh starter culture and identified through molecular analysis, was employed to ferment glucose in a pilot experiment. A concentration of 1×10^7 spores/mL was inoculated into 150 mL shake flasks containing 1.2 g/mL glucose and incubated at 37 °C with shaking at 100 rpm for a duration of 1 to 7 days, yielding 1.037 g/g [84]. As a result, this research is significantly impactful, and it will focus on cellulose and hemicellulose fermentation, as well as morphological control for LA generation, which has been underreported. Table 5 is the compilation of recent findings of LA production from this species.

Table 5: Recent Findings on the production of LA from *Rhizopus sp.*

Fungi Species	Carbon Source	Pretreatment	Morphology Analysis	Parameters Used	LA Production	Key Findings	Ref.
R. oryzae	Glucose, xylose, ribose, Yam peel hydrolysate	Acid Hydrolysis - 12 wt. % NH4OH	Filamentous vs. kinetic growth	Surface vs submerged fermentation	80 & 100 g/L	- Highest yield of 7.04 g/g in submerged fermentation with 200 rpm of agitation - Growth associated fermentation	[5]
R. oryzae	*Sophora flavescens* residues	Alkali - 120 °C with 1.5 % NaOH	None	SSF (full words?), inoculation volume: 10%, pH	46.78 g/L & 0.97 g/L/h	- Added 50 g/L $CaCO_3$ at 0 h of fermentation - Added 10	[78]

Fungi Species	Carbon Source	Pretreatment	Morphology Analysis	Parameters Used	LA Production	Key Findings	Ref.
				adjusted by adding $CaCO_3$ in stages during SSF		g/L $CaCO_3$ at 0, 12, 24, 48, and 72 h - 28 °C and 180 rpm	
R. oryzae LA-UN-1	Glucose on PDA	None	Pellet > Filamentous form	Growth on malt agar to evaluate morphological characteristics, pH range: 1-7, Temperature range: 30-55 °C	Pellet = 63.5 g/L, Filamentous = 41.5 g/L	- Fungus formed pellets at an initial pH of 3.0 - Fungus grew in filamentous form at an initial pH of 5.0 - Pellet morphology favored LA production	[85])
R. oryzae NCIM 1299	Grape stalk	Aqueous extraction	None	First Order, and First Order Plus Dead Time models were employed to model biomass increase	0.570 ± 0.018 g LA/g. h	The Luedeking-Piret model was employed to simulate lactic acid generation, with a R^2 of 90.05%	[80]
R. oryzae NRRL395	Cassava pulp hydrolysate and glucose	Steam pretreatment, NaOH explosion, HCl treatment, enzymatic hydrolysis	Immobilized fungal cells increase LA yield	Batch vs continuous fermentation in static bed fermentor	75.28 g/L	- Feasibility and effectiveness of using immobilized cells in static bed fermentors for continuous operation - Batch fermentation: 96 hours, 1.05 g/L/h - Continuous fermentation: 215 hours, 0.72 g/L/h	[82]

Fungi Species	Carbon Source	Pretreatment	Morphology Analysis	Parameters Used	LA Production	Key Findings	Ref.
R. oryzae NBRC 5384	Paper sludge	Alkali and Acid – NaOH followed by HCl	None	Aerobic fermentation, temperature: 40 °C	80 g/L	- Bioconversion of various sugars into LA - Optimal temperature: 40 °C - Productivity: 1.11 g/L/h at 72 hours	[83]
R. microsporus	Glucose	None	None	Pilot flask setup at 1 × 10^7 spores/mL was inoculated into150 mL shake flasks with 1.2 g/mL glucose, incubated at 37°C for 1 to 7 days with 100 rpm shaking.	1.037 g/g	Molecular analysis confirmed that the *Rhizopus* strain isolated from the Ragi Tempeh commercial starter culture falls under the classification of *R. microsporus*.	[84]

Very few explorations have been made in this topic in recent years due to their technical complexity, methodological challenges, limited applicability to specific industries, or resource limitations, which have hindered more extensive exploration in this area. Researchers might need to develop new experimental techniques or secure funding for comprehensive studies. However, this can also mean that research is precious in niche applications.

