Rice waste streams as a promising source of biofuels: feedstocks, technologies and future perspectives

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Highlights

- **Rice waste has potential to produce many gigalitres of biofuels worldwide.**
- **Selection of suitable pretreatment is the key to extract maximum biofuels.**
- **Production strategy makes biofuel economically viable.**
- **Biorefinery approach is essential to consider while producing biofuels.**

Abstract

Increased environmental concern over climate change due to higher oil usage has made human being to shift to cleaner and greener alternatives. The utilization of abundant agricultural waste streams as renewable feedstock for biofuels production can be a pivotal strategy. Among others, rice is one of the most largely grown crops, generating huge amounts of waste which can be usefully processed into biofuels. Bioethanol is one of the most important applications, along with biobutanol and biodiesel. Whereas biogas and biohydrogen are the most promising gaseous biofuels, also electricity is an important energy for modern electronic vehicles. This paper reviews the biotechnological approaches to convert rice waste, such as rice husk, rice straw, broken rice, discolored rice, unripe rice into biofuels. The physical, chemical, enzymatic or microbial pretreatments, which play a key role in making carbon available for hydrolysis and fermentation, are discussed. Insights on the advantages and limitations of biorefinery approaches processing rice waste streams into a cluster of value added products are also provided.

Word count: 7935

KEYWORDS

Rice biowaste, Bioethanol, Biogas, Microbial fuel cells, Biorefinery, Microbial cell factories

Abbreviations

ABE- Acetone Butanol Ethanol

ADSS- Anaerobically digested sewage sludge

AFEX- ammonia fiber expansion

AFP- Acid fungal protease

AFP- Acid fungal protease

AS- Anaerobic sludge

AS- Anaerobic sludge

ASS-Activated Sewage Sludge

ASSP- Anaerobic sludge from sediment of pond

ATCC- American type culture collection

BR- Broken rice

BTU- British thermal unit

C/N-Carbon to nitrogen ratio

CBP- Consolidated bioprocessing

CD- Cow dung

CDSM- Cellulose degrading soil microflora

CDTD- Combinative dispersion thermochemical disintegration

CDTD- Combinative dispersion thermochemical disintegration

CDW- Cell dry weight

CRF- Cow rumen fluid

dAT- Deacetylation acid pretreatment

DDGS - Distillers' dried grains with solubles

DM- Dairy manure

DR- Discolored rice

DRB- De-oiled rice bran

DS- Digested sludge

FAME- Fatty acid methyl ester

FW- Food waste

GHG- Green house gases
GL- Gigalitres
GMO- Genetically modified organism
GSHE- Granular starch hydrolyzing enzyme
GSHE- Granular starch hydrolyzing enzyme
HC- Hydrodynamic cavitation
HRT- Hydraulic retension time
IEA- International Energy Agency
Mg- Megagram
MFC- Microbial fuel cell
MTCC- Microbial type culture collection
MWTPS- Municipal wastewater treatment plant sludge
NMMO- N-methyl morpholine N-oxide
NMR- Nuclear magnetic resonance
nr- not reported
OLR- Organic loading rate
PB- Pond bottom
PEM- Proton echchange membrane
PFS- Paddy field soil
RB- Rice bran
RBDW- Rice Bran De-oiled wastewater
RH- Rice husk
RRC- Rice residues from canteen
RS- Rice straw
RWW-Rice washing water
S/I- Substrate to inoculum ratio
SHF- Simultaneous hydrolysis and fermentation
SHS- Slaughterhouse Sludge
SM- Swine manure

SRSH- Synthetic rice straw

SS- Sewage sludge

SSF- Separate saccharification and fermentation

Tg- Teragram

TS- Total solids

UR- Unripe rice

VFA- Volatile fatty acids

VS- volatile solids

[BMIM][OAc]- 1-butyl-3-methylimidazolium acetate

1. Introduction

Fuel is a basic requirement of the developing world. Industrial development and population growth are the main drivers for energy demand. Considering the highest economic growth, the energy consumption in 2050 is expected to increase by almost 70% [1], with an overall energy demand rising to almost 680 quadrillions BTU by 2030 [2]. Up to 85% of this demand will be fulfilled by fossil fuels, thus continuing to contribute to environmental pollution by the release of greenhouse gases (GHG) in the atmosphere (50% higher than in 2011) [3]. Globally, a share of 34% of the primary energy supply is covered by crude oil, which is higher than any other energy source [5]. Moreover, the instability of oil prices has major impact on the economy in long run [6]. To overcome the continuous increase of energy demand, alternative solutions for cleaner and more environmentally friendly fuels than the available fossil ones, are needed [7].

