Yeast-Mediated Biomass Valorization for Biofuel Production: A Literature Review

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Abstract: The European Union has recommended that about 10–50% of the global energy requirement should be supplemented by waste biomass resources by 2050 in order to achieve the objective of having net-zero-emission economies. This has led to intensive research being conducted on developing appropriate biofuel production technologies using advanced or integrated systems to tackle local, national, and global energy challenges using waste feedstock. Researchers have realized the potential of microbes (e.g., yeast strains) for bioenergy production. For this paper, both non-oleaginous and oleaginous yeasts were reviewed, with a specific focus being placed on their diversity in metabolism and tolerance to the various challenges that arise from the use of waste feedstock and influence bioprocessing. Gathering in-depth knowledge and information on yeast metabolism has paved the way for newer and better technologies to employ them for consolidated biorefineries to not only produce biofuels but also to cut down process expenses and decrease the risks of net carbon emissions. The rationale for using yeast strains improved by metabolic engineering and genetic manipulation that can substantially meet the challenges of alternate fuel resources is also described in this paper. This literature review presents the advantages and disadvantages of yeast-based biofuel production and highlights the advancements in technologies and how they contrast to conventional methods. Over the last decade, scientific publications have endorsed the idea of biorefineries for environmentally friendly, cost-effective, and sustainable biofuel production.

Keywords: yeast; biofuel; biorefinery; waste; net-zero emissions; biorefinery

1. Introduction

The use of biofuels such as cellulosic ethanol, biodiesel, and biogas in the transport sector has been accepted as a key step towards achieving low C emissions. The governments of many countries are promoting the large-scale deployment of biofuels through a favourable policy framework [1]. Biofuels are the most preferred drop-in fuel at this juncture, not only because they are C-neutral, help with respect to climate protection through producing fewer greenhouse gas emissions in the transport sector, ensure energy security by reducing dependence on imports, and help to conserve fossil fuel resources, but also because they are more efficient than conventional fuels and provide opportunities for developing rural economies. The contribution of yeast in the production of various commercial products, e.g., xylitol, biodiesel, bioethanol, etc., has been explored, and the application of yeast using anaerobic digestion for biogas and methane production is gaining attraction among researchers around the world [1].
Wastes from the agricultural, food processing, and forestry sectors, which are abundantly and ubiquitously available, can be targeted as potential feedstocks for biofuel production and consequent waste remediation. The second-generation biofuels produced from lignocellulosic biomass can reduce greenhouse gas emissions, and the carbon intensity of these fuels is approaching net-zero (Figure 1). The processing of lignocellulosic waste for biofuel production could pave the way for developing economic and environmentally friendly biofuels to significantly restore our global C footprint [2]. Therefore, emphasis should be placed on developing innovative biorefinery approaches producing not only biofuels but also other platforms or value-added chemicals, eventually paving the way for a bio-based sustainable economy [3]. Recently published literature reviews on the advancements in yeast-mediated biofuel production have shown that the use of microbes, e.g., yeast for the exhaustive utilization of waste residues, can lead to the synthesis of useful metabolites via eco-friendly means. The cost effectiveness of these methods have been predicted via biorefinery models that predict the development of an indispensable circular economy. This review explores the potential of diverse yeast strains to produce important biofuels, especially bioethanol, biodiesel, and biogas, and also investigates the relevant technologies, as well as their associated advancements and challenges, involved in using various biomass feedstocks. Both oleaginous and non-oleaginous yeast strains are discussed in the context of biodiesel and bioethanol production, respectively, and their metabolic differences are compared to better understand the effects of process parameters on product formation. The various fermentation technologies for bioethanol/biodiesel production, including strain improvement, technical advancements, innovations, and environmental impact, are discussed. The application of yeast for anaerobic metabolism during biogas production and as an effective catalyst for biomethane production is also explored.

**Figure 1.** Biofuel production from waste biomass via an environmentally friendly approach for attaining net-zero emissions and achieving a circular economy.
2. The Application of Yeast for Bioethanol Production

The use of ethanol could help to reduce greenhouse gas emissions by 70 to 90% (excluding C emissions from land-use change) as it is free of sulphur and has higher octane ratings than petrol. The production of bioethanol has been spurred by concerns about the environment and economic sustainability; however, the deployment of 2G ethanol from lignocellulosic biomass has faced challenges due to biomass recalcitrance, resulting in process hurdles. Moreover, the low value of bioethanol and the associated logistical challenges, e.g., the cost and transportation of low-density feedstocks, severely limit its economic feasibility. Biorefineries developed for the production of 2G ethanol along with other valuable by-products face a wide range of issues, e.g., the high cost associated with treating multiple and recalcitrant feedstock, slow processing, difficulties in commercial deployment, constant changes in the transport and communication market, etc. Despite these hurdles, its sheer abundance, renewability, and C neutrality make lignocellulosic biomass the most attractive alternate resource for energy production [4].

It is important to first unlock the complex polysaccharides present in lignocellulosic biomass to convert them into simple, assimilable, fermentable sugars for consumption by the fermenting organisms. The first challenge in the commercial production of cellulosic ethanol production relates to logistics, including ensuring the safe transport and year-round supply of large quantities of low-density raw materials, which is largely being addressed by the formation of a favourable policy framework by state and structured supply chains. The next challenge in technology development involves the integration and optimization of all of the stages of the process in an environmentally friendly and cost-effective manner. All of the steps in the conversion process are strongly associated and inter-dependent; specifically, the pretreatment step determines the efficiency of subsequent steps like enzymatic hydrolysis and fermentation [5]. During fermentation, trade-offs have to be made to attain the highest possible substrate loadings at a sufficient gravity to obtain higher ethanol titres, thus limiting the energy consumption necessary for distillation and minimizing the toxicity of the inhibitors emanating from pretreatment prior to yeast cultivation. While protocols to produce 2G biofuels are available, many of them are being evaluated on a pilot scale and demonstration plants. The main crux of the present research and development investments is on improving economics by increasing pretreatment efficiency, reducing enzyme costs, valorizing lignin, and improving the yeast strains through recent technological advancements [6].

2.1. The Selection of Yeast Strains

Until recently, *Saccharomyces cerevisiae* was preferred for almost all ethanolic fermentation processes on an industrial scale because of its robustness and high tolerance to ethanol and inhibitors. The only limiting factor affecting the efficiency of yeast is its inability to utilize xylose.