The findings underscore the significant influence of carbon sources and fungal morphology on LA production. The highest yields were generally associated with more refined substrates and specific growth conditions that favor efficient metabolic activity. The variation in production rates also highlights the potential for optimizing growth conditions and substrate selection to maximize LA yields in industrial applications.

5.1 Fungal pellet versus filamentous morphology

One of the most extensively studied areas in fungal biotechnology research is the creation of mycelial pellets by filamentous fungus. Pelletized fungi are more favorable in producing high LA yield [85] as it possesses several benefits and may offer a way to circumvent the issues that arise with filamentous fungus growth in large-scale industrial bioreactors [86]. Filamentous fungal fermentations are challenging to operate in large-scale bioreactors due to their mycelial shape and high broth viscosity. The heavily branched fungal mycelia produce complex large cells and viscous broth rheology, making mixing and aeration complex in typical stirred-tank fermenters. In large-scale filamentous fungal fermentation, pellet formation as self-immobilization is preferred because the cells produced in this form—also known as self-

immobilization—have benefits such as reduced medium viscosity [87]. Various cultivation factors, including inoculum size, pH, dissolved oxygen level, agitation system, nucleating agents, additives, trace metals, CO_2, temperature, reactor types, carbon substrate, rheology, culture modes, fermenter geometry, nitrogen, and phosphate levels, etc., all have an impact on the formation of pellets [88].

Significant parameters in the study on LA production using *Rhizopus sp* include inoculum size, pH, temperature, agitation system, carbon substrate (pretreated biomass), culture modes (batch and fed batch), aeration, and nutrient levels (nitrogen and phosphate).

It has been recorded that *R. oryzae* were observed to grow only as pellets in all the pH 3, 5.5, and 6.5, with small fluffy pellets at pH 3 and large spherical pellets at pH 5.5 and 6.5 [88]. [85] also examined pH alterations between two distinct morphologies. When the culture medium's initial pH was 3.0 or 5.0, *R. oryzae* showed pellet and filamentous morphology, respectively. In contrast to the 41.5 g/L produced by filamentous *R. oryzae,* the samples with the pellet morphology had a higher LA content of 63.5 g/ L [85]. These findings suggested that *R. oryzae* hyphae with pellet morphology, has more carbon flow devoted towards LA production. Hence, in the LA fermentation processes of *R. oryzae*, pelletized forms were the preferred morphology, and the production of LA was closely connected with it. In this report, morphological control has been done using neutralizing agents ($CaCO_3$, $NaHCO_3$, $NH_4(HCO_3)$, NH_4NO_3 & $(NH_4)_2SO_4$) in fermentation medium to optimize LA yield and fungi growth. Figure 3 is the *Rhizopus oryzae's* morphology.

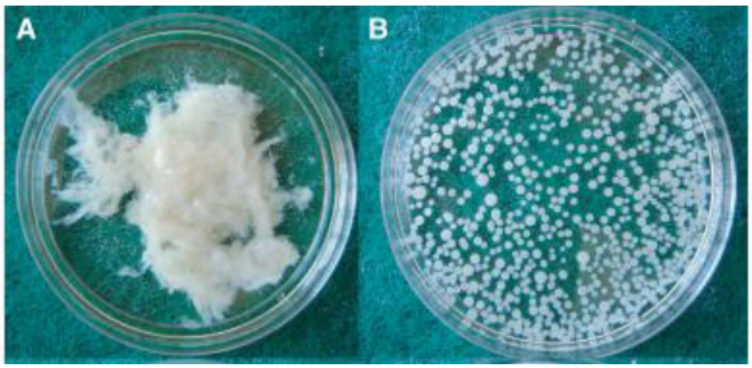

Figure 3 *Rhizopus oryzae's* morphology. Where A) Clump form and B) Pellet form [85].