The most promising alternative is represented by the use, when available, of waste/residual materials to be converted into biofuels, such as bioethanol, biogas, biobutanol, biohydrogen etc. [12]. This could be achieved by selected and/or improved microorganisms, once appropriate pretreatments are applied to the raw substrate.

Three main categories of raw materials are available and can be utilized for biofuel production: sugars-, starch- and lignocellulose- rich feedstocks [13]. Sugar and starch are found in grains, seeds, tubers, fruits etc., but unfortunately, their use for biofuels production can create food-versus-fuel competition [14,15]. The attention has therefore been turned toward the so-called “second generation” strategy by looking at inexpensive starchy and lignocellulosic residues originated from the industrial sectors [16–19].

Lignocellulose is by far the main component of farm residues like bagasse, straw, husks, brans and it is the most abundantly available raw material on the Earth. It contains an aromatic polymer (lignin) and 80% of polymeric carbohydrates (cellulose, hemicellulose) [20], suitable for the production of biofuels. Moreover, since 140 x 10^3 teragrams (Tg) of agricultural biomass is generated every year worldwide, the improper management of such organic material could lead to pollution. For instance, the excess of biomass burned in the open [21] results in an important loss of resources potentially available for fuel production. In fact, the yearly generated lignocellulosic biomass is theoretically equivalent to 50 x 10^3 Tg of oil [22]. Thus, developing technologies aimed at converting such excess biomass into biofuels could contribute to reduce the dependence from oil-producing countries and, at the same time, to safeguard the environment. Some surveys have been developed and published on the evaluation and characterization of agro-food residues for bioethanol production [23–28] and, among a number of different starchy and lignocellulosic residues, rice waste biomass has been indicated as one of the most abundant and promising feedstock [2]. The present review is focused on the latest biotechnological approaches devoted to biofuels production from rice waste streams.

2. Rice waste biomass: global availability and composition

3. Rice is one of the most important crops with a worldwide production of almost 1000 Tg in 2018 [37]. About 88% of the globally produced rice is used for human consumption and 2.6% for animal feed. Besides food, feed and seed, more than 4.8% of total rice grains go to waste [38]. For instance, in North America, 12% of produced rice is wasted and in Asia around 22 Tg of dry rice are discharged. The proper utilization of total wasted rice could allow obtaining 12.3 gigaliter (GL) bioethanol potentially replacing 8.9 GL of gasoline [48].

Biomass production in the rice industry includes both lignocellulosic and starch-rich residues (Table 1). Their potential in bioethanol, here chosen as representative of other biofuels potentially obtainable from, was also assessed. Lignocellulosic waste streams are the most abundant with up to 836 Tg. Rice straw (RS), the crop residue available on the field when the product is harvested (approximately 22% wet weight), accounts worldwide for 685 Tg, with a potential ethanol of nearly 194 Tg. Rice husk (RH), which is the seed cover obtained as an agro-industrial waste during grain processing (40% wet weight) can be converted into up to 41 Tg ethanol (Table 1).

Several starch-rich residual biomasses from rice could be also utilized for fuels production (Table 1). Their starch levels vary from 29 to 80% of dry matter with a high content of proteins which were shown to support nitrogen requirements of microbial strains involved in their fermentation [49]. Broken rice (BR) is a promising feedstock with an availability of up to 45 Tg an ethanol potential of 16 Tg. Rice bran (RB), unripe (UR) and discolored rice (DS) are also largely available, with significant ethanol applications [50–53].