Waste biomass and especially pretreated and hydrolysed lignocellulosic waste feedstock contains hexose and pentose sugars, along with sugar alcohols, etc. The inability of *Saccharomyces cerevisiae* to ferment xylose, which forms about 25–30% sugars in biomass hydrolysates, is a matter of great concern during bioethanol production. To circumvent this drawback, it was pertinent to look beyond *S. cerevisiae*, bioprospecting non-conventional yeast strains that utilize both glucose and xylose. The limitations of *S. cerevisiae* have become more evident in the current scenario, wherein improving the economic viability of second-generation bioethanol production has become a necessity. On the contrary, non-conventional yeasts that have evolved independently from *S. cerevisiae* in diverse ecological niches are sources of gene pools which have been sparsely exploited. Monocultures of some non-*Saccharomyces* yeasts have shown prolonged growth and diverse sugar fermentation ability compared to *Saccharomyces cerevisiae*. [7,8]. Several other genera of yeasts, including *Pichia, Candida*, etc., can ferment a wide range of sugars present in hydrolysates for ethanol production [9]. Native pentose-fermenting yeasts, such as *Pichia*
stipitis, Candida tropicalis, and Rhodotorula glutinis, have been shown to grow well and produce ethanol when cultivated in rice straw hydrolysate. They efficiently utilize xylose along with the glucose and produced ethanol. The fermentation industry also faces the challenge of inhibitors in addition to the efficient utilization of diverse sugars present in biomass hydrolysates. In one study, it was shown that non-Saccharomyces strains could tolerate furfural and yield higher ethanol levels than engineered strains of Saccharomyces cerevisiae when cultivated on different biomass hydrolysates [9]. Two Kodamaea ohmeri strains isolated from Lagenaria siceraria flowers through enrichment on xylose efficiently fermented glucose (61%) in a medium supplemented with minerals. The level of supplementation (0.1%) of the yeast extract and peptone stimulated the co-utilization of sugars and facilitated 0.25 g g⁻¹ ethanol production on mixed sugars with ~50% fermentation efficiency, and this was the case for both of the strains [10]. Meyerozyma caribbaca demonstrated its potential for application in the bioethanol industry. This strain demonstrated some exceptional characteristics in terms of ethanol productivity and tolerance to temperature, ethanol, and inhibitors. Strains of M. caribbaca and Wickerhamomyces anomalus showed high tolerance to ethanol 18% (v/v), whereas Meyerozyma caribbica MJTm3 survived 20% ethanol. The actual ethanol concentration was 26 g L⁻¹ (12.7% (v/v)). Also, these strains showed a temperature optima of about 45 °C and a pH optima ranging from 2 to 10. They could also survive in a high specific gravity medium containing 50% sugar [11]. Meyerozyma and Lodderomyces yeast strains isolated from rotten apples showed highly efficient mixed sugar utilization and ethanol production, consuming 100% glucose and 33% xylose within 24 h, yielding ethanol at the rate of 0.344 g g⁻¹ and 0.327 g g⁻¹, respectively, with an efficiency of about 65%. The strains were tolerant to inhibitors like 5-hydroxymethyl furfural and furfural at concentrations commonly found in pre-treated hydrolysates [12]. A biofilm of M. caribbaca YLP01GX, an endophytic yeast (isolated from oil palm leaf), formed a biofilm onto a bio-based epoxy foam, which enhanced ethanol formation when cultivated on the hydrolysed empty fruit bunch of oil-palm [13]. The only limitation of these strains is their lower ethanol tolerance in comparison to S. cerevisiae. Yeast strains utilizing a wide range of sugars and other carbon sources may therefore be successfully employed for enhanced bioethanol production in addition to other high-value commodities from lignocellulosic biomasses.

2.2. Yeasts from Extreme Environments

Minimising the steps involved is an option for lowering the production costs of second-generation bioethanol production to promote a simultaneous saccharification and fermentation process. However, its applicability is affected by the inhibitory effects of pretreatment on the yeast strains, impairing the total bioethanol productivity. Therefore, using robust highly tolerant yeasts instead of prevalent mesophilic yeast strains is desirable. Thermophilic yeasts grown at 40 °C or higher are well-suited for this process since they can help reduce operational costs by eliminating pumping and cooling costs. This leads to efficient saccharification in the form of concentrated sugar syrups that enhance ethanol titre and productivity and simultaneously minimize the risk of contamination [14]. Thermotolerant yeasts also benefit large-scale alcohol production at high temperatures since they can metabolize sugars at higher temperatures. The operational problems associated with lower processing temperatures, e.g., rheological constraints, are thereby resolved. Also, the lower solubility of oxygen and other gases in the fermentation broth decreases at higher temperatures, thus facilitating anaerobic conditions. Additionally, the energy requirement for agitation is lower, and the heat generated due to the metabolic activity of microbes and frictional effects of agitation help in maintaining the fermenter at the required temperature optima. In one study, extremophilic ethanologenic symbiotic cellulo-lytic yeasts were isolated from dung beetles fed on lignocellulosic substrates, and the yeast strains also acquired the ability to withstand extreme stress conditions [14].

Thermotolerant strains of S. cerevisiae Pv-2, Tari-2, and Df-1; Pichia kudriavzevii Mlw-1 and Bp-2; Candida tropicalis Pv-1; P. guilliermondii; and Candida rugosa isolated from
different habitats produced 6.5 to 6.7% ethanol at 42 °C and 37 °C [15]. Strains of *Candida tropicalis*, *Meyerozyma guilliermondii*, and *Saccharomyces cerevisiae* isolated from lychees could tolerate temperatures up to 45 °C, 12% (v/v) ethanol, 10 g/L acetic acid, and 5 g/L furfural, respectively. About 47.96 to 70.18 g/L of ethanol was produced from 160 g/L glucose at 40 °C during 48 h of fermentation, where *M. guilliermondii* H1 utilized xylose and arabinose. Under statistically optimized conditions, *M. guilliermondii* H1 produced 11.12 g/L of ethanol from non-detoxified sugarcane bagasse hydrolysate (hydrolysate) at 40 °C [16]. Such thermotolerant yeasts could be isolated from different ecological habitats for application in bioethanol production from cellulosic feedstock.

2.3. *Simultaneous Saccharification and Fermentation (SSF)*

The use of thermotolerant yeasts has enabled the smooth operation of SSF processes and substantially reduced the associated costs of the processes, which are otherwise foreseen while using separate hydrolysis and fermentation (SHF), where each step requires a specific pH and temperature optima. The higher bioethanol yields generated using SSF rather than SHF make this strategy interesting for bioethanol production from lignocellulosic feedstocks [14]. The simultaneous saccharification, fermentation, and ethanol recovery (SSFE) process prolongs hydrolysis in the case of polysaccharides but at the same time relieves product inhibition effects associated with the high specific gravity of the growth medium. Sugars produced by saccharification are simultaneously fermented to ethanol, leading to reductions in costs and opex, and the increased utilization of polysaccharides takes place because of prolonged hydrolysis. SSF enables the fermentation of a highly concentrated and viscous hydrolysate slurry (up to 25% of dry matter), as it relieves product-inhibitions and facilitates the simultaneous removal of sugars via fermentation, keeping glucose and cellobiose concentration low in the broth. Such conditions lead to higher ethanol productivity. A low ethanol titre is maintained to prevent the inhibitory effects of ethanol and organic acid on the yeast strain, and ethanol is recovered via continuous evaporation from the fermenter while it is being produced [17].

2.4. *Consolidated Bioprocessing (CBP)*

Second-generation biofuel production through consolidated bioprocessing (CBP) is a strategy whereby the same organism performs all activities, e.g., degradation of lignocellulose and ethanol fermentation. Such organisms are obtained either by engineering lignocellulolytic enzymes into an ethanologenic pathway or vice versa. Thus, in CBP, the four biological events involved in the conversion of biomass to ethanol (the production of hydrolytic enzymes, saccharification of the carbohydrates present in pretreated biomass, fermentation of hexose and pentose sugars) can be accomplished in a single reactor. It has the potential for low-cost biomass processing and reconfigures the biorefinery concept by minimizing the infrastructure and use of toxic chemicals, thus making it economically and environmentally friendly. However, the commercial deployment of this strategy is limited due to its low conversion efficiency. It is not expected that a single native organism will possess all the characteristics for CBP. To obtain many of the desired traits in the same consolidated process, either a naturally occurring monoculture, engineered microorganism, or a consortium can be used. The major challenge is to identify a set of bifunctional catalysts that assist in utilizing and valorizing the substrates with high efficiency [18–20].

Specially, CBP focuses on cellulose and hemicellulose degradation and their utilization by engineering the enzymes involved. Kiesenhofer et al. [21] suggested four routes for the degradation of lignocellulosics by *S. cerevisiae*: (1) the extracellular expression of lignocellulose-degrading enzymes, (2) the expression of lignocellulose-degrading enzymes on the cell surface via the membrane anchoring method, (3) the uptake and assimilation of breakdown products (e.g., celloolx, and (4) packaging into cellulosomes. The current CBP approach makes reference to *S. cerevisiae*, enabling its use via robust lignocellulolytic machinery. Nevertheless, filamentous fungi are established organisms that are naturally equipped for plant biomass degradation; thus, they have enormous potential for CBP if
they can produce ethanol. The limiting factors associated with CBP include the selection of adequate genes and the challenges encountered in heterologous protein expression and processing, the placement of proteins, and real-time activity [22].

In the CBP approach, two aspects are important: Firstly, the host organism should be capable of expressing high levels of extracellular biomass-hydrolyzing enzymes. Secondly, it is important to execute the extracellular hydrolysis of lignocellulose to yield a high level of D-glucose. As *Saccharomyces cerevisiae* is incapable of using xylose efficiently, it has been subjected to engineering for the expression of hemicellulolytic enzymes on the cell surface and optimized xylose uptake and assimilation to obtain whole-cell biocatalysts for the consolidated bioprocessing of corn cob hemicellulose directly to ethanol using non-detoxified hydrolysates. About 11.1 g/L of ethanol was produced (0.328 g/g) from xylose and glucose [23]. CBP was more efficient in the corn cobs pretreated for ethanol production from hemicellulose than simultaneous saccharification and fermentation using commercial xylanases [23].

Several other organisms, like *Bacillus subtilis*, which has an intrinsic ability to use different carbohydrates, have been engineered for the CBP of lignocellulosics for ethanol production. They were engineered with an alcohol dehydrogenase gene (*adh*) and a pyruvate decarboxylase gene (*pdc*) from *Z. mobilis* and *S. cerevisiae* (*adh*) to create ethanologenic operons in lactate-deficient (∆*adh*) *B. subtilis*, resulting in NZ and NQS strains. Using the CBP approach, 16.3 g/L and 21.5 g/L of ethanol was produced by these NZ and NQS strains, respectively, after 96 h of fermentation [24]. The CBP of lignocellulose into bioethanol can also be carried out using fungi. The metabolic network of thermophilic cellulolytic fungus *Myceliophthora thermophila* was rewired to achieve the direct fermentation of cellulose into ethanol, and this was achieved during the aerobic ethanol fermentation of yeast (the Crabtree effect). The rearrangements involved optimized the synthetic pathway, enhancing the glycolytic rate, inhibiting mitochondrial NADH shuttles, and knocking out the ethanol consumption pathway. The final engineered strain produced 52.8 g/L of ethanol by utilizing cellulose and 39.8 g/L using corn cob biomass. This fungal-consolidated bioprocessing technology simplified the production process significantly and increased the yield of ethanol [25].

Cellulolytic, thermophilic anaerobic *Clostridia* have been shown to be appropriate for consolidated bioprocessing for second-generation biofuels production. A cellulolytic, thermophilic anaerobic *Clostridium* sp. DBT-IOC-C19 strain isolated from a Himalayan hot spring through enrichment showed a broad substrate range and was capable of predominantly converting different substrates containing cellulose and hemicellulose into ethanol, along with acetate and lactate [26].

In a study that used a novel consolidated bioprocessing approach, another anaerobic, cellulolytic thermophile *Caldicellulosiruptor bescii*, which could degrade raw biomass, was engineered by deleting lactate dehydrogenase and introducing heterologous *Clostridium thermocellum* bifunctional acetaldehyde/alcohol dehydrogenase genes directly so that it could produce ethanol from untreated raw switch grass feedstock. The predominant fermentation product in the engineered strain was ethanol (12.8 mM), which was produced directly from 2% (w/v) switchgrass with a decrease in the production of acetate compared to the wild-type strain [27].

2.5. Metabolic and Evolutionary Engineering

Industrially relevant microorganisms for biofuel production should be capable of high substrate utilization and processing capacities, fast sugar uptake and assimilation, tolerant to inhibitors and resistant to product inhibition, and contain deregulated pathways for directing high metabolic fluxes towards a single fermentation product. Recent advancements in research have enabled a deeper understanding of basic biology and the technology that can be applied to metabolic engineering and justify the optimism around cellulolic biofuel potential [ERROR! REFERENCE SOURCE NOT FOUND., ERROR! REFERENCE SOURCE NOT FOUND.].
The main strategies for improving strains through metabolic and evolutionary engineering are directed towards (a) the development of ethanol-fermenting yeast with a high tolerance to inhibitory compounds and for both pentose and hexose sugar fermentation via a genetic engineering approach, (b) the genetic engineering of a cellulosytic fungal strain for ethanol production, (c) the development of an efficient cellulosytic thermo-tolerant fungal strain for high-level enzyme production via a metabolic engineering approach, and (d) the genetic engineering of a cellulosytic fungal strain for ethanol production [29].

Several organisms, including yeast strains, bacteria, and fungi, have been subjected to metabolic engineering to achieve objectives like efficient and broad substrate utilization, ethanol and inhibitor tolerance, etc. The achievements and advancements made in the last decade in this respect have been critically analysed and are summarized in Table 1 [30–39].

Table 1. Some recent studies on the metabolic engineering of strains for improved bioethanol and lipid production.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Strategy for Enhancing Biomass Utilization or Biofuel Production</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hansenula polymorpha</em>, <em>Kluyveromyces lactis</em>, <em>Pichia pastoris</em>, <em>Yarrowia lipolytica</em></td>
<td>CRISPR-Cas enhancing biofuel production in non-conventional yeast</td>
<td>[30]</td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em></td>
<td>CRISPR/Cas9 for application in CBP</td>
<td>[31]</td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em></td>
<td>Engineering yeast for xylose utilization and ethanol production</td>
<td>[32]</td>
</tr>
<tr>
<td><em>Zymomonas mobilis</em></td>
<td>Metabolic engineering to broaden substrate range, remove competing pathways, and enhance tolerance to ethanol and lignocellulosic hydrolysate inhibitors</td>
<td>[33]</td>
</tr>
<tr>
<td><em>S. cerevisiae</em></td>
<td>Mining and identification of regulatory elements of bioethanol synthesis pathways, such as non-coding RNAs</td>
<td>[34]</td>
</tr>
<tr>
<td><em>S. cerevisiae</em></td>
<td>Genetic engineering, heterologous expression of cellulase genes, xylose transporters, knock-out, and the overexpression of key genes and promoters may be closely related to bioethanol yield</td>
<td>[35]</td>
</tr>
<tr>
<td><em>S. cerevisiae</em></td>
<td>Direction design optimization based on machine learning can effectively regulate the pretreatment parameters</td>
<td>[36]</td>
</tr>
<tr>
<td><em>S. cerevisiae</em></td>
<td>Improving ethanol yield via the addition of quorum-sensing molecules that deter growth of <em>S. cerevisiae</em> cells</td>
<td>[37]</td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em>, <em>Pichia stipitis</em></td>
<td>Metabolic engineering for enhanced bioethanol production</td>
<td>[38]</td>
</tr>
<tr>
<td><em>S. cerevisiae</em> extract</td>
<td>Constructing a cell-free system using Synthetic Biology tools</td>
<td>[39]</td>
</tr>
<tr>
<td><em>Yarrowia lipolytica</em></td>
<td>Overexpression of Diacylglycerol acyltransferases the I (DGA) gene(s) to promote lipid accumulation</td>
<td>[40]</td>
</tr>
<tr>
<td><em>Rhodosporidium toruloides</em></td>
<td>Overexpressing malic enzyme and acetyl-CoA carboxylase to redirect central C metabolism to enhance the availability of precursors toward lipogenic activity</td>
<td>[40]</td>
</tr>
<tr>
<td>Yeast Strain</td>
<td>Description</td>
<td>Reference(s)</td>
</tr>
<tr>
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<tr>
<td>Y. lipolytica</td>
<td>Co-overexpressing the glyceraldehyde-3-phosphate dehydrogenase and malate dehydrogenase for increasing lipid accumulation</td>
<td>[40]</td>
</tr>
<tr>
<td>Y. lipolytica</td>
<td>The overexpression of aldehyde dehydrogenase endogenous genes to enhance the conversion of furfural to furoic acid</td>
<td>[40]</td>
</tr>
<tr>
<td>R. toruloides L1–1</td>
<td>Breeding strategy to improve lipid accumulation along with stress tolerance</td>
<td>[40]</td>
</tr>
<tr>
<td>Y. lipolytica</td>
<td>Elimination of lipid catabolism by deleting lipid-assimilating genes, e.g., the acyl-CoA oxidases (POX) or peroxisomal biogenesis (PEX) genes</td>
<td>[40]</td>
</tr>
</tbody>
</table>

3. The Application of Yeast for Biodiesel Production

Initially, first- and second-generation biodiesel production from plant-based oils via transesterification processes was the subject of wide research; however, food insecurity, rising food prices, lack of cost effectiveness, and other reasons led to the development of other non-conventional alternatives. As a solution to this problem, third-generation biodiesel production was explored, which involved the usage of oleaginous microbes (isolated from agricultural/industrial waste, sewage water, etc.) for the synthesis and accumulation of lipids (triacylglycerol-TAGs) [41]. These cellular lipids were further processed via transesterification for biodiesel production. Oleaginous microalgae also accumulate lipids, but yeast strains are superior to algae with respect to lipid production due to their ease of bulk production followed by their lipid extraction steps. In relation to algae, its extraction process is difficult due to its presence of various pigments and other secondary metabolites, e.g., phenolics. Due to the high potential and eco-friendliness of oleaginous yeasts in producing fatty acids (FAs) resembling that of vegetable oils, they are suitable candidates for biodiesel production after effective transesterification [41,42].

3.1. The Selection of Yeast Strains

Selecting a suitable yeast strain is important for obtaining the lipids that are rich in TAGs and beneficial for biodiesel production. Oleaginous yeast can be cultured in media containing varied carbon sources such as sugarcane molasses, crude glycerol, glycerol (by-products of transesterification of lipids), xylose, etc. Various yeast strains, e.g., Rhodosporidium toruloides, Rhodotorula glutinis, Yarrowia lipolytica, Lipomyces starkeyi, Cryptococcus curvatus or Apiotrichum curvatum, Rhodotorula graminis, Rhodotorula gracilis, Rhodotorula mucilaginosa, etc., accumulate lipids consisting of long-chain fatty acids such as stearic acid (C18:0), palmitic acid (C16:0), oleic acid (C18:1), and linoleic acid (C18:2), which are highly potent in biodiesel production [43–46]. Oleaginous yeast species can be cultivated on a wide range of ‘C’ sources, such as glycerol, xylose, glucose, sugar alcohols, etc., and synthesize/accumulate lipids above 50% of their body weight. Various oleaginous yeast strains have been cultivated on agro-industrial waste, liquid waste, or fruit/vegetable waste to obtain large quantities of single-cell oil using optimized bioprocessing [47–51].

Despite sharing underlying similarities in their biochemical pathways, there are stark differences between oleaginous and non-oleaginous yeast strains. S. cerevisiae, Candida utilis, etc., are non-oleaginous, and they can store only about fifteen percent lipid with respect to its body mass [43,52,53]. Lipid accumulation in an oleaginous microorganism begins when it completely utilizes a nutrient component from the medium (usually nitrogen), but an excess of carbon (in the form of glucose) is still assimilated by the cells and is converted into triacylglycerols (TAG), while lipid is synthesized during the balance phase of growth at nearly the same rate. As the supply of nitrogen is limited, cells do not proliferate, and the lipid already produced is stored within the existing cells, which can no longer divide, leading to lipid accumulation. Lipid biosynthesis in oleaginous microbes occurs in the cytosol, and the flux of carbon, utilization of reductants, and involvement of few
key enzymes promote lipogenesis and its accumulation [54]. Several pathways, like tricarboxylic acid (TCA), the hexose monophosphate/pentose phosphate pathways (HMP/PPP), and the fatty acid synthesis pathway, as well as amino acid metabolism, are linked to TAG synthesis, especially in *R. toruloides*, *L. starkeyi*, and *Y. lipolytica* [52,54–56]. Malate enzyme and ATP citrate lyase (ACL) are two essential enzymes that have been studied extensively for regulating lipid accumulation; however, there are exceptions to this rule. In *Yarrowia lipolytica*, an oleaginous yeast, ACL activity was absent; on the other hand, the presence of ACL activity did not trigger lipid accumulation in the ethanologenic *Saccharomyces* sp. [53,55]. In non-oleaginous but ethanologenic yeast, the C flux is diverted toward pyruvate decarboxylase (PDC) and alcohol dehydrogenase activity. Oleaginous yeast also handles the effect of inhibitors, e.g., acetate inhibition when grown in lignocellulosic biomass hydrolysate [57].

### 3.2. Cultivation of Yeast for Lipid Production and Transesterification

Conventional and advanced fermentation techniques have been employed for biodiesel production from various oleaginous yeast strains. The optimization of fermentation conditions, as well as the methods for transesterification, is critical for determining the efficiency and quality of biodiesel production [47,51]. Methods of cultivation on various feedstock not only determine the characteristics of the lipid formed but also govern the cost effectiveness of the process [48,58]. Various fermentation methods have been optimized for cultivating oleaginous yeast on cheaper feedstock to facilitate economic bioprocessing, as mentioned below.

#### 3.2.1. Submerged Fermentation

Various parameters govern the accumulation of lipids during submerged fermentation. The carbon sources, along with the C/N (carbon/nitrogen) ratio, dissolved oxygen, pH, and temperature, are the major factors [40]. The type or quantity of carbon sources, e.g., sugars, glycerol, sugar alcohols, and other constituents of the culture medium, e.g., micronutrients, inhibitors, etc., regulate the lipogenic pathways. Differences were observed in the metabolism of oleaginous and non-oleaginous yeast strains during submerged fermentation under different nutrient conditions. During the growth of *S. cerevisiae* strain D5A (oleaginous strain) under N deprivation, the genes for lipid synthesis were up-regulated compared to BY4741 (non-oleaginous strain) [52]. This treatment did not lead to any significant difference in phospholipid biosynthesis between D5A and BY4741, indicating that phospholipid conversion did not always contribute to higher lipid content. On the other hand, the removal of organic nitrogen sources from the culture medium resulted in large amounts of biomass having lower lipid accumulation. The selection of a suitable nitrogen source and the C/N ratio also influenced the lipid accumulation [54,59].

#### 3.2.2. Solid-State Fermentation

In this type of method, the microbial culture is grown directly onto the solid substrate, similar to lignocellulosic biomass, agricultural waste, or any other suitable solids under optimal growth conditions. Although it is a simpler and cheaper technique because it utilizes the abundant nutritional sources from waste materials, downstreaming and the operation of lipid extraction processes after the removal of the solid substrate pose huge challenges [60]. The oleaginous fungus *M. circinelloides* Q531 and yeast *Mortierella isabellina* exhibited good product yield through SSF of lignocellulosic substrates, e.g., sorghum, rice hulls, pear pomace. *M. circinelloides* Q531 produced 42.43 ± 4.01 mg/g through SSF of mulberry branches, where unsaturated FA content was about 75.95% more than saturated FAs, which is recommendable for biodiesel production.
3.2.3. Two-Stage Fermentation

This method involves two stages: Firstly, the yeast strain is grown in a highly nutrient-rich medium to enhance biomass production by multiple folds. Subsequently, the medium is transferred to a restricted O2 and N2 supply to enhance lipogenesis. This method simplifies the optimization processes because biomass production and lipogenesis are controlled individually [61,62]. A two-stage fed-batch process at low temperatures (20 °C) and a suitable feeding strategy yielded high-cell-density cultures of Rhodotorula glutinis var. rubescens LOCKR13, with significant levels of oleic acid [63]. Oleic acid is a preferred TAG for biodiesel production, and achieving this via single-stage fermentation was not feasible since low temperatures do not support higher biomass production.

3.2.4. Co-Culture System

Different strains of yeast have been cultivated together with bacteria or microalgae to promote metabolite-based mutualism among strains, which in turn facilitates the utilization of suitable substrates in waste feedstock and enhances net biomass yield under optimized culturing conditions [64]. Yeast strains are carefully selected so that they complement each other’s metabolism and properties, thereby enhancing growth on various mixed feedstocks that are usually present in waste hydrolysates. The co-cultivation of oleaginous yeast (Trichosporonoides spatulata) with microalgae (Chlorella vulgaris var. vulgaris TISTR 8261 and Lipomyces starkeyi) and bacterium (Bacillus cereus) successfully enhanced lipid production compared to pure yeast cultivation under optimized conditions [64].

3.3. Genetic Engineering of Yeast Strains

Genetic modification/engineering techniques have been employed to ‘design’ yeast strains to improve lipid production by increasing their capability to utilize a wider range of carbon sources and tolerate inhibitors or nutrient-limited conditions (Table 1) [40]. Metabolically engineered yeast strains where the key players that determine ethanol biosynthesis were deleted or engineered to shift the C flux toward storage [65,66]. However, all yeast strains cannot be engineered to improve fatty acid synthesis. Engineering approaches were undertaken to redvert flux for the production of fatty acids by completely blocking ethanol production. However, such trials failed, as they led to severe growth defects in S. cerevisiae [67]. After these challenges are resolved, genetic engineering approaches will hold huge promise for both researchers and the industry [68,69].

The basic idea behind biodiesel production is the transesterification process (transformation of oils (fatty acids) into fatty acid methyl esters, commonly abbreviated as FAMEs) [45,70]. Once the lipids have been extracted from oleaginous yeast using cell membrane-disruptive techniques, the transesterification process converts them to biodiesel. The suitability of the biodiesel properties depends on the various methods of transesterification. The catalysts employed may be acidic, basic, or enzymatic, and the advanced processes use nanoparticle-based processes [70,71]. We have limited the discussion on these techniques according to the focus of this review.

3.4. Characteristics of the Biodiesel Produced from Yeast Lipids

Both the characteristics and quality of the biodiesel produced are directly correlated with the characteristics of the triacylglycerides (TAGs) produced by the yeasts, which in turn is dependent on a lot of factors, such as culture conditions/duration, type of raw materials/substrates used, yeast strain, etc. [70–73]. The fatty acids are unsaturated or saturated fatty acids, commonly palmitic acids (C16:0), stearic acid (C18:0), linoleic acid (C18:2), linolenic acid (C18:3), etc. An analysis of fatty acid methyl esters of biodiesel from lipids of R. toruloides strain ATCC 20409 and R. kratochvilovae HIMPA1 cultivated on different waste feedstock showed that the monounsaturated fatty acid (MUFA) content was higher than the number of polyunsaturated fatty acids (PUFAs) [43,74]. Unsaturated fatty acids contributed to improved biodiesel quality under low temperatures characterized by...
a low cold filter plugging point (CFPP), good cetane number, and an oxidative stability adhering to the EN 14214 and ASTM D6751-02 guidelines [74].

Also, to ensure the biofuel produced was at the international standard, an evaluation of properties such as viscosity, specific gravity, cetane number, cloud point, iodine value, chain length, chain branching, extent of unsaturation, etc., must be performed. Many nations have tried to set up and standardize these quality standards, e.g., EN 14214 (European Union), ASTM 6751-3 (American Standards for Testing Materials), etc. [75–77]. Calorific value, cetane numbers, viscosity, density, oxidative stability, etc., are some of the important fuel properties of yeast lipids that are reviewed in this paper.

3.4.1. Cetane Number

Unlike octane number, which is used for petrol, cetane number quantifies the delay in time for the fuel to ignite and can be correlated with both the quality and the performance of the biodiesel. The cetane number of biodiesels extracted from various kinds of yeast may vary roughly from 40 to 50. For example, the cetane number of biodiesels produced from Saccharomyces cerevisiae and Lipomyces starkeyi were found to be between 45 and 55. Similarly, a biodiesel produced from lipids of Cryptococcus curvatus showed a cetane No. in the range of 48–52 [75,78].

3.4.2. Density

The density of biodiesels is usually found to be greater than that of conventional diesels. For example, a biodiesel produced with the help of the Candida species showed a density between 0.87 and 0.89 g/cm³ [68]. The ASTM density range for biofuels is 0.86–0.90 g/cm³, and yeast biodiesel is close to this range, whereas biodiesels from sources other than yeast differed from these values [43,79].

3.4.3. Viscosity

The viscosity of biodiesels is also usually greater in value than conventional ones, and biodiesels can be used in normal diesel engines. Unlike the cetane number, the viscosity increases with the carbon chain length and saturation. The presence of C23:0 (28.71%) in yeast biodiesel raises its kinematic viscosity and contributes to problems, e.g., engine deposits [80,81].

3.4.4. Chemical Composition

The chemical compositions of biodiesels are dependent on various factors, such as the kind of yeast used, the environmental conditions, substrates used, etc. Biodiesels are composed of fatty acid alkyl esters formed from TAGs, where the presence of saturated and unsaturated fatty acids (FAs) is both important for biodiesel production as they influence its properties. Studies indicate that the saturated and unsaturated fatty acids exhibited by R. toruloides are desirable for biodiesel production; they are predicted to possess good flow property at low temperatures (due to unsaturated FAs), and this is balanced by the presence of saturated FAs, which resolve the oxidation problems caused by unsaturated FAs [43]. The physico-chemical characteristics of biodiesel produced from lipids/fats of various sources were compared by Singh et al. [43], who highlighted the suitability of yeast lipids as feed for biodiesel production, adhering to ASTM standards.

3.4.5. Calorific Value

Biodiesels show lower calorific values compared to conventional fuels, falling in the range of 35–45 MJ/kg. A biodiesel produced from Cryptococcus curvatus was found to have calorific values between 36 and 40 MJ/kg, but a biodiesel derived from R. toruloides showed about 4 MJ/kg [43,82,83]. Further research is warranted on effective catalysis during transesterification to enhance fuel properties.
4. Application of Yeast for Biogas and Bio-Methane Production

Biogas is a reliable, environmentally friendly, economically feasible, and sustainable fuel as it is produced from low-cost organic waste feedstocks [84]. Initially, its application was restricted to cooking, but now it has been extended to heating and power generation. Aside from its conventional applications, biogas is used to generate electricity by using diesel and petrol engines, turbines, and stirling engines. The global adaptation of biogas-based electricity has now spiked up by about 90%. The biggest advantage of using internal combustion engines with biogas is that only slight modifications are needed to operate them in dual mode. In addition to fuel, it also acts as a precursor for producing fuels, e.g., hydrogen and methanol [85]. Biogas is mainly composed of methane (approximately 60%) and carbon dioxide (40%), along with some fractions of hydrogen sulphide and water. To improve the energy content and for more precise applications, biogas is processed or upgraded to attain a methane content of 97% or higher, and this renewable gaseous fuel is biomethane.

Comparatively, electrochemical conversion, biotransformation, and fermentation have much higher environmental feasibility as well as lower energy investment compared to chemical production since it involves mild operating temperatures and biocatalysts, which are easier to apply in comparison to metal or heterogenous catalysts. Microbial fermentation facilitates the bio-methanation of a diverse range of substrates, like cheese whey, municipal waste water, swine manure, dairy wastewater, corn stover, and grass [84]. Among various microorganisms, yeast has attracted attention due to its high osmotic pressure and acid resistance, metabolically efficient system, and ability to withstand high concentrations of organic and toxic waste. Even during anaerobic digestion processes, yeast efficiently synthesizes ethanol from degradable organic matter, which can be used by methanogens for biomethanation [86]. The contributions of yeast in waste biotransformation and biomethane production have been categorized into three groups, i.e., during pretreatment, as feed or a co-substrate, and as a biocatalyst.

4.1. Biogas Production from Waste Biomass

Biogas is produced via different pretreatment methods and a variety of raw materials, viz., livestock waste, fruits seeds and shells, wastewater, food waste, agricultural waste (rice husk, plant stalks, straws, molasses), etc. The different disposal systems presently used for these waste residues are giving rise to environmental concerns, but they can be utilized successfully to produce biogas [87]. Biomass is converted into energy via two key pathways: (a) the Thermochemical pathway, which includes the direct conversion of biomass into gas or fuel by combustion, gasification, pyrolysis, and hydrothermal liquefaction and (b) the Bio-chemical pathway, which involves fermentation, aerobic, and anaerobic digestion [88]. The large-scale production of biomethane from biomass gasification process is the major advantage over anaerobic digestion. On the other hand, excessive investment as well as operation costs and more complex system configuration due to additional water–gas shift and methanation processes decreases the overall productivity of gasification [89]. To overcome the disadvantages of thermal treatment, bio-production methods that can be operated at mild operating conditions and reduce the discharge of by-products such as residues from anaerobic digestion, which can be used as bio-fertilizer for crops, have been considered. To increase the digestion, the co-fermentation strategy has been adapted in which manure and slurry were added to the agricultural biomass that uplifted not only digestion rates but also biogas yield. Anaerobic digestion (AD) process converts wet biomass/feedstocks, e.g., industrial or organic waste and manure into high-density gaseous fuels. This process has been popularly used for decades, and it has several advantages, such as the stabilization of waste, pollution control, and enhanced manure quality, as well as biogas production [90].
The AD process is interceded by both thermophilic and mesophilic methanogenic microorganisms. A complex process of biogas production from organic matter utilising AD includes four major stages: (a) hydrolysis, (b) acidogenesis, (c) acetogenesis/dehydrogenation, and (d) methanogenesis [91]. Each step involves microorganisms breaking down polymers into soluble metabolic products for methane production. Methane production is carried out by both hydrogenotrophic methanogenic microbes utilizing H2 and CO2 or by aceticlastic methanogenic microbes via the consumption of acetic acid. The high sensitivity of anaerobes towards ambient conditions, viz. pH, temperature, toxicity, alkalinity, makes it necessary to maintain the controlled environment to achieve the efficient metabolic activity of microbes during AD [92,93].

Other than anaerobic bacteria, yeast is extensively used in the treatment of high-refractory wastewater with a high organic waste concentration and toxic pollutants due to its characteristics of acid resistance, osmotic pressure resistance, and high metabolic efficacy [94]. Based on this, it has been shown that yeast can stimulate the hydrolysis of food waste by accelerating the rate of reaction of organic matter during the hydrolysis stage [95]. As the food waste is composed of a sufficient number of amino acids, peptides, and trace elements resulting from the hydrolysis of food, it delivers adequate nutrition for the yeasts to proliferate. The effect of yeast on the anaerobic digestion of food waste and production of neutral ethanol from degradable organic matter instead of organic acids results in a reduction in volatile fatty acids [96–98]. The utilization of yeast for the pre-fermentation of ethanol from food waste before AD produces optimal quantities of ethanol, which is subsequently converted into acetic acid. Further, methanogens utilize this acetic acid and produce an enhanced amount of methane by decreasing the effect of acidification on the system and increasing the AD stability [99]. Similarly, Gao et al. [86] revealed that the addition of yeast reinstated and promoted the performance of biogas production in an AD set up and may be considered as a viable approach for maintaining a steady AD system. In a study that employed fermentative yeast (S. cerevisiae) for biogas production from various substrates, e.g., fresh market garbage, solid potatoes, etc., using an AD system enhanced the quantity of biogas generation. Syaichurrozi et al. [100] studied the simultaneous activity of anaerobic bacteria from rumen fluid and yeast Saccharomyces cerevisiae during a four-stage AD process for biogas production. For this, a specialized anaerobic fermenter called a digester or bio-digester was deployed to enhance methane production using a wide range of substrates. However, the optimization of specific conditions and the operating parameters of AD systems is necessary when the type of raw material used is altered because substrates with high solid contents need special conditions [101]. Alterations in the substrate deters the hydrolysis processes and acidogenesis, thereby adversely impacting subsequent stages, resulting in a decrease in methane production during the final stages. Therefore, inoculum/substrate ratio and residence time must be given sufficient attention as they impact the feasibility and rates of bioprocess during AD.

4.2. Yeast Biomass as Feed and Biocatalysts

Gao et al. [86] have evaluated biogas production systems in the presence and absence of yeast. They reported that the addition of 2% yeast improved biogas production compared to control set ups without yeast. A comparative analysis suggested that overall biogas production in a yeast-supplemented system showed 33.2% higher biogas production compared to the control group. In addition to yield, the concentration of volatile organic acids and propionic acid, as well as system stability, was higher in the yeast supplementation group [86].

Yeast strains contribute to biogas production by assisting AD processes; alternatively, yeast biomass can be used as a protein supplement for other microbes during AD. For either application, further research is needed to establish the optimized bioprocesses as, at the time of writing, very few studies on this have been reported [102]. Yeast biomass has been suggested to be rich in nutrients such as proteins; hence, it can be utilized as a protein source during growth as well as fermentation. Suitable homogenization
techniques are needed to disrupt yeast cell walls and enhance the protein richness of the yeast extract. Ekpeni et al. [102] evaluated the effect of high-pressure homogenizer-assisted pretreatment on the protein content of bakers’ yeast (Saccharomyces cerevisiae) extract, where the frozen biomass was homogenized at 90 MPa at 20 °C for five cycles; a maximum protein release of 1.4 mg/mL was recorded [102].

It has been found that not only yeast biomass but its source of generation is also important for achieving effective valorization and product yield. A biochemical evaluation of methanogenesis via yeast biomass using substrates collected from breweries showed that the substrate on which yeast is cultivated also significantly affects the rates of methanogenesis. Yeast collected from pure barley brewing has shown slow degradability and a lag phase of 10.72–19.7 days. The substrate offered methane production rates of 14.59–4.63 mL/day. In comparison, yeast biomass collected from the brewing of white wheat malt and barley malt has a lag phase of <1 day and offered 17% of TCOD conversion to methane [103]. The presence of yeast biomass for use as a co-substrate or as feed has been shown to improve the productivity of the system and lead to a higher digestion rate. Zupančič et al. [104] combined brewer’s yeast and anaerobically treated brewery wastewater in a pilot-scale anaerobic sequencing batch reactor for biogas production, and the system achieved a maximum organic loading rate of 13.6 kg/m³ day/cycle and offered a biogas productivity of more than 0.430 m³/kg with over 90% total COD removal efficiency. It was suggested that addition of brewer’s yeast biomass increased biogas production by 50%. Hashemi et al. [105] also cultivated baker’s yeast wastewater to produce a protein-rich fungal biomass (along with a biogas) and developed a bioprocess to target COD removal with nutrient recovery. Hashmi et al. evidenced that red yeast and fungal strains have demonstrated the potential of COD removal, along with the production of other valuable commodities, e.g., carotenoids, which supplement and decrease fuel production costs. This strategy was also investigated by Moeller et al. [106] to produce citric acid as a by-product along with AD, which led to a maximum biogas production of 1.45 m³N/kgVS (methane concentration 66 ± 4%) [106]. To prevent over-acidification, tube clogging was performed, and operation took place at a high organic loading rate; concentrated yeast biomass was co-digested with waste frying fat (1:20). The experiment was stable for up to the 70th day of the fermentation period and allowed an organic loading of 2 kg/m³ d). Co-digestion offered a biogas yield of 1.42 m³/kgVS, with a methane concentration of 67 ± 4% [107]. Table 2 [108–114] summarizes the few studies on the application of yeast for biogas production.

### Table 2. The valorization of yeast for biogas production as a substrate as well as a catalyst.

<table>
<thead>
<tr>
<th>Role</th>
<th>Yeast/Source</th>
<th>Operating Conditions</th>
<th>Effect/Yield</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biocatalyst</td>
<td><em>Saccharomyces cerevisiae</em> (Yeast Sacc 1026, Alltech Inc., Nicholasville, KY, USA)</td>
<td>In vivo model of Jersey cows (Addition of NO₃⁻ (electron acceptor) and live yeast culture)</td>
<td>Methane production reduced from 22.6 mol/day to 18.8 mol/day</td>
<td>[107]</td>
</tr>
<tr>
<td>Co-substrate</td>
<td>Bakers’ yeast and craft yeast <em>Saccharomyces cerevisiae</em> (Brewery in Asheville, NC, USA)</td>
<td>10 mL rumen fluid mixture with cell suspension buffer was mixed with culture media (cornmeal and silage feed) inoculated with yeast at 39 °C in a CO₂ environment</td>
<td>Baker’s yeast: 4760 ppm Craft yeast: 3530 ppm</td>
<td>[108]</td>
</tr>
</tbody>
</table>
| Biocatalysts       | Yeast isolate 1 (Commercial probiotic; Angela yeast Co., Guangzhou, China)  
<p>|                    | <em>Saccharomyces cerevisiae</em> YST2 (Bakery; Kuala Lumpur, Malaysia) | In vitro fermentation Large intestinal content as inoculum with freeze-dried yeast powder is inoculated in fermentation medium at 39 °C for 24 h | Methane production reduction potential &gt;25%                                                      | [109]|
|                    |                                                   |                                                                                                                             |                                                                                                    |      |</p>
<table>
<thead>
<tr>
<th>Biocatalyst</th>
<th>Source of enzyme for waste pretreatment</th>
<th>Enzyme mixture from Candida rougsa</th>
<th>Simultaneous hydrolysis and the anaerobic digestion of lipid-rich waste water from the dairy industry</th>
<th>Methane yield increased by 140% [110]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co-biocatalyst</td>
<td>Saccharomyces cerevisiae (Ragi)</td>
<td>Tofu wastewater; pH 8; 1 atm pressure; rumen fluid as inoculum</td>
<td>421 mL [100]</td>
<td></td>
</tr>
<tr>
<td>Co-substrate</td>
<td>Brewer’s yeast</td>
<td>Wastewater Biosolids + brewer’s yeast; sewage sludge inoculum; period of 21 days at 37 °C</td>
<td>338.2 NmL CH₄/g volatile solids (VS) [110]</td>
<td></td>
</tr>
<tr>
<td>Co-catalyst</td>
<td>Meyerozyma gullerimondi (rhizosphere)</td>
<td>Providencia rettgeri and Meyerozyma gullerimondi (6 × 10⁴ colonies/mL)</td>
<td>Methane content 63.81% [112]</td>
<td></td>
</tr>
<tr>
<td>Co-catalyst</td>
<td>S. cerevisiae D2</td>
<td>Simultaneous saccharification and fermentation</td>
<td>Ethanol 16.98 ± 0.00 g/L Biogas 330 L/kg dry organic matter [113]</td>
<td></td>
</tr>
<tr>
<td>Catalyst</td>
<td>Yeast</td>
<td>Case 1: 2 g yeast + 4 g coconut fibre</td>
<td>Case 2: 2 g yeast + 4 g cocoa pods</td>
<td>Case 3: 2 g yeast + 4 g maize husk</td>
</tr>
</tbody>
</table>

5. Challenges for Yeast-Mediated Biofuel Production from Waste Feedstock

The primary challenge encountered for biofuel production using waste feedstock is the hydrolysis and breakdown of waste matter to simpler sugars that is utilisable by yeast. The plant lignocellulosic biomass is mainly composed of cellulose, hemicellulose, and lignin, which have different crystallinity and degradation patterns. Lignin is known as a protective and inert molecule for plant biomass, which also makes the biomass recalcitrant in nature. In addition, the biodegradation of lignocellulose generates some inhibitors like volatile fatty acids and phenolics, which not only slows down the degradation but also reduces product formation. Delignification is one of the most common approaches used to prevent the effect of lignin and phenolics, but over-delignification (above 50%) results in the collapse of the cellulose matrix [115]. Pretreatment by physico-chemical, biological, or combined methods prevent the negative effects of lignin that deter product yield. The chemical hydrolysis of waste also generates furfurals, acetic acid, hydroxymethyl furfurals, etc., which hinder microbial digestion, and so the detoxification of hydrolysate with activated carbon treatment and or membrane filtration methods has been adopted. The use of activated carbon has been more successful than membrane filtration techniques, where above 90% of inhibitors could be removed by adsorption [116–118].
Solid waste material undergoes AD processes using microbial consortia or sludge. Even with the presence of sufficient nutritive material, the yield of methane was merely 50 L/Kg biomass due to the recalcitrant nature of the substrate, which delayed bio-methanogenesis [119]. Another problem with lignocellulosic biomass is its low nitrogen content and high C/N ratio, which lower digestion rates and productivity. This issue can be overcome by adding waste with a higher nitrogen content or a suitable nitrogen supplement [118]. In solid-state fermentation, poor mass transfer is also one of the biggest challenges, leading to higher retention time [119]. The co-digestion of lignocellulosic biomass, i.e., rice straw, cow dung, and recycled slurry, shows that a richer microbial load can improve the rates and production of biogas [120].

Biogas has been produced using sludge from wastewater treatment plants (WWTPs) using the up-flow anaerobic sludge blanket (UASB) reactor. Generally, it is considered environmentally friendly; however, these conventional anaerobic processes have disadvantages, including long start-up period, odour problems, and problems associated with post-treatment techniques. Recently, chemical engineering technologies using an anaerobic membrane bioreactor (An-MBR) combining conventional AD with membrane prevents the leaching of methanogens. The significance of this technique has been endorsed by over 5000 published records on ScienceDirect in the last decade on An-MBR technology [121]. The method mitigates fouling when advanced membrane systems are utilized. Other valuable feedstocks for biorefining processes for obtaining biofuels include fish waste, coffee waste, etc., via anaerobic co-fermentation processes. These waste materials can be transformed into various products, including methane. However, the economic potential of such biorefineries may be impacted by production yields, market values, and government policies, e.g., subsidies. The success of such strategies requires further research and the support of regulatory frameworks and investment policies that are fundamental indicators of a sustainable circular bioeconomy. However, cellulosic biorefinery still entails challenges pertaining to suitable technology and high costs of operation for achieving market-ready biofuels, along with other value-added [122].

6. Conclusions

Biofuels produced by using waste resources via eco-friendly approaches are an answer to the energy challenges posed by developed and developing countries alike due to an upsurge in anthropogenic activities and industrialization. Currently, various biofuels (e.g., bioethanol, methanol, biodiesel, biomethane, bio-oil, biohydrogen) are being produced from biomasses via the activity of various microbial strains. Mining the literature has revealed the application of both oleaginous and non-oleaginous yeast strains for various types of biofuels, where the role played by yeast has been either in production or as a biomass/catalyst. Our review of the latest strategies of genetic or metabolic engineering that circumvent the existing problems related to the use of waste feedstock for biofuel production has demonstrated an upsurge of interest from the research community and industry alike. It is vital that future research endeavours to consolidate bioprocesses and frame superior biorefinery models for producing affordable, green, and sustainable biofuels. Government policies and regulations should also support biorefineries for the concomitant production of biofuels and other valuable products.

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