In a study by [85], LA generation and the form of pellet morphology were examined in relation to the effects of four different neutralizing agents, including $CaCO_3$, sodium hydroxide (NaOH), ammoniacal solution, and $NaHCO_3$. It was found that $CaCO_3$ produced the pellet morphology that gave out the greatest lactic acid concentration (43.3 g/L) obtained in the batch utilizing 60 g/L of sweet potato starch as feedstock [85] as summarized in Table 4.

Table 6 Detailed information on *Rhizopus Oryzae* grown in potato starch by [85] using various neutralizing agents.

	$CaCO_3$	Ammoniacal	NaOH	$NaHCO_3$
The maximum lactic acid concentration (g/L)	43.3	25	41.2	35.5
The LA productivity (g/L h)	1.23	0.82	0.48	1.14
Morphology	Pellet	Cotton-like floc	Mycelium	Pellet

In a recent finding, pelletised *Rhizopus microsporus* was created to optimise fungal biomass separation and augment LA production. Optimal fermentation parameters, comprising temperature (37°C), neutralising agent ($CaCO_3$), twof old inoculum size (2×10^7 spores/ml), and agitation (100 rpm), yielded a maximum LA yield of 0.063 g/g, with a LA production of 3.15 g/L [19].

Studies show a complex relationship between fungi morphology and fermentation product production. This review highlights how lactic acid production and morphology control are interconnected. The effects can be positive and negative, emphasizing the need for more comprehensive research to address methodological concerns and understand the diverse influence of each parameter.

6.0 *Rhizopus sp.,* a LA-Producing Fungus

Within the context of LA production and substrate utilization *Rhizopus* species have emerged as a focal point of research. Understanding the factors influencing this topic is fundamental to optimizing LA production processes. However, to harness the full potential of this bioprocess, a comprehensive understanding of the growth patterns of *Rhizopus,* the dynamics of LA production, and the efficient utilization of substrates is essential. By delving into the synergistic mechanism of these processes, this review aims to uncover critical insights that can improve LA yield, higher productivity, and optimized resource utilization. Such knowledge contributes to the advancement of bioprocess engineering and aligns with the broader goal of developing sustainable and green technologies, which are increasingly imperative in our environmentally conscious world.

Based on the previous studies, this topic is broad, and it comes with several specific inferences:

1. Diverse research approaches: Researchers study *Rhizopus* for LA production using various approaches, including different *Rhizopus* species, substrates, fermentation conditions, and morphology modifications [89]
2. Sustainability focus: Many studies prioritize sustainability by using waste materials or renewable resources as substrates, aligning with global trends toward green technologies
3. Optimization Goals: The primary aim is to optimize LA production, focusing on higher yields, increased productivity, and efficient resource use through understanding growth, substrate utilization, and production rates.
4. Importance of pH and environmental factors: pH levels and other environmental factors are crucial for *Rhizopus* growth and LA production, with research often manipulating these to achieve optimal results.

7.0 Conclusion

Ultimately, a diverse subject with increasing importance in sustainable biotechnology is study on the fermentation of *Rhizopus* for LA synthesis. The important effect of lignocellulosic agro-waste substrates, pretreatment techniques—especially EDA delignification—and morphological regulation of Rhizopus on LA yield is underlined in this review. Technical complexity, methodological difficulties, and limited industrial adaption mean that despite its potential this field is still understudied. Still, this also offers a special chance for strong niche uses. Advanced morphological engineering should be the main emphasis of future research to maximise pellet formation; integrated pretreatment techniques should improve substrate digestibility; and techno-economic analyses should assess process feasibility. Furthermore crucial will be widening the substrate base, raising bioreactor scale-up, and investigating genetic or metabolic changes in *Rhizopus* strains. Accelerating development is urged using multidisciplinary approaches combining data analytics, material science, and process

engineering. Promoting these sectors will significantly help to meet objectives of the circular economy and facilitate the more general shift towards green technology in industrial bioproduction.

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