Table 1: Average composition and availability of rice waste

Waste	**Average composition (% dry matter)**						**World biomass availability** (Tg)	**Bioethanol potential** (Tg)	**References**
	Starch	**Cellulose**	**Hemicellulose**	**Lignin**	**Protein**	**Ash**			
RH	6.9	40.1	20.6	22.3	3.4	18.2	151.1	41.4	[2,34,50,54–56]
RS	11.8	34.3	25.1	18.6	1.3	15.0	685.0	193.7	[2,32,34,54,57–62]
BR	77.7	0.2	0.5	-	8.3	0.5	45.3	16.0	[50]
DR	84.6	0.1	0.9	-	8.0	0.5	7.5	2.9	[50]
RB	29.6	6.9	15.7	4.1	14.5	8.0	52.9	11.5	[57,63]
UR	68.6	1.8	3.7	-	9.9	1.5	30.2	9.9	[50]

Lignocellulosic rice byproducts (RH- Rice husk, RS- Rice straw) and starchy waste streams (BR- Broken rice, DR- Discolored rice, RB- Rice bran, UR- Unripe rice), Yearly ethanol potential (Tg) from each feedstock has been calculated as previously described [27] considering both the availability and average composition.

4. Pretreatment of rice biomass

Pretreatment of rice waste streams is one of the most important and cost determining steps for their conversion into biofuels. This is necessary for the separation of lignin and hemicellulose, to reduce the crystallinity of cellulose and to increase the accessibility of hydrolytic enzymes [64]. Pretreatments should meet the following criteria: 1. obtain high efficiency of sugars formation either by the chemical, physical or enzymatic way [65]; 2. reduce loss of carbohydrates; 3. reduce inhibitory byproducts formation; 4. be cost-effective [66]. In principle, the treatment of lignocellulosic feedstocks is more complex than the processing of starch-rich substrates. Many efficient pretreatments of lignocellulosic and starchy rice byproducts have been recently developed to optimize the production of various biofuels and added valued compounds. Table 2 reports a selection of the most used physical, enzymatic and chemical methods.

Considering RS as raw material, a number of attempts have been reported to improve the efficiency of the enzymatic hydrolysis. For instance, a novel lime-pretreatment process was proposed without solid-liquid-separation. In the same vessel, xylan, starch and sucrose are present together and inhibitory effects on saccharification and fermentation were found to be not significant [58]. When the same pretreatment was applied on RH, no generation of detectable furfural and hydroxymethyl furfural was also observed [67]. Castro *et al.* focused on deacetylation of RS using alkali which resulted in a reduced concentration of inhibitors in pretreated hydrolysate [68]. NaOH combined with urea helped to increase the availability of cellulose and hemicellulose by effectively disrupting the structure of RS and increased maximum hydrogen production by over 160% than control [69]. Zhu *et al.* combined microwaves along with NaOH to reduce reaction time and enzyme loading. This combination yielded around 5% more ethanol than only alkali pretreatment [70]. Two-step pretreatment process consisting of aqueous ammonia and sulfuric acid helped in selective removal of lignin and hemicellulose respectively [71]. Teghammar *et al.* used N-methyl morpholine N-oxide (NMMO) for pretreatment of RS which increased the methane production by seven times than that of untreated RS. Also, 98% of the solvent used during pretreatment was recovered, making this pretreatment method environmentally friendly and economically feasible [72]. When the same method was adopted for bioethanol production and compared with 1-buthyl-3-methyl imidazolium acetate, NMMO was found to be more efficient in producing bioethanol [73].

Glycerol, a byproduct of the bioethanol and biodiesel industry, was used in two forms (i.e., acidified aqueous glycerol and glycerol carbonate) for pretreatment of RH. Results showed that glycerol carbonate showed better bioethanol production than acidic counterpart [74]. Saha *et al.*[67] treated milled RH with 1.5% NaOH at 121°C along with a cocktail of three commercial enzymes (i.e cellulase, b-glucosidase and hemicellulase), whereas Ebrahimi *et al.* [75] used ammonium carbonate to improve the ethanol yield from 10 to 47% in the 72h fermentation. This indicates that usage of alkali for pretreatment of RH is helpful to boost bioethanol production. Treating RH at 900°C produced ash that provided the economic and efficient source of proton exchange membrane (PEM) for the production of electricity [76].

Starchy-rich rice waste is usually more prone to pretreatment than the lignocellulosic one (Table 2). However, efficient enzymatic hydrolysis is needed to release glucose and thus a cluster of mostly commercial amylolytic blends was tested.

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Overall, towards the efficient processing of rice by-products into biofuels, with the large varieties of pretreatment technologies available, an in-depth assessment should consider the economic trade-off associated with pretreatment handling and transportation costs.

Table 2: Selection of the most used and efficient physical, chemical and/or enzymatic pretreatment recently adopted for rice waste streams.

Feedstock	Pretreatment			Product	References
	Physical	**Chemical**	**Enzymatic or microbial**		
Lignocellulosic materials					
RH	Wet air oxidation	-	-	Bioethanol	[56]
RH	Milling, Autoclaving	2% H_2SO_4, 3% NaOH	-	Bioethanol	[77]
RH	Milling	$(NH_4)_2CO_3$	Cellulase	Bioethanol	[75]
RH	Thermal	-	-	Electricity	[76]
RH	Milling	Acidified aqueous glycerol	Cellulase	Bioethanol	[74]
RH	Milling	Glycerol carbonate	Cellulase	Bioethanol	[74]
RH	Milling, Autoclaving	1.5%NaOH	Cellulase, β-glucosidase, hemicellulase	Bioethanol	[67]
RS	-	3.5% H_2SO_4	-	Biolipids	[78]
RS	Steam explosion	10% NaOH	-	Glucose	[79]
RS	Thermal	2% $Ca(OH)_2$	-	Biogas	[80]
RS	Extrusion	-	-	Biogas	[81]
RS	Extrusion	3% H_2SO_4	-	Bioethanol	[82]
RS	Autoclaving	-	-	Biogas	[83]
RS	Ozone	aqueous ammonia	-	Biogas	[84]
RS	Gamma irradiation	1% NaOH	-	Biogas	[85]
RS	Milling, Autoclaving	0.4% NaOH	*Trametes hirsute*	Bioethanol	[86]
RS	Milling, Autoclaving	-	*Pleurotus ostreatus*	Biogas	[87]
RS	Autoclaving	-	*Pleurotus ostreatus* *Trichoderma reesei*	Biogas	[88]
RS	Milling, Autoclaving	2.5-3 % HCl	Cellulase	Biohydrogen, Bioethanol	[89]
RS	CDTD	-	-	Biohydrogen	[90]
Starchy materials					
BR	-	-	α-amylase, amyloglucosidase	Bioethanol	[91]
BR	-	-	AFP, GSHE	Bioethanol	[92–94]
BR	-	-	Hyper active α-amylase	Bioethanol	[53]
BR, DS, RB, UR	-	-	GSHE	Bioethanol	[49,50]

AFP- Acid fungal protease, GSHE- Granular starch hydrolyzing enzyme, CDTD- Combinative dispersion thermochemical disintegration.

5. Biofuels production from rice waste streams

4.1 The key role of microorganisms as cell factories

In general, the microbial conversion of a waste into a product is an approach that is becoming increasingly popular as microorganisms can be considered powerful cell factories, capable of metabolizing raw materials and producing useful substances at industrial level [98–100]. Moreover, microorganisms can be further improved by genetic as well as evolutionary engineering approaches to maximize the desired product(s) yields and productivities. In this perspective, microorganisms can play an essential role in the transition from fossil fuels to biofuels from rice waste streams. Essentially, after the optimization of the pretreatments, two approaches have been developed in converting pretreated rice products into biofuels, namely the utilization of microbial consortia or the use of single bacterial or yeast strains (Fig. 1).

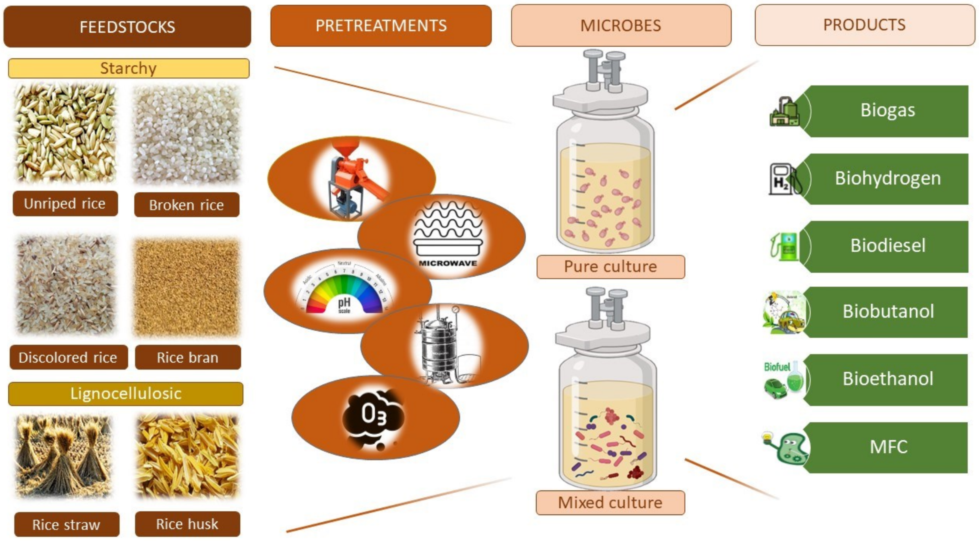

Figure 1: Biofuels production from different rice waste streams. Once subjected to a single or a combination of pretreatment(s), rice byproducts can be processed into different gaseous or liquid biofuels and electricity by using a pure or a mixed culture approach. MFC-Microbial fuel cell.

Mixed cultures are typically adopted for biohydrogen and biogas applications. The production of these biofuels provides that the process conditions select specific groups of microorganisms, naturally present in the inoculum or the feedstocks, acting sequentially to convert complex substrates into hydrogen or methane. Thus, the research is mostly focused on pretreatments optimization of the feedstocks as well as on the fine-tuning of process conditions aimed to select and facilitate the most efficient microbial populations.

Pure cultures are mainly used to obtain bioethanol, biobutanol biodiesel and electricity. This approach considers the utilization of single strains and specific efforts were spent towards efficient biotechnological routes by exploiting properly selected and/or genetically modified bacterial and yeast strains.

4.2 Biogas

Anaerobic digestion is one of the proven technologies of converting organic waste into biogas. The generation of biogas, mainly a mixture of methane and carbon dioxide, is considered eco-friendly and contributes to the reduction of soil and water pollution [101], thus encouraging the circular economy [102].different feedstocks.Methanogenesis is a complex process (Figurthat needs multiple reactions conducted by bacterial and archeal consortia under anaerobic conditions [102]. Insoluble organic compounds, mainly carbohydrates, proteins, and fats, are hydrolysed into soluble molecules, monosaccharides, amino acids, and fatty acids by extracellular enzymes synthesized by specific hydrolytic bacteria. Then, lactate, ethanol, propionate, butyrate, and higher volatile fatty acids (VFA) can accumulate and are converted to hydrogen by a specific microflora. In the following acetogenesis process, the acetate bacteria convert the acid phase products into acetic acid and hydrogen, used by methanogenic bacteria to produce methane [105,106]. Thus the syntrophic degradation of complex organic compounds to methane and carbon dioxide is a difficult process and requires the cooperation of diverse groups of microorganisms occurring in the natural environments and usually introduced in the industrial plants through specific inocula. Once biogas is generated, methane must be separated from carbon dioxide. As it is cost imposing process, methane yield in biogas is equally important.

The use of rice wastes to feed biogas plants has been proven feasible and sustainable, although anaerobic bacteria can hardly degrade lignocellulosic materials such as those contained in RS and RH (Table 1), due to the high C/N ratio, cellulose crystallinity, and great lignin content. As previously discussed, since the hydrolytic stage is usually considered the bottleneck mostly affecting the conversion rate of RS, many studies were focused on physical, chemical, and biological pretreatments, alone or in combination, aimed to improve hydrolysis (Table 3).

As an example, Chen *et al.* [81] evaluated the extrusion of RS compared to the milling. The authors demonstrated that the extrusion changed some physical properties of lignocellulose such as bulk density or porosity, thus enhancing the efficiency of bacterial cellulose and hemicellulose degradation. As a consequence, the digestion time of RS was shorter and methane yields increased.

A biological approach treating RS with suspensions of Pleurotus ostreatus DSM 11191 and Trichoderma reesei QM9414 gave interesting outputs [88]. Although moisture content and incubation time affected the efficiency of the treatments, the fungal incubation significantly improved lignin removal as well as biogas and methane yields.

In the work of Yan *et al.* [62], RS was firstly composted to facilitate the biodegradability of complex substrates and, then, treated in a solid-state anaerobic digester with anaerobic sludge as inoculum. After optimization of initial substrate concentration, temperature and C/N ratio, composted RS resulted to be more effectively degraded, thus increasing biogas yields.

Although biological pretreatments have undeniable advantages such as fewer energy requirements, specificity, or generation of fewer toxic compounds, they are expensive and need a long time and complex operating conditions [66]. Thus, to decrease operation time and enhance the biogas conversion efficiency of rice wastes, the utilization of acids or alkali, alone or in combination with physical pretreatments, is preferred. For example, Du *et al.* [80] reported that the alkaline thermal pretreatment of RS at mild temperature was more efficient than the hydrothermal in terms of lignocellulose decomposition and methane production. Kim and colleagues compared autoclaving the RS after the addition of H_2SO_4, with pretreatment with hot water and alkali [83]. However, although the highest lignocellulose decomposition was obtained by autoclaving after H_2SO_4 addition, the methane production potential was very low probably due to the inhibitory effect of the sulfate ion on methanogenesis, as reported previously [122].

The optimal process parameters for a combined synergistic pretreatment of RS with ammonia hydrochloride and ozone were also defined [84]. The combination of chemical and physical factors enhanced the enzymatic release of fermentable sugar and consequently biogas production.

Gu *et al.* [123] considered the role of inocula and found that digested manures (from dairy, swine and poultry) were more suitable than digested municipal, granular or paper mill sludges in increasing biogas production from RS.

Co-degestion of farmwaste is the most applied method at an individual level, wherein farmers can co-digest their farmwaste with other organic waste for production of biogas [95]. The possibility of improving biogas yield by the co-digestion of RS or RH with other biological wastes such as animal manure has been also investigated. As an example, Ye *et al.* [52] suggested the co-digestion of RS with kitchen waste and pig manure as a promising approach to balance the low C/N ratio of lignocellulose biomass. Haider *et al.* [96] assessed the co-digestion of RH with food waste, using fresh cow dung as inoculum pointing out the substrate to inoculum ratio (S/I) as one of the key parameters.

The effect of macro- and micro-nutrients on the performance of anaerobic digestion of RS [124] and RH [125] was also studied. In small scale experiments, using cow rumen liquid and acclimated anaerobic sludge as inoculum, the supplementation with heavy metals, such as Ni^{2+}, Zn^{2+} and Cu^{2+}, improved biogas yield from RH [125], while methane production rate from RS was accelerated by optimizing phosphate levels (465 mg-P/L) [124].

The effect of organic loading rate (OLR) on the conversion of RS to biogas was explored in a 300 m^3 mixed bioreactor [126]. An increase in biogas was observed when OLR was below 2.00 kg $VS_{substrate}/m^3d$ while the maximum production rate was 323 m^3/t dry substrate. The monitoring of prokaryotic community structure in the plant during biogas production confirmed that the hydrogenotrophic and acetoclastic pathways are the most common in the digestion of lignocellulosic wastes to methane [127,128].

Table 3: Biogas production from rice wastes: main pretreatments, inocula and yields.

Feedstock	Pretreatments			Inoculum	Temperature (°C)	Biogas Yield[a] mL/g VS	Methane %	Reference
	Physical	**Chemical**	**Enzymatic or microbial**					
RH	-	-	-	CRF	30	382	78	[125]
RH and FW	-	-	-	Acclimatized CD	37	584	-	[119]
RH and FW	Milling	-	-	AS and Pig manure	37	674	57	[61]
RS	-	Ozone, aqueous ammonia	Mixed Cellulases	DS	37	396	-	[84]
RS	Hydrothermal	Alkali	-	ADSS	37	411	49	[80]
RS	Milling	-	*Pleurotus ostreatus*	AS	37	353	73	[88]
RS	Autoclaving	Alkali or Acid	-	DS	35	932	-	[83]
RS	Milling	-	-	AS	37	227	-	[81]
RS	-	-	Composting	AS	35.6	447	-	[62]
RS	Milling	-	-	-	39	349	52	[126]
RS	Milling	-	*Pleurotus ostreatus* DSM 11191	AS	37	367	72	[88]
RS	Milling	-	*Trichoderma reesei* QM9414	AS	37	299	72	[88]
RS	Milling	-	-	DM	37	325	55	[123]
RS	Milling	-	-	Acclimatized AS	22 ± 2	340	77	[124]

[a] -Highest values of biogas reported (or calculated from available data) when available. FW- Food waste, DS- Digested sludge, ADSS- Anaerobically digested sewage sludge, AS- Anaerobic sludge, CD- Cow dung, CRF- Cow rumen fluid, DM- Dairy manure.

4.3 Biohydrogen

Biohydrogen can be obtained from carbohydrate-rich biomass by anaerobic (dark fermentation) and photoheterotrophic (light fermentation) microbes [129]. In recent years, biohydrogen has gained popularity as a clean fuel to reduce toxic gas release. Like all other fuels, biohydrogen must be cost-effective as well. Though biohydrogen production can be performed by dark-, Photo- and combined (dark- and photo-), to the best of the author's knowledge, only the dark fermentation route was exploited to obtain hydrogen from rice waste streams. Baeyens *et al.* provided detailed insights of the different pathways adopted by bacteria for the producrion of biohydrogen [130]. Recent studies on combinative pretreatments of RS have to be considered as an emerging cost-effective, alternative energy technology [90]. The difference in composition of RS, RH, RB and cooked rice leftover waste require a comparison between the effects of different temperatures on biohydrogen production potential, since for all rice biowaste, except for leftover cooked rice, a significant increase in biohydrogen yields was observed as the temperature increased [131]. Moreover, the concentration and particle size of the substrate were found to represent key parameters for determining the processing time. Similarly, hydrolysis time and concentration of additives were found to play an key role during the biohydrogen production from RS [89].

A further important aspect is concerning the nature and treatment of inocula, which are quite frequently obtained from anaerobic digestors. During anaerobic digestion, hydrogen is produced as an intermediate metabolite with hydrogen-producing and -consuming bacteria working together to obtain methane. To maximize hydrogen yield through dark fermentation, methanogens and hydrogen-consuming bacteria have to be inhibited. Several methods have been proposed to achieve this aim, including heat treatment, acidification, basification, freezing or dehydration [132–135]. Table 4 gives a summary of pretreatments of feedstocks, inocula and the corresponding biohydrogen yields. Along with biohydrogen yield, it is important to monitor the percentage of biohydrogen in the biogas, which ranged between 25-70%.

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Studies of heat treatment of inoculum were performed on activated sewage sludge and optimal results were obtained at 100°C for 60 min [136]. However, at a C/N ratio of 25, the use of non-heat treated sewage sludge resulted in a biohydrogen production from RS higher than the yield obtained by heat-treated sewage sludge [137]. On the contrary, other studies suggest the importance of heat treatment of sludge in terms of the selection of hydrogen-producing microflora over methanogenic organisms. As an example, Chen and colleagues explored heat treatments of different sludges and cow dung compost used as inocula for untreated RS [138]. Maximum biohydrogen yields were obtained using heat-treated sludges from municipal waste treatment plants. Moreover, they demonstrated that the heat treatment enriched the inocula in both hydrolytic and fermentative bacteria [138]. This study further highlights the importance of heat treatment of sludge in terms of the selection of hydrogen-producing microflora over methanogenic organisms.

Unlike pre-treated mixed inocula, also single cultures approaches have been pursued to convert rice waste streams into hydrogen. Cellulolytic bacteria isolated from soil and observed that pure culture of *Clostridium butyricum* CGS5 gave efficient biohydrogen production using enzymatically hydrolysed RH as substrate [139]. A pure culture of *Clostridium acetobutylicum* YM1 was also adopted on an acid-treated starchy waste such as DRB (de-oiled rice bran) [140].

In concentrated acid-treated RS hydrolysate and wastewater from the food industry, the presence of *Clostridium pