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Review

Membrane Bioreactors: A Promising Approach to Enhanced Enzymatic Hydrolysis of Cellulose

Saleha Al-Mardeai [1], Emad Elnajjar [2], Raed Hashaikeh [3], Boguslaw Kruczek [4], Bart Van der Bruggen [5] and Sulaiman Al-Zuhair [1,*]

[1] Chemical and Petroleum Engineering, UAE University, Al Ain 15551, United Arab Emirates
[2] Mechanical Engineering, UAE University, Al Ain 15551, United Arab Emirates
[3] Mechanical Engineering, New York University, Abu Dhabi 129188, United Arab Emirates
[4] Chemical and Biological Engineering, University of Ottawa, Ottawa, ON K1N 6N5, Canada
[5] Department of Chemical Engineering, KU Leuven, 3000 Leuven, Belgium
* Correspondence: s.alzuhair@uaeu.ac.ae; Tel.: +971-3-713-5319

Abstract: The depletion of fossil fuel resources and the negative impact of their use on the climate have resulted in the need for alternative sources of clean, sustainable energy. One available alternative, bioethanol, is a potential substitute for, or additive to, petroleum-derived gasoline. In the lignocellulose-to-bioethanol process, the cellulose hydrolysis step represents a major hurdle that hinders commercialization. To achieve economical production of bioethanol from lignocellulosic materials, the rate and yield of the enzymatic hydrolysis of cellulose, which is preferred over other chemically catalyzed processes, must be enhanced. To achieve this, product inhibition and enzyme loss, which are two major challenges, must be overcome. The implementation of membranes, which can permeate molecules selectively based on their size, offers a solution to this problem. Membrane bioreactors (MBRs) can enhance enzymatic hydrolysis yields and lower costs by retaining enzymes for repeated usage while permeating the products. This paper presents a critical discussion of the use of MBRs as a promising approach to the enhanced enzymatic hydrolysis of cellulosic materials. Various MBR configurations and factors that affect their performance are presented.

Keywords: enzymatic hydrolysis; lignocellulose; membrane bioreactor; pretreatment; product separation

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1. Introduction

Concurrent increases in population and industrialization have resulted in energy demand escalation, with fossil fuels being the main source of energy [1]. However, fossil fuels are nonrenewable energy resources whose use has an adverse impact on the environment [2]. Therefore, there is a need for greener, more sustainable energy sources [3]. Biofuels, which are renewable energy sources produced from biomass, has received increased attention as potential substitutes for fossil fuels, particularly in the transport sector, because of their sustainability and low environmental impact [3]. Bioethanol is a biofuel that is used as a petrol-derived gasoline additive in conventional engines to reduce the harmful impact of combustion emissions; it can also be used pure in slightly modified engines. For example, Brazil depends heavily on bioethanol produced from sugarcane as an energy source. It accounts for 18% of the nation's total energy consumption [4]. However, sugarcane and other conventional feedstocks that are also food stock are not preferred for use in energy production. Lignocellulosic biomass is considered more appropriate, as it is generally not used as a direct feed source. It is typically considered biomass waste and is, therefore, a sustainable feedstock for biofuel production [5–7]. However, this type of biomass is considered recalcitrant because the lignin within it is linked strongly to cellulose, such that a protective shield is formed. In addition, the highly crystalline cellulose structure in most lignocelluloses renders incomplete enzymatic hydrolysis inevitable [8,9]. Various

approaches have been used to separate cellulose from other components in the lignocellulosic structure before converting it to sugars that can be converted to bioethanol via fermentation easily [10]. The enzymatic hydrolysis of cellulose, which is preferable to other chemical hydrolysis approaches, has relatively slow reaction rates due to the inhibition of enzymes by the resulting sugars and losses in traditional continuous reactors [11,12]. Membrane bioreactors (MBRs) have been suggested to overcome these challenges and enhance bioethanol yield [13]. This review is oriented towards comprehensively addressing the biochemical conversion process for bioethanol production. Different current technologies for each step of the process are discussed. In addition, this review presents a deep discussion on the opportunities and challenges associated with the use of various MBR configurations and factors affecting their performance in enhancing bioethanol production.

2. Ethanol Feedstock

Biomass feedstock is a material of biological origin that can be converted to various bio-based products, such as ethanol, in a biorefinery. Biomass feedstocks are categorized into first- and second-generation feedstocks based on their composition and origin [11].

2.1. First-Generation Feedstocks

First-generation feedstocks are edible biomass materials that are easy to process. The most common types of first-generation feedstock used for ethanol production are starch-rich and sugar crops. Sugar crops such as sugar cane, sugar beet, and sugar sorghum are biomass composed mainly of mono or disaccharides. Starch-rich crops, such as corn and wheat, are composed primarily of starch. Due to their composition, the conversion of these feedstocks to bioethanol is easy and inexpensive [12].

2.2. Second-Generation Feedstocks

Lignocellulose is an example of second-generation feedstocks, which comprise non-food-part residues. Unlike first-generation biomass, lignocellulosic biomass is the inedible part of the plant, which is composed mainly of cellulose, hemicellulose, and lignin. Agricultural waste such as straw, corn stover, corn cob, and bagasse; forestry wastes such as wood chips; and municipal and industrial wastes are all examples of lignocellulosic biomass that can be used for ethanol production [12].

2.3. Third- and Fourth-Generation Feedstocks

Algae and recombinant microorganisms are considered third- and fourth-generation feedstocks. The triglycerides stored in microalgae can be utilized to produce other energy products in addition to carbohydrates that can be used for ethanol production [12]. However, extracting the triglycerides is considered a costly and energy-demanding step, rendering the process for large production unfeasible [14]. An example of fourth-generation feedstock is cyanobacteria that is genetically modified to increase oil production [15]. However, there are some environmental concerns related to this type of feedstock. Most importantly, the resultant wastewater from the process might contain plasmids and chromosomes, which lead to different mutations and lateral gene transfer in the environment [14].

3. Lignocellulose

Lignocellulosic biomass is considered an environmentally friendly, sustainable energy production resource [8]. The various lignocellulosic biomass sources include industrial and agricultural waste, as well as forestry lignocellulosic biomass. Different biomass sources have different lignocellulose compositions [16].

Structure

Lignocellulose has three main components: cellulose, hemicellulose, and lignin; other components, such as extractives and ash, are also found in smaller amounts in their structure. Lignocellulose is predominantly cellulose (40–50% of the total structure), followed

by hemicellulose, which is estimated to be 25–30%, and lignin, which is 15–20% [13]. A schematic diagram of the lignocellulosic structure is shown in Figure 1.

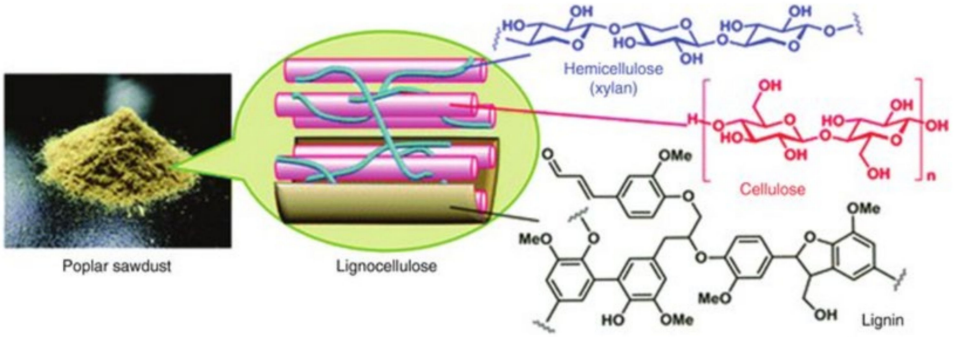

Figure 1. Schematic diagram of the lignocellulosic structure, which is composed of cellulose, hemicellulose, and lignin [17].

Cellulose is composed of repeating cellobiose units, which comprise two glucose molecules joined together by a β-1,4 glycosidic linkage. The repeating glucose units give rise to a glucan unit, which is estimated to be between 2000 and 2700 units, composing cellulose [16]. The degree of polymerization is determined by the glucan units contained in the cellulose structure [18]. The polymer chains that comprise cellulose are linked via covalent and non-covalent bonds, such as hydrogen and van der Waals bonds, to form microfibrils [19]. Cellulose is dominated by a crystalline structure in which the microfibrils are arranged in parallel, whereas the remainder of the structure is amorphous [20].

Hemicellulose is made of repeat units of various sugar monomers, including xylose, arabinose, mannose, galactose, and glucose. The sugar monomer that comprises the chain determines the type of hemicellulose, which can be either linear or branched, surrounding cellulose [21]. Lignin, on the other hand, is composed of phenylpropane units. These are composed of coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol. Lignin is known to have a three-dimensional structure that is linked to the remaining components, hemicellulose and cellulose, and thus provides rigidity to the cell wall and protection from the microorganism-related activity and environmental factors [22].

4. Conversion of Lignocellulose to Bioethanol

A representative diagram of ethanol production from lignocellulosic feedstock is shown in Figure 2. The process includes mechanical size reduction, followed by pretreatment in which the biomass structure is disrupted and separated further. Hydrolysis, which is the focus of this work, occurs next. Polysaccharides are broken down into monomers such as glucose, xylose, and arabinose. During fermentation, simple sugars are converted to ethanol.

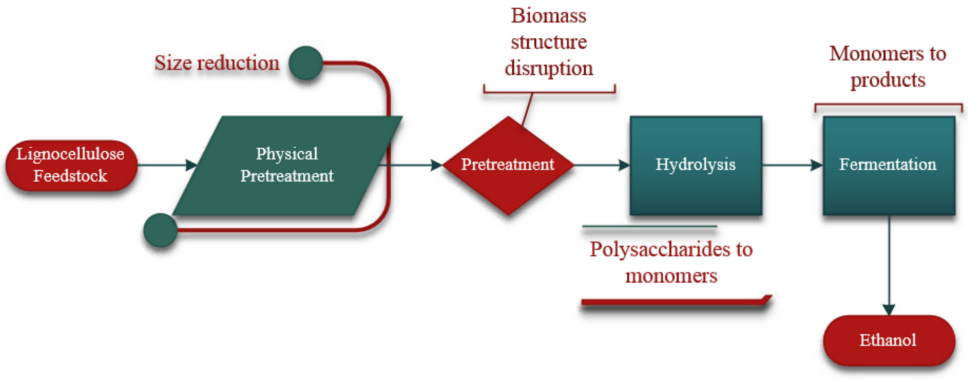

Figure 2. Biorefinery process of ethanol production from lignocellulosic feedstock.

4.1. Pretreatment

Pretreatment is the step in which lignocellulosic biomass is converted from being recalcitrant to a form ready for enzymatic hydrolysis [23]. An efficient pretreatment process must be able to recover complete lignocellulosic components and produce less degradation byproducts. In addition, it must be feasible and effective on various biomass quantities and types [23]. The different types of pretreatment methods used with lignocellulosic biomass can be classified into physical, chemical, and biological pretreatments.

4.1.1. Physical Pretreatment

Most biochemical conversion processes use physical pretreatment as a first step. This step serves to reduce the particle size, which increases the surface area to volume ratio and reduces the degree of polymerization and crystallinity of the biomass, thus enhancing the conversion rate [24]. The enzymatic hydrolysis of cotton cellulose was enhanced to produce glucose yields of up to 99.8% over 50 h when the particle size was reduced from 25 to 0.78 μm [25]. The several types of physical treatments, such as chipping, shredding, milling, and grinding, produce different final particle sizes. In general, decreasing the particle size below 0.3 mm has been proven to increase the glucose conversion yield [26].

4.1.2. Chemical Pretreatment

Acidic Pretreatment

Hemicellulose and cellulose are partially solubilized by both dilute and concentrated acids. However, concentrated acids are not favored due to their severe effects on the biomass and the process. Their effects include cellulose degradation and inhibitor production [27]. Dilute acids are used within a concentration range of 0.5% to 2.5% and a temperature range of 100 to 200 °C [27]. Various studies have investigated the enhancement of the enzymatic hydrolysis step via dilute sulfuric acid pretreatment [28,29]. Pretreating rice straw with 1% (*w*/*w*) sulfuric acid enhanced the efficiency of enzymatic hydrolysis to 70% by increasing the biomass pore volume. This resulted in a combined glucose and xylose yield of 83% after 72 h [30]. Although enzymatic hydrolysis is enhanced by this type of pretreatment, the partial hydrolysis of cellulose results in more crystalline cellulose. This produces lower conversion yields than other pretreatment methods [31].

Alkaline Pretreatment

In contrast to acid pretreatment, the alkaline reagent used in alkaline pretreatment interacts with, breaks down, and isolates only lignin from the biomass during alkaline pretreatment. This can provide a simple, inexpensive way of enhancing enzymatic hydrolysis [32]. The use of alkaline compounds, such as sodium hydroxide, sodium carbonate, and ammonia, makes this pretreatment method superior to other methods because these compounds are non-corrosive chemicals that require mild conditions [32]. In addition, this method is highly selective for lignin removal and retains cellulose and hemicellulose intact. The absence of inhibitor production enhances the fermentation step. It was found that pretreating rice straw with ultrasound-assisted alkaline (NaOH) improved the digestible cellulose yield by a factor of 3.5 compared to untreated biomass [33]. The suggested pretreatment method was found to increase the surface area accessible for cellulase and to increase the porosity compared to the same biomass treated with heat only. However, this pretreatment method generally increases the crystallinity index. This is due primarily to lignin removal rather than to structural changes within the cellulose [32]. However, the degree of polymerization was found to decrease when pretreatment was performed using a NaOH concentration below 8 wt%. Such low concentrations caused separation in the cellulose lattice [34]. In addition, pretreating sugarcane bagasse with 20% aqueous ammonia for 48 h at 50 °C was found to result in 57.3% total sugar release during the subsequent enzymatic hydrolysis [35].

Oxidative Pretreatment

Oxidizing agents such as oxygen, ozone, and hydrogen peroxide are used to remove lignin. However, partial breakdown of some of the hemicellulose is observed. In addition, the aforementioned agents are not selective for lignin. They can attack cellulose and lead to the production of byproducts such as aliphatic aldehydes and aliphatic organic acids, which inhibit the subsequent enzymatic hydrolysis [36]. This pretreatment method can be combined with other methods to improve the degradation of lignocellulose and enhance enzymatic hydrolysis. For example, corn stover was pretreated in a two-stage process; the first stage was pretreatment with dilute hydrochloric acid (1 wt%) for 40 min at 120 °C. This was followed by alkaline wet oxidative pretreatment with 12.6 wt% ammonium hydroxide under pressurized oxygen (3 MPa) at 130 °C for the same duration. This two-stage process was found to remove approximately 86% of lignin and produce xylan and glucan yields of 82.8% and 71.5%, respectively [37].

4.1.3. Physicochemical Pretreatment

This class of pretreatment changes the structure of the biomass both physically and chemically. Several types, such as solvent fractionation, steam explosion, liquid hot water, and carbon dioxide explosion, are used.

Solvent Fractionation

Solvent fractionation is the partial solubilization of lignocellulosic components by breaking down hydrogen bonds between fibrils. This occurs because different lignocellulosic components have different solubilities in different solvents [24]. This type of pretreatment involves the use of organic solvents, ionic liquids, or phosphoric acid.

Organic solvents, such as ethanol in the presence of an acid catalyst, are used to extract lignin from the biomass and thus reduce the crystallinity [38]. However, some properties of these solvents restrict their application as a pretreatment method. For example, the use of organic solvents with low boiling points, such as ethanol, acetone, methanol, and ethyl acetate [38], necessitates operating at high pressure. In addition, safety issues should be considered when using flammable solvents [39]. However, the fractionation of corn stover biomass using ethanol resulted in a 91% glucan content after lignin removal, while the fractionation of giant miscanthus and wheat straw with ethanol was not efficient [40]. In addition, the use of organic amines, such as polyamine, as ethanol fractionation catalysts has been shown to aid in boosting the delignification of corn stover biomass. This produced a lignin removal of 82% and a sugar yield of 83% [41]. Furthermore, combining sulfuric acid and ethanol to perform wheat straw pretreatment was found to enhance the extraction of fermentable sugars to 89%, which is better than the other organic solvents tested, such as methanol, butanol, acetone, and diethylene glycol [42]. Although different solvents have been reported for lignocellulose fractionation, their residence time, biomass loading, byproduct production, and structural disruption are all factors to consider when selecting the most convenient pretreatment method. For example, using a cellulose solvent such as concentrated phosphoric acid produced better structural disruption and a 97% glucan yield in 24 h. This can be compared to dilute sulfuric acid, which achieved 84% in 72 h. However, the effect of using such concentrated acids on inhibitor production was not reported [43,44].

Ionic liquids (ILs) such as 1-allyl-3-methylimidazolium chloride [AMIMCl], -allyl-3-methylimidazolium acetate [EMIM][AC], and 1-butyl-3-methylimidazolium chloride [BMIMCl] have been shown to solubilize cellulose from biomass effectively [45]. Due to the presence of anions, such as chloride, cellulose can bind with the ionic liquid via hydrogen. Cellulose can then be recovered using an antisolvent, such as water, which breaks down these bonds and enables recovery of the used IL [45]. Thus, pretreatment with ILs is an area of research interest [46]. Sugar yields of 89% and 87% from sugarcane bagasse and wheat straw, respectively, were achieved when both biomasses were pretreated with [EMIM][AC] [47]. Pretreatment of wheat straw with 1-ethyl-3-methyl-imidazolium acetate produced competitive results when followed by xylanases before cellulose hydrolysis.

These two pretreatment steps helped to improve the accessibility of cellulose to enzymes and enabled cellulosic degradation of up to 99% and a xylose yield of 97.6% [48].

Steam Explosion

This commonly used pretreatment method involves the application of high pressure and temperature, followed by a sudden pressure decrease. This process leads to lignocellulosic structure breakdown [49]. Better biomass disruption was observed when a steam explosion was combined with other pretreatment methods. For example, elephant grass was treated with different concentrations of sulfuric acid to yield around 52% digestible cellulose, while barley straw biomass pretreated with steam explosion and extrusion yielded up to 84% glucan [50–54]. However, like dilute acid pretreatment, this pretreatment method results in the production of byproducts that inhibit enzymatic hydrolysis. Thus, a detoxification step is required before enzymatic hydrolysis [55]. The formation of acetic acid, furfural, 5-HMF, and vanillin produced insignificant cellulase inhibition, but formic acid inactivated the enzymes, and this effect increased with the solid loading [56].

Hydrothermal Pretreatment

Hydrothermal pretreatment (HTP) is another example of physicochemical pretreatments. It involves the use of highly pressurized water at high temperatures to disrupt the lignocellulosic structure. HTP can be divided into subcritical and supercritical processes [49]. Due to environmentally friendly conditions, such as no catalyst used, less corrosion, and a high output process, HTP is favored in combination with other pretreatment methods. For example, it has been used in combination with alkaline pretreatment using NaOH, resulting in 86% lignin removal from wheat straw. In addition, it is recognized to be able to enhance the biomass surface area, which enhances the sugar yield in the subsequent steps and enhances the dissolution and recovery of hemicellulose [57].

4.1.4. Biological Pretreatment

Biological pretreatment involves the use of bacterial and fungal strains, such as *Bacillus* sp., *Trichoderma reesei*, *Thermomonospora* sp., and *Phanerochaete chrysosporium*, to degrade the lignocellulose structure [58,59]. Due to the ability of these organisms to release enzymes, such as lignin peroxidase and laccases, lignin is removed from the lignocellulosic structure [60]. The most commonly applied fungus, which is known for its ability to degrade lignocelluloses, is the white-rot fungus. Using *Ceriporiopsis subvermispora* to pretreat sugarcane bagasse at 27 °C for 60 days resulted in a 47% sugar yield [61]. Biological pretreatment represents an advance over other pretreatment technology in that it does not require energy input and is environmentally friendly and cost-efficient. However, it is time-consuming and produces low yields; thus, it is infeasible for biorefinery process implementation [62].

4.2. Hydrolysis

There are several ways in which cellulose can be hydrolyzed to fermentable sugars. Chemical, biological, and other methods such as gamma-ray, electron-beam, and microwave irradiation have been reported. Chemical and biological hydrolysis are the most commonly used due to their feasibility and effectiveness [63–65].

4.2.1. Chemical Hydrolysis

Chemical hydrolysis involves the use of chemicals such as diluted and concentrated acids. The use of concentrated acid helps to enhance the sugar yield, and such treatments can be performed using lower temperatures than those used in dilute acid hydrolysis. However, acid consumption is high, and further downstream processing, such as detoxification, is required. In addition, this process requires long residence times and recovering acid for reuse is costly. In contrast, dilute acid hydrolysis produces lower yields, requires high temperatures, and generates non-useful byproducts [66,67].

4.2.2. Enzymatic Hydrolysis

The massive application of different cellulases in various fields has attracted attention to their use in bioenergy production. The use of enzymes in those applications helps in reducing the environmental influences and can contribute to enhancing the quality of the production. This is mainly due to the mild conditions required in enzymatic processes, which results in less energy consumption. Moreover, the lower toxins produced, as compared to the chemically catalyzed processes, make enzymatic approaches a good alternative that lowers the required post-treatment steps [14]. However, the high cost of enzymes, associated with their high production cost, remains the main obstacle facing their large-scale application. Therefore, different enzyme-producing companies are leading studies to economically develop enzymes that can be used in various applications, including biofuel production [14].

The main sources of commercial cellulases are *Trichoderma reesei* and *Aspergillus niger* [68]. Cellulase plays a key role in enzymatic hydrolysis. It is a multi-component system that breaks polymer chains into fermentable sugars, as shown in Figure 3 [69]. It is composed of three types of enzymes: endoglucanase, cellobiohydrolases (exoglucanase), and β-glucosidases. Endoglucanases work synergistically with exoglucanase. Hydrolysis initiates with endoglucanase, which attacks the polymer chain at random sites to create reducing and non-reducing ends. Exoglucanase then acts on those ends to convert them to shorter polysaccharide chains that consist of two glucose units called cellobiose. The last component, β-glucosidase, breaks down cellobiose from the mid-point to produce two glucose units. The latter is considered the rate-limiting step for the hydrolysis reaction because the sensitivity of the enzymes toward the end product, glucose, leads to product inhibition [70]. Studies have shown that each cellulase-producing microorganism lacks one or more types of cellulase, which leads to inefficient hydrolysis. *A. niger* and *Trichoderma atroviride* are mostly β-glucosidase producers and lack the other two cellulases [70]. Thus, the use of different cellulase recipes from different sources is critical to enhancing the conversion rate. It has been reported that the catalytic activities of commercial cellulases derived from *T. reesei* and *A. niger* can be improved via the addition of crude cellulases from five different fungal strains, namely *Chaetomium thermophilum, Thielavia terrestris, Thermoascus aurantiacus, Corynascus thermophilus,* and *Myceliophthora thermophile*, when hydrolyzing pretreated barley straw [71].

4.3. Enzyme Kinetics and Modeling

The mechanism of the enzymatic hydrolysis of cellulose is similar to other enzymatic reactions. Figure 4 illustrates the mechanism and kinetics of each step. In the first step, the enzyme is adsorbed onto the substrate surface. After this, two pathways are possible; the enzyme binds either to an active site, denoted as productive binding, or to a non-active site, denoted as non-productive binding. In the former pathway, an enzyme–substrate complex is formed and can proceed to the catalytic reaction step, during which the glycosidic bond is broken. The enzyme-product complex is formed and then separated to release the product from the enzyme, and the enzyme active site is free again for another binding. Therefore, the rate of the catalytic reaction is directly proportional to the rate of substrate productive binding to the active sites [73]. However, if the substrate is adsorbed via a non-productive pathway, the substrate acts as an inhibitor, and the catalytic process is inhibited. No product is formed in this pathway, and the enzyme is inactive [73]. This demonstrates that the substrate surface changes dynamically as enzymatic hydrolysis progresses [74]. Cellulose is composed of hydrolyzable and non-hydrolyzable (inert) parts. At the enzyme–substrate surface, the enzyme breaks down cellulose leaving the inert at that layer, proceeding to the next layer, which also contains cellulose and inert [74]. As the reaction continues, the enzyme is adsorbed deeper into the substrate, shrinking the available substrate surface area and thus limiting the substrate available for reaction.

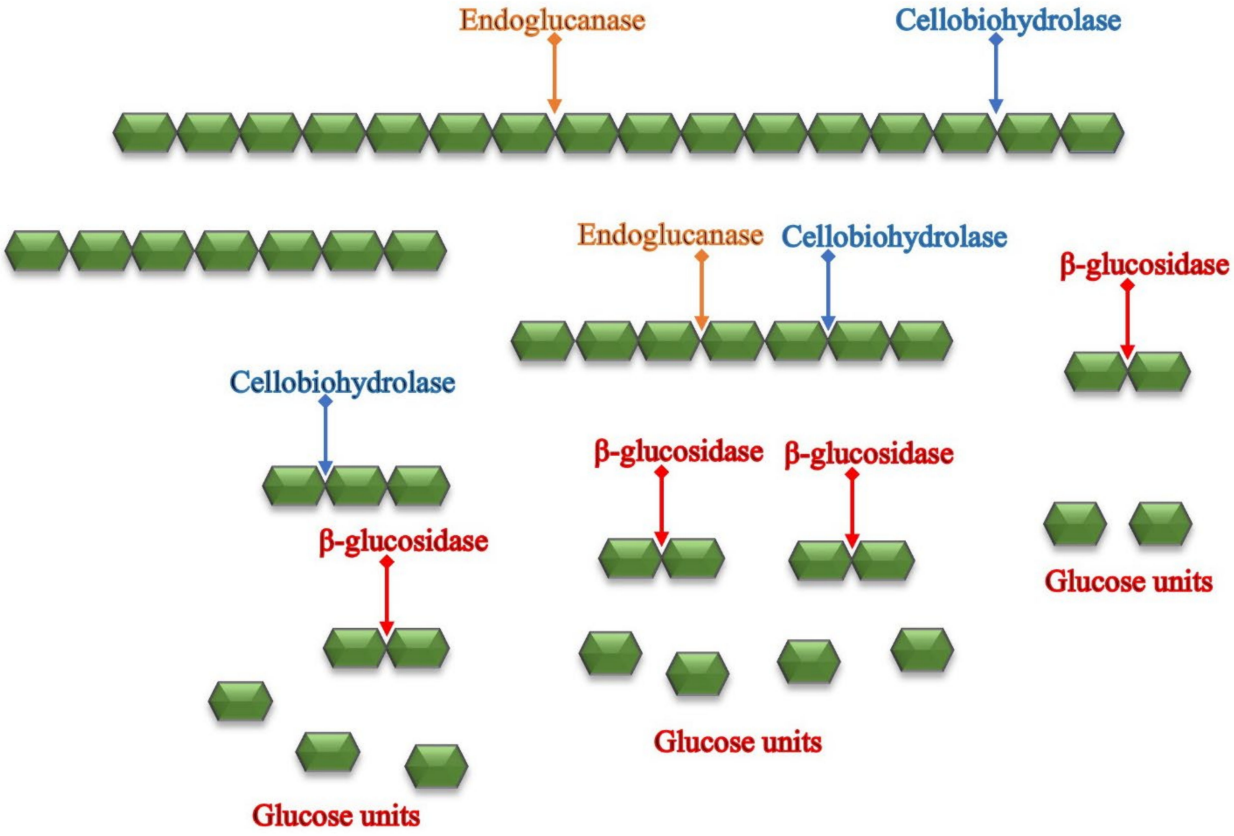

Figure 3. A schematic diagram of the bioconversion of lignocellulosic biomass to fermentable glucose [72].

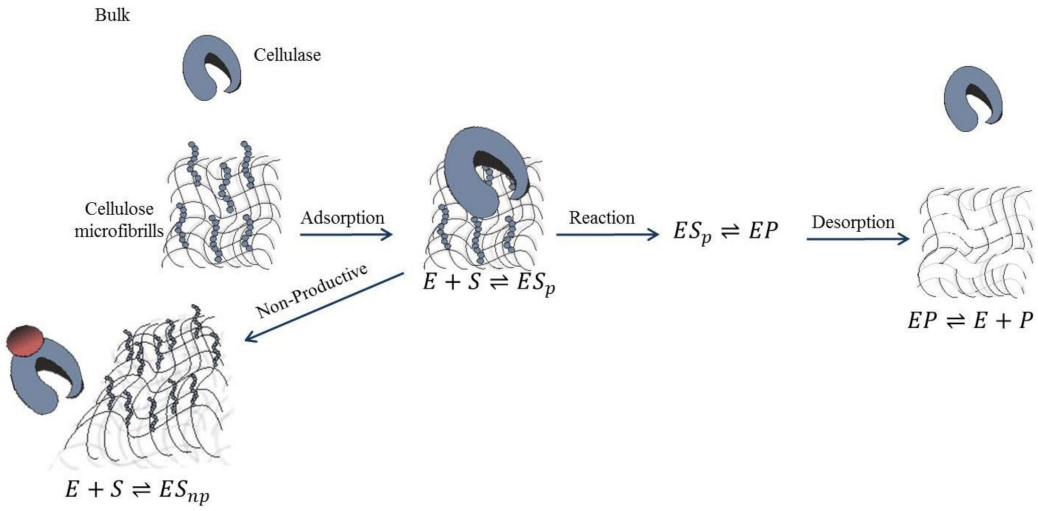

Figure 4. Reaction mechanism of enzymatic hydrolysis of cellulose.

Various cellulase kinetic mechanisms have been proposed by researchers who have sought to understand the process fully. The mechanism shown in Equation (1) was proposed to explain the burst phase for a soluble substrate and non-processive enzyme, which cleaves cellulose randomly and non-processively. When the processive action of an enzyme occurs, the enzyme binds to the cellulose and cleaves it continuously for multiple cycles before it dissociates [75].

$$E + S \underset{k_{-1}}{\overset{k_1}{\leftrightarrow}} ES \xrightarrow{k_2} EP_2 + P_1 \xrightarrow{k_3} E + P_2 \quad (1)$$

The model suggests that an enzymatic reaction produces two products. The first, P_1, is produced rapidly at the beginning of the reaction when k_1 and k_2 are larger than k_3. The enzyme then accumulates as an EP_2 complex, which dissociates slowly over time to produce P_2. This can be proven by monitoring the concentrations of P_1 and P_2 as functions of time. P_1 increases during the first stage of the reaction, before the reaction reaches steady-state [75–77]. However, key factors that affect the enzyme mechanisms, such as product inhibition, are not considered.

To account for the processive action of the enzyme, Equation (1) was modified to include multiple cycles of the catalytic action of cellobiohydrolase, as shown in Equation (2).

$$\begin{array}{ccccccccc} & & k_1 & & k_2 & & k_2 & & k_2 \\ E + C_n & \underset{\downarrow k_3}{\rightarrow} & EC_n & \underset{\downarrow k_3}{\rightarrow} & EC_{n-1} + C & \underset{\downarrow k_3}{\rightarrow} & EC_{n-2} + C \ldots . & \underset{\downarrow k_3}{\rightarrow} & EC_x \\ & E + C_n & & E + C_{n-1} & & E + C_{n-2} & & E + C_x & \end{array} \quad (2)$$

In this model of cellulose cleavage, the enzyme cleaves the cellulose strand in consecutive steps in a processive manner and goes through many cycles of consecutive reactions before it dissociates. Hydrolysis initiates via the binding of cellobiohydrolase, E, to a cellulose strand that contains n cellobiose units, C_n, to form an enzyme–substrate complex, EC_n. After this, it can either produce one cellobiose unit, C, with a rate constant k_2 or dissociate back to the enzyme and cellulose with the dissociation rate constant k_3. The reaction continues via the same steps but with shorter cellulose strands in each cycle and with the possibility of enzyme dissociation. After multiple cycles of cellobiose unit cleavage, the desorption rate slows because inhibition by cellobiose leads to enzyme–substrate complex (EC_x) accumulation. This occurs because k_2 is larger than k_3, and a further decline in kinetics is observed [75–77].

The drawback of this model is that it assumes a constant substrate concentration. This is valid only at the beginning of the reaction when the substrate concentration is higher than the enzyme concentration. Hence, the accessibility and affinity of the enzyme to bind to the remainder of the cellulose strand is not considered [78]. In addition, the inhibition effect is considered only with respect to cellobiose; mono-sugars are not considered. This model has been modified further to consider enzyme deactivation via irreversible binding to the substrate, as in Equation (3).

$$\begin{array}{ccccccccccc} & & & & IC_{n-1} & & IC_{n-2} & & & & IC_x \\ & k_1 & EC_n & k_2 & \uparrow k_4 & k_2 & \uparrow k_4 & & k_2 & & \uparrow k_4 \\ E + C_n & \leftrightarrow & \downarrow k_3 & \rightarrow & EC_{n-1} + C & \rightarrow & EC_{n-1} + C \ldots . & \rightarrow & & & EC_x \\ & k_{-1} & E + C_n & & \downarrow k_3 & & \downarrow k_3 & & & & \downarrow k_3 \\ & & & & E + C_{n-1} & & E + C_{n-2} & & & & E + C_x \end{array} \quad (3)$$

Enzyme inhibition during processive hydrolysis is described in Equation (3) and was proposed via assessment of the hydrolysis of microcrystalline cellulose, which is composed of amorphous, completely hydrolyzable cellulose. Researchers reported that the decline in the kinetics occurred due to irreversible enzyme–substrate binding [79]. However, the model does not consider enzyme inhibition by the end product and ignores mass transfer limitations, which are significant in heterogeneous reactions.

The product inhibition effect was included in a model developed by Huang [80] and Peitersen et al. [81], in which the cellulose substrate binds reversibly to the enzyme to form either productive or non-productive enzyme–substrate complexes. The productive complex may then undergo the forward reaction to produce the product, P. Once the product is released, product inhibition, which is irreversible in this model, might occur upon binding to the enzyme. The mechanistic steps of this model are presented in Equations (4)–(7).

$$E + S_c \overset{k_{c_1}}{\underset{k_{c_2}}{\leftrightarrow}} E^* S_c \quad (4)$$

$$E^*S_c \xrightarrow{k_P} E + P \quad (5)$$

$$E + S_x \underset{k_{Sx_2}}{\overset{k_{Sx_1}}{\leftrightarrow}} E^*S_x \quad (6)$$

$$E + P \underset{k_{EP_2}}{\overset{k_{EP_1}}{\leftrightarrow}} EP \quad (7)$$

In this mechanism, the substrate is presented in two fractions, a hydrolyzable substrate that can produce a product upon binding with the enzyme (S_c) and a non-hydrolyzable substrate (S_x) that results in non-productive binding and deactivates enzymes. Reversible binding of the enzyme to the hydrolyzable and non-hydrolyzable substrates and to the product is accounted for in this model. In addition, unlike previous models, this model considers the active surface concentration, which is represented by S_c, instead of the total mass concentration. This provides insight into the quality of the substrate and better represents the reaction mechanism [74,82,83]. Moreover, this presentation incorporates the dynamic changes that occur on the substrate surface during enzymatic hydrolysis. This helps to predict the conversion decline as the reaction proceeds. However, the different types of cellulases are not distinguished but rather assumed to be one type with the same function for simplicity, unlike in the models proposed by Zyl et al. [84] and Zhang [85], which distinguish the actions of endoglucanase and exoglucanase using separate steps. In the model described in Equations (4)–(7), product inhibition is represented by the reversible formation of an enzyme–product complex. Models that are more recent account for various additions, such as heterogeneous substrates, the degree of polymerization, inhibition effects, rapid declines in initial rates, and the effects of pretreatment on the hydrolysis yield. However, this model is used as the basis for modeling in the present paper. Modifications are made to estimate the kinetic parameters involved in the enzymatic hydrolysis of selected lignocellulosic biomass materials.

5. Lignocellulose Enzymatic Hydrolysis Challenges and Potential Solutions

The use of lignocellulose for ethanol production increases farmer incomes, provides jobs, and reduces gas emissions by increasing the green lands [86]. However, due to the difficulty of lignocellulose hydrolysis, the cost of production, estimated at 0.60 EUR/L, is higher than the market price of ethanol, which is 0.23 EUR/L. This makes the overall process economically infeasible [87–89]. Overcoming barriers that slow the hydrolysis process and enhancing the yield should change the situation and make large-scale production more feasible.

5.1. Heterogeneous Mixture

Lignocellulose is a difficult substrate because it is composed of several components, some of which are resistant to enzyme degradation. In addition, the crystalline cellulose substrate forms a heterogeneous mixture, wherein the enzyme, which must be used in its free form, binds to the heterogeneous substrate for the reaction to proceed. The slow kinetics of enzymatic cellulose hydrolysis are correlated with the heterogeneous nature of the substrate, which affects enzyme diffusion to the substrate. In addition, the requirement that the enzyme is used in a soluble form imposes another difficulty in continuous reactor systems, as the enzymes are continuously lost with the effluent. This makes the process infeasible. Due to its low conversion and high cost, the expense of enzymatic hydrolysis can exceed 50% of the total bioethanol production cost [90–93].

5.2. Enzyme Inhibition

There are three pathways by which the enzyme can be inhibited: competitive, non-competitive, and uncompetitive inhibition. In competitive inhibition, the inhibitor competes with the substrate for active sites and binds only to free enzymes. In non-competitive inhibition, the inhibitor binds only to the enzyme–substrate complex. In contrast, in

uncompetitive inhibition, the inhibitor binds to either the free enzyme or the enzyme–substrate complex [94]. The effect of competitive inhibition can be reduced by increasing the substrate concentration. This decreases the chance that the inhibitor will bind to the enzyme and the Ki value remains unchanged. However, the Km value increases with substrate loading. In contrast, a non-competitive inhibitor acts on the enzyme–substrate complex. Hence, the addition of more substrate does not help improve inhibition [95].

Surface accessibility is among the factors that effects the conversion rate. It results in declining the adsorption of enzymes to the substrate over time [96]. At first glance, it seems that increasing the solid loading in the reaction could be a strategy to overcome this problem. However, different solid loading levels produce similar trends of an instant conversion increase followed by a decline. Nevertheless, the increment in the initial phase of the reaction is higher when a higher solid loading is used. The instant increase can be attributed to the instant occupation of most of the active sites on the enzyme, and productive binding occurs when more substrate is available [97]. However, as enzymatic hydrolysis progresses, the substrate surface changes dynamically, and the non-hydrolyzable parts are gradually exposed over time for the enzyme to bind with. Thus, the non-productive enzyme–substrate complex grows over time. In addition, there are three interconnecting causes of the decrease that is observed during hydrolysis: mass transfer, mixing speed, and product concentration. The main inhibitors of cellulase are the hydrolysis products. Among the hydrolysis products, xylose exhibits non-competitive inhibition with cellulase, whereas glucose and cellobiose both exhibit competitive inhibition [82,98]. Much less inhibition has been reported for galactose and mannose [97].

5.3. Immobilization: A Solution to the Challenges of Heterogeneous Mixtures

Enzymatic hydrolysis of lignocellulose has been suggested as a potentially sustainable approach to ethanol production. However, challenges such as the high cost and recyclability of the enzyme render large-scale production infeasible. During the reaction, cellulase is lost with the product, and a purification unit is thus required to purify the product and recycle the enzyme. To simplify the separation and reuse of the enzyme, immobilization via entrapment within a matrix using methods such as covalent binding and crosslinking has been intensively investigated [99–102]. Several reports have emphasized the positive effect of immobilization on cellulase stability. For example, the immobilization of cellulase into a polyacrylic acid nanogel enhanced its thermal stability such that 75% of the enzyme activity was maintained at 80 °C [103]. The use of magnetic nanoparticles for enzyme immobilization has also attracted interest due to their large surface areas and high enzyme loading capacities [104]. The immobilized enzyme capacity of the magnetic nanospheres can increase with the surface charge, which can stabilize the catalytic activity of the enzymes and enhance thermal and pH stability [105–107]. Although the immobilization of cellulase on polyvinyl alcohol/Fe_2O_3 magnetic nanoparticles enhanced the conversion yield more than the use of free enzymes, enzyme activity was reduced to 40% within four cycles [108].

Despite the advantages of immobilization in enhancing cellulase stability and simplifying separation, the use of immobilized cellulase with highly crystallized lignocellulose biomass that remains insoluble in an aqueous solution is not practical. Immobilization results in fixing enzymes on or within solid support, which limits their accessibility to the heterogeneous substrate. This adds resistance, which further reduces the hydrolysis rate. Furthermore, since cellulose contains hydrolyzable and non-hydrolyzable parts, using the enzyme in immobilized form would prevent it from reaching deep within the cellulose matrix to reach hydrolyzable cellulose as the reaction proceeds, and the surface hydrolyzable parts are consumed [109–111]. Therefore, the use of the enzyme in the soluble form is required to achieve an appreciable reaction rate. This necessitates finding another way to separate the enzyme from the product and facilitate its reuse.

5.4. Membrane Technology: A Solution to the Challenges of Product Inhibition

Convectional bioreactors with various designs have been used in the industry to achieve bioethanol production from cellulosic materials. However, they all face one or more of the problems explained in Section 4.3. For example, stirred tank bioreactors (STRs) are commonly used in the industry due to their high solid loading advantage. However, enzyme deactivation is inevitable in these types of reactors because of product inhibition and the shear stress generated by vigorous agitation [112]. In addition, since the enzyme must be used in a soluble form due to the heterogeneous nature of the reactant, it is used for a single pass only in STRs and is then lost with the effluent. To overcome the shear deactivation problem in the reactor, a horizontal rotating tubular bioreactor has been suggested, and various STR rotation agitation impellers have been adopted. However, a reduction in the shear stress imposed on the enzymes was observed only when enzyme loss and deactivation due to product inhibition were not eliminated. Therefore, large-scale production using current reactor designs remains infeasible [113].

Membrane bioreactors (MBRs) have been proposed as promising solutions to the product inhibition effect, as they can separate the produced inhibitors selectively. In addition, using membranes with proper cut-offs enables large enzyme molecules to be retained. This prevents their loss with the effluent and enables their repeated use during longer reaction times. At the same time, smaller product molecules are separated from the reaction without the need for additional purification [114]. This eliminates product inhibition and maintains enzyme activity. The shear stress imposed on the enzymes can also be reduced in MBRs. This further maintains the enzyme activity.

The superiority of MBRs over STRs can be seen when comparing enzymatic hydrolysis yields achieved using the two reaction systems. For example, the hydrolysis of parchment coffee composed mainly of xylan in an MBR and an STR under the same conditions, including a low solid loading of 1 mg/mL, produced 97% conversion in each reactor within 3 h. However, the superiority of the MBR over the STR became evident upon increasing the solid reactant loading to 10 mg/mL. The former reactor achieved a conversion of 78%, whereas the latter achieved only 53% [115]. The conversion decreases observed in the two reactor systems occurred because of increases in the concentrations of produced sugars, which resulted in product inhibition. In addition, given the same agitation, increasing the solid substrate concentration reduced the mixing efficiency. Although the effect of solid loading was expected to be the same in both reactor systems, the reduced product inhibition effect in the MBR system was the reason for its better performance.

5.4.1. MBRs Configurations

Generally, filtration can be integrated with a bioreactor system in either of two configurations. The membrane can be either in a separate unit, submerged, or in contact with the reaction vessel [116]. Figure 5 shows schematic diagrams of various MBR configurations adopted in various studies. In the hybrid membrane reactor shown in Figure 5A,B, the reaction is performed in one vessel and then the reaction slurry is passed to a different unit where the filtration membrane is placed. This configuration is easier to scale up. The enzymatic hydrolysis of olive mill solid residues in a continuous MBR coupled with separate ultrafiltration (Carbosep M5) in crossflow filtration mode (10 kDa), similar to the configuration in Figure 5A, produces a better conversion than the same reaction in a batch system. Under the same conditions, a glucose yield of 45% was achieved in the MBR within 14 h, whereas the batch system required 24 h to achieve the same yield [117]. However, this enhanced performance was not observed when an MBR coupled with an ultrafiltration polyethersulfone membrane with a 50 kDa molecular weight cut-off (MWCO) was used for the saccharification of washed corn stover using 20 FPU/g cellulases (Trichoderma longibrachiatum). This was attributed to the loss in enzymes from the system [118]. In addition, a techno-economical assessment of an MBR coupled in crossflow filtration mode with an ultrafiltration unit of 10 kDa MWCO used for the hydrolysis of α-cellulose pretreated with an ionic liquid showed that the process is economically infeasible. In this study, the

end products, glucose and cellobiose, that permeated from the ultrafiltration unit were purified further in a nanofiltration unit to separate the intermediate cellobiose. In the last stage, an electrodialysis unit was used to remove the ionic liquid used in the pretreatment step. The overall cost of glucose was estimated to be 2.75 EUR/kg, which is relatively expensive [119]. Despite its advantage in retaining the enzyme and enabling its repeated use, when a separate MBR-filtration configuration is used, the reaction slurry must be pumped to the filtration unit and recycled back to the reaction vessel. This adds to the production cost and may cause enzyme deactivation and loss. In addition, the advantages of simultaneous product separation are not provided in such configurations. Furthermore, fouling is more pronounced, as recycling the reaction slurry between the reaction vessel and the ultrafiltration unit requires applying pressurized filtration to maintain a constant permeate flux. This problem is more severe in crossflow filtration, where filter-cake layer formation is high. Pumping the reaction slurry to the ultrafiltration unit quickly may reduce this accumulation, but this requires large amounts of energy. A decrease in membrane permeation was observed even when an agitation of 500 rpm was used to eliminate cake-layer formation at a filtration pressure of 1 bar [119].

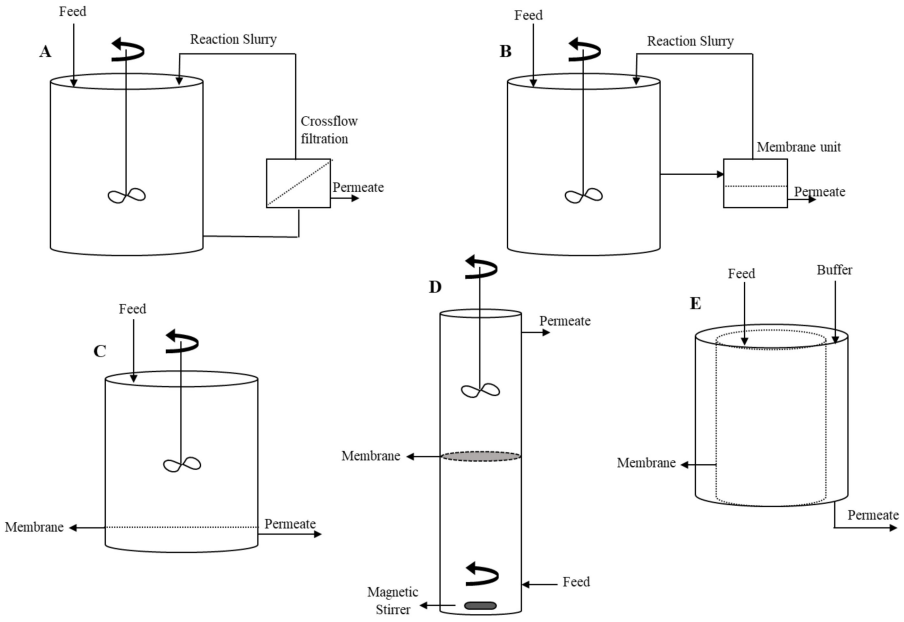

Figure 5. Schematic diagrams of different MBR configurations. (**A**) External filtration unit coupled with an STR in the crossflow module, (**B**) external filtration in the dead-end module, (**C**) dead-end filtration MBR, (**D**) inverted dead-end filtration MBR, and (**E**) tubular MBR.

Designed to avoid using multiple systems and their attendant pumping requirements, integrated MBRs, in which the products are separated simultaneously, have attracted attention [120]. In addition to using fewer units and not requiring inter-pumping, which reduces the overall cost, simultaneous product separation pushes the reaction forward and reduces enzyme inhibition. Examples of various integrated MBRs reported in the literature are shown in Table 1.

In most of the investigated integrated MBRs, in which reaction and separation are performed in one unit, the dead-end filtration concept is adopted. When this filtration concept is used, the flow direction is perpendicular to the membrane [116]. In these MBRs, the membrane is placed at the bottom of an STR, as shown in Figure 5C. The reactants are placed above the membrane and the low-molecular-weight products, glucose and cellobiose, permeate to the bottom stream. Like other MBRs, dead-end filtration MBRs exhibit better cellulose conversion than conventional STRs. For example, an α-cellulose hydrolysis conversion of 53% was achieved in a dead-end filtration MBR with a 10 kDa MWCO flat-sheet polysulfone membrane, whereas the conversion was only 35% in an

STR under the same reaction conditions [98]. The superior performance of the dead-end filtration MBR was also shown via enzymatic hydrolysis of corn stover pretreated with combined acid and base. Hydrolysis conversion in the MBR was 94% compared to only 77% in a continuous bioreactor (CBR) under the same reaction conditions [121]. Despite their favorable product inhibition reduction and enzyme retention characteristics, dead-end filtration MBRs have several limitations that restrict their large-scale application to the enzymatic hydrolysis of cellulose. For example, their limited solid substrate loading negatively impacts yield. This is mainly because high substrate concentrations result in insufficient mixing and increased surface deposition and filter-cake formation. These problems reduce membrane permeability and result in membrane damage via molecular deposition [122]. Vigorous mixing near the membrane surface can be used to minimize these effects, but this results in increased shear stress, which reduces enzyme activity. A modified configuration with a multiple-membrane system was proposed to overcome substrate cake formation on the ultrafiltration surface. A sealed nylon bag containing pretreated cellulose is submerged in a reaction vessel that contains buffer and enzyme. The bag has relatively large openings that allow the enzyme to diffuse in but prevents the cellulose from diffusing out and thus traps the substrate, preventing its deposition on the ultrafiltration membrane. The glucose molecules produced inside the sealed bag diffuse to an outer vessel and are separated using a separate ultrafiltration unit [123]. Although the concept of this modified configuration is promising on the small bench scale, applying it to large-scale production might be difficult. In addition, when the concept was tested, the accumulation of substrate on the ultrafiltration membrane surface was indeed eliminated, but protein molecules accumulated on the membrane to form a gel layer that reduced membrane permeability [124].

Recently, a novel MBR with inverse dead-end filtration was proposed and tested. This approach was proposed as a method of enhancing enzymatic hydrolysis while maintaining good mixing and eliminating cake deposition and membrane fouling. The MBR consists of two zones separated by a 10 kDa MWCO polyethersulfone flat-sheet ultrafiltration membrane, as shown in Figure 5D. The reactants are added to the reaction zone through an inlet in the bottom zone, while the glucose-containing permeate is collected from an outlet placed in the upper zone. Simultaneous reaction and separation occur, and the glucose molecules produced pass against the direction of gravity, through the membrane, and to the upper zone. The enzymes and substrate are retained in the reaction zone. Placing the membrane above the reaction vessel eliminates substrate deposition on the membrane surface via the gravity effect. The height of the reaction cell is designed carefully to enable precipitation of the suspended substrate before it reaches the membrane surface. Filter-cake formation is reduced further by applying tangential agitation using a magnetic stirrer. This causes movement tangential to the membrane, which disrupts any aggregation. The magnetic stirrer used for agitation is placed in the bottom zone, where it enables sufficient mixing without imposing shear stress on the enzymes. Shear stress can result in enzyme deactivation. A maximum yield of 86.7% was achieved after 8 h of continuous reaction and separation. Membrane characterization showed a complete absence of membrane fouling and deposition on the membrane surface [125].

The tubular reactor MBR configuration has also been tested, as shown in Figure 5E. The tubular membrane at the center of the MBR provides a large surface area for separation. This results in more efficient product separation, which produces a faster conversion rate and enables a reduction in the reactor volume. In addition, the enzyme–substrate diffusion resistance is expected to be low in such a configuration. This configuration was also simulated theoretically [126]. In a recent study, the performance of a tubular MBR with a PES membrane with a 10 kDa MWCO was compared to that of an inverted dead-end MBR with a similar membrane. The superior performance of the tubular MBR was evident using pretreated date seeds as the substrate. The tubular MBR achieved 60% conversion within 8 h, whereas the inverted dead-end MBR achieved only 10.8% under the same conditions [127]. This clearly demonstrates the positive effect of increased product removal

in the tubular reactor, which occurs because it has a higher membrane surface area per reaction volume.

5.4.2. Membrane Selection

The ability of an MBR to retain enzymes effectively while permeating the product easily depends on the type and properties of the membrane used in the reactor [116]. Ultrafiltration (UF) and nanofiltration (NF) membranes are two types that are commonly reported for enzymatic hydrolysis applications [116]. However, UF membranes, which have an average pore size in the range of 0.5 to 100 kDa, are typically used to retain large enzyme molecules in the main hydrolysis reactor selectively, which is the focus of this review paper. NF membranes, on the other hand, which have an average pore size of 150 to 1000 Da, are used for product concentration [116,130], which is essential for enhancing downstream processes and reducing production costs [131].

UF membranes can be fabricated from various materials, including polysulfone (PS), polyethersulfone (PES), cellulose acetate (CA), nylon (NY), and ceramics [132]. The membranes most commonly used in MBRs to enhance enzymatic cellulose hydrolysis are PES membranes, with MWCOs of 10 to 50 kDa. This is mainly due to their hydrophobicity, which gives them the ability to effectively reject cellulase enzymes without interfering with the reaction [116]. However, PES hydrophobicity has been found to increase membrane fouling. Therefore, it has been suggested that hydrophilic polymers such as polyvinyl pyrrolidine (PVP) be added to the membrane as antifouling agents [133,134]. Unfortunately, the high water solubilities of the added hydrophilic polymers result in their leaching during the process and thus the loss of their antifouling properties after multiple membrane uses [133]. Ceramic membranes appear to be more interesting for industrial applications because of their high physical and mechanical strengths, which PES membranes lack, that allow them to withstand high permeation fluxes suitable for large-scale production [135]. The performance of a ceramic membrane was examined in a hybrid MBR in crossflow filtration mode. A reaction performed in an STR was compared with one performed using a PES membrane [136]. An MBR with a tubular ceramic membrane (5 kDa MWCO) that contained three channels was operated under continuous mode with a permeation flow rate of 215 mL/min and 0.5 bar of back pressure. An MBR with a PES membrane (5 and 10 kDa MWCO) was operated under semi-continuous mode with a flow rate of 120 mL/min and 1.2 bar of back pressure. Although both membranes achieved high enzyme retention of over 98%, their ability to maintain enzyme activity and the permeation flux for multiple cycles varied. The 5-kDa PES membrane maintained active enzymes for six cycles, based upon a consistent hydrolysis yield of 94%. However, a decline in permeation flux was observed. This was attributed to pore blockage by unhydrolyzed glucose oligomers. The performance was improved when a PES membrane with a larger MWCO of 10 kDa was used. The activity and permeation flux was maintained for nine cycles. The performance of the 5-kDa ceramic membrane was found to be similar to that of the 10-kDa PES membrane, which suggests that it could be a superior alternative to PES membranes because of its higher mechanical strength [136].

Table 1. Applications of integrated membrane bioreactors (MBRs) in enzymatic hydrolysis of cellulose.

MBRs Configurations	Membrane			Substrate		Enzyme	Operational Conditions								Conversion	Ref.
	Type	Composite	MWCO	Type	Pretreatment		Flux	Substrate (g/L)	Enzyme (g/L)	T (°C)	pH	Press (bar)	t (h)	Mixing (rpm)		
Dead-end filtration	UF	Polysulfone	10 kDa	Alpha-cellulose fiber	-	C8546 *T. reesei*	7–9 L/m^2 h	25	0.1	40	4.7	0.7	48	- [a]	53%	[98]
		Cellulose acetate	10 kDa	Xylan extracted from coffee parchment	-	Xylanase, *A. niger*	nd	1	0.11	40	4.6	nd	3	200	97%	[115]
		PES	10 kDa	Microcrystalline Cellulose	NaOH	Cellic CTec2-with high level of β-glucosidase	10 mL/min	100	2.4	50	5	nd	8	200	7.6%	[123]
		Polysulfone	10 kDa	Corn Stover	Aquas ammonia (SAA)	(A) Spezyme CP, *T. reesei* (B) Novozyme 188	-	5	(A) 60 FPU/g (B) 30 CBU/g	45	4.8	0.6	20	120	82%	[121]
					Dilute sulfuric acid-sodium hydroxide			10							94%	
Submerged filtration	Dialysis	Spectra/Pro6	1 kDa	Wheat straw	Heat	(A) Celluclast 1.5 L *T. reesei* (B) Novozyme 188 *A. niger*	-	1	(A) 4.1, and (B) 1.08	50	5	-	72	350	28%	[128]
Tubular filtration	UF	Non-woven textile-polyethylene (PE)	nd	Solka Floc powder	-	Celluclast *T. reesei*	80 mL/min	25	3	50	4.8	-	25	-	50%	[129]
				Mavicell cellulose pellets	Heat								10		70%	

[a] 300 V electric pulse was subjected on membrane for 20 s.

5.4.3. Key Factors Affecting the Performance of MBRs

MBR performance is influenced by various parameters that are related to the enzymatic hydrolysis reaction, the reactor configuration, and the membrane properties. The temperature and pH of the reaction system are important factors to optimize activity and enzyme stability in order to achieve good hydrolysis rates and yields [137]. The activity and stability of cellulase also depend on the microbial strain used in the enzymatic cocktail recipe [114]. Other factors that affect enzymatic activity but have received much less attention are salts and the ionic strength of the reaction medium [138]. For example, the presence of sodium ions from the sodium acetate buffer used in many studies to adjust the pH of the reaction was found to enhance endoglucanase action but suppress exoglycanase [138]. The two effects can be balanced at a certain sodium ion concentration. This concentration depends on the source of the cellulase used.

In addition to the enzyme-related factors discussed previously, the performance of an MBR during enzymatic cellulose hydrolysis is affected by substrate-related factors, which are correlated directly with the pretreatment step [137]. Lignocellulosic biomass pretreatment is a crucial step that determines not only the success of the hydrolysis step but also the subsequent steps. The efficiency of the pretreatment method, which depends on the type of lignocellulosic material [139], is measured by the digestibility of the resultant cellulose. A sugar yield that exceeds 90% should be achieved in less than 3 days using an enzyme loading lower than 10 FPU/g cellulose [140]. The crystallinity index of the pretreated lignocellulose, which measures the recalcitrance of cellulose, has been linked to hydrolysis efficiency. Efficient pretreatment results in a lower crystallinity index, which enables better enzyme accessibility through the amorphous cellulose matrix [139]. For example, pretreating rice straw with ultrasound-assisted alkaline (NaOH) improved the digestible cellulose yield by a factor of 3.5, as compared to untreated biomass [33]. This pretreatment method was found to increase the porosity and decrease the crystallinity index of the biomass, which enhanced the accessibility of cellulase, as compared to the same biomass treated only with heat. Pretreatment with less than 8 wt% NaOH was also shown to cause separation in the cellulose lattice and decrease polymerization [34]. On the other hand, despite its strong ability to remove lignin and hemicellulose, an acid-alkali lignocellulose pretreatment (0.1 M hydrochloric acid and 0.1 M sodium hydroxide) produced increased crystallinity. This was attributed mainly to the ability of the acid to remove amorphous cellulose and leave behind only the recalcitrant cellulose. Therefore, it has been suggested that delignification using alkaline pretreatment is adequate for enhancing the enzyme accessibility of the treated substrate [127]. Sodium hydroxide and mechanical pretreatment (ball milling) of wheat straw cellulose were compared; the alkaline pretreatment enhanced the hydrolysis rate more than the mechanical treatment. Complete hydrolysis of biomass pretreated with NaOH was attained in 10 h, whereas the biomass pretreated with ball milling required 24 h. This shows that the lignin removal attained via alkaline treatment was better at enhancing hydrolysis than the particle-size reduction attained via ball milling. However, better hydrolysis was attained when both pretreatments were combined [141].

Enzyme and substrate loading are other important parameters that affect the technical and economic feasibility of the cellulose hydrolysis process. Although increasing the substrate concentration is expected to increase the hydrolysis yield, studies have shown that this is correct only up to a certain concentration. Substrate inhibition and poor mixing due to the viscous slurry occur above this concentration, resulting in reduced hydrolysis rates [142]. In addition, a high substrate concentration in some MBR configurations increases membrane fouling, which strongly influences the feasibility of the process [143]. Increasing the enzyme concentration also contributes to membrane fouling; the former contributes to external fouling, and the latter contributes more to internal fouling. Figure 6 shows filter-cake formation that increases the hydraulic resistance manifested as a permeate flux decline. Physical and chemical cleaning are usually performed to remove the accumulated molecules from the membrane surface. Physical cleaning can be achieved via either backflushing, in which the water flux is reversed for a short period of time to disrupt the cake layer or via relaxation, in

which the membrane is scoured with air bubbles [144]. On the other hand, chemical cleaning with a dissolving reagent is used when the fouling is irreversible. However, neither method enables complete permeability retention. As a result, the membranes require replacement. Fouling is more severe in crossflow filtration systems. Such systems should be operated below a critical flux, above which fouling starts to build up. Nevertheless, fouling is inevitable and is observed even at low fluxes. Pumping of a reaction slurry has been suggested as a method of slowing substrate accumulation by redistributing the molecules on the membrane surface and thus controlling the rate of accumulation. However, such an approach adds to energy requirements and increases operating expenses [145].

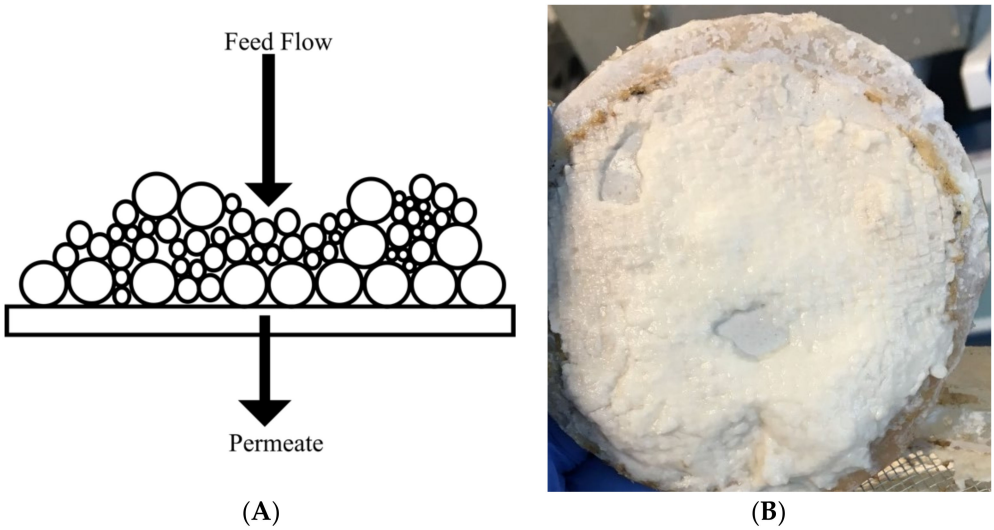

Figure 6. Filter-cake formation in dead-end MBR on the membrane surface. (**A**) Schematic diagram of the deposition of solutes on the membrane surface and (**B**) real accumulation of standard cellulose molecules on a PES membrane.

In an attempt to minimize membrane fouling, an electrical pulse was directed into the membrane by installing a cathode in the form of a stainless-steel mesh that physically supported the membrane from the bottom and an anode placed above the membrane at a distance of 1 mm. After fouling, the membrane surface was subjected to an electric pulse of 300 V for 20 s, which increased the permeation positively by six-fold. However, this improvement was sustained for only 120 s before the permeation declined again. In addition, it resulted in conformational changes within the enzyme, which affected its activity [98]. In a recent novel MBR design, a PES membrane was placed above the reaction to eliminate molecular deposition on the membrane via the gravity effect. The height of the reaction cell was designed carefully to enable precipitation of the suspended substrate before it reached the membrane surface. Filter-cake formation was reduced further by applying tangential agitation, which disrupted any aggregation [125]. The examined membrane exhibited no accumulation on its surface, and thus, fouling was eliminated in this design.

Agitation is another parameter that can be adjusted to increase cellulose conversion and MBR performance. The effect of the agitation speed on carboxymethyl cellulose conversion was investigated in an STR. Agitation was facilitated using a hanging-bar impeller, and a 10-kDa PES membrane was installed at the reactor bottom. The conversion improved to approximately 90% within 1 h at 55 °C when the highest agitation speed of 1200 rpm was used. The lowest tested speed of 300 rpm resulted in a conversion of almost 80% under the same conditions [138]. This enhancement was attributed to sufficient mixing in the reaction cell, which enabled better mass transfer, as well as enhanced disruption of local product accumulation around the enzymes. This facilitated substrate-enzyme adsorption [127,146]. More importantly, high agitation was found to reduce fouling by disrupting filter-cake formation on the membrane surface [125]. However, it should be

noted that the use of an extremely high agitation speed could generate excessive shear stress on the enzymes, which results in activity loss [147].

6. MBRs Prospects

To improve the performance of the lignocellulose-to-ethanol production process and bring it closer to commercialization, it is essential to enhance the cellulose hydrolysis rate and yield while operating at a high substrate loading to increase the concentrations of the produced sugars [148]. To achieve this, an effective substrate pretreatment method that results in improved substrate-cellulase productive binding should be adopted [149]. The enhancement of enzymatic hydrolysis and the subsequent fermentation step both require high solid loading levels. This is a major problem in most conventional stirred reactors. As previously mentioned, high solid loading levels increase the viscosity of the reaction medium. This affects the mixing efficiency negatively and results in low productive binding between enzymes and hydrolyzable cellulose and an increase in the energy required for agitation [148]. Table 2 shows a summary of the main factors affecting enzymatic hydrolysis. Several approaches to this problem have been suggested. They include fed-batch mode operation and increasing the dry matter content by enhancing the pretreatment step [125,148]. Another major problem encountered in conventional reactors is the accumulation of products that inhibit the enzyme and reduce the conversion yield. Therefore, there is a need for a novel reactor system that can solve these challenges. Various MBR configurations have been used successfully to overcome the product-inhibition challenge. However, membrane fouling remains a major problem in industrial applications, where it requires frequent membrane replacement and thus increases production costs. To resolve this, a novel MBR design that adapted the inverted dead-end filtration concept was recently suggested. Although membrane fouling was eliminated, the high solid loading requirement proved to be a harder challenge to resolve. The advantages and disadvantages of the MBRs discussed in this paper are presented in Table 3. However, a complete analysis of various factors that affect enzymatic hydrolysis in MBRs is still missing. A novel tubular MBR that uses a PES membrane was tested recently. It exhibited promising enhanced enzymatic hydrolysis results and enabled high solid loading [127]. However, membranes with high physical and mechanical strengths that can withstand higher separation fluxes are needed for industrial applications. As explained in Section 5.4.2, ceramic membranes have exhibited promising results. This suggests that they can be a better alternative to PES membranes. Nevertheless, the use of ceramic membranes in MBRs to achieve enhanced enzymatic hydrolysis of cellulose is scarce in the literature. Further investigations that use this type of membrane are needed for large-scale production.

Table 2. Summary of factors to be considered for enhanced production yield in MBRs.

Enzymatic hydrolysis	Enzyme-related factors	- pH - Temperature - Product inhibition - Enzyme loading - Enzyme source - Salt and ionic strength
	Substrate-related factors	- Substrate inhibition - Mixing efficiency - Solid loading - Pretreatment type
Membrane performance	Membrane-related factors	- Reactor design - Membrane material - MWCO - Membrane maintenance - Membrane fouling

Table 3. Summary of advantages and disadvantageous of different MBR designs.

MBR Design		Advantages	Disadvantages
Hybrid MBRs	Reaction and filtration are separated	- Membrane advantages - Easy to scale up	- Multiple units in the system - Enzyme loss and deactivation - Pressurized pumping - Energy consumption - Membrane fouling - Economically unfeasible
Integrated MBRs Reaction and filtration combined	Dead-end filtration MBR	- Simple set-up	- Solid loading limitation - Enzyme deactivation due to shear stress - Severe membrane fouling
	Inverted dead-end filtration MBR	- Membrane fouling elimination - Enhanced conversion yield	- Solid loading limitation
	Tubular MBR	- Enhanced membrane surface area - Low enzyme–substrate resistance - Small reactor volume	- Limited investigations

Global annual bioethanol production is estimated to reach 140 billion liters in 2022, with a compound annual growth rate of 7.6%. These figures are low compared to other industrial applications. This is mainly due to the challenges faced in large-scale bioethanol production [150]. Therefore, an important issue that remains to be considered is the use of MBRs for simultaneous enzymatic hydrolysis and fermentation, which can have a positive effect on process simplification and total production cost reduction.

7. Conclusions

MBRs offer a cellulose bioconversion enhancement solution that eliminates enzyme deactivation and loss of enzymes with the effluent, which are drawbacks that are encountered using conventional STRs. The ability of the membrane to retain enzymes and separate the product from the reaction system gives MBRs a superior performance. In this review paper, two main MBR configurations divided based on the reaction and separation steps

for hybrid and integrated MBRs were discussed. Because of their simple designs, hybrid MBRs are easy to scale up. This adds to the general advantages of using membranes for the selective separation of products. However, the drawbacks encountered with this reactor type limit its potential for economical application. Integrated MBRs, on the other hand, offer various sub-designs, including dead-end filtration, inverted dead-end filtration, and tubular MBRs, with the last exhibiting good commercial application potential. The cellulose conversion yield must be enhanced to enable feasible bioethanol production that makes bioethanol a competitive replacement for fossil fuels. This can be achieved not only by improving the hydrolysis reaction but also by enhancing the MBR performance by implementing an efficient configuration coupled with a membrane with appropriate properties and cut-offs. Additional studies on the use of MBRs for the improved enzymatic hydrolysis of cellulose are still needed.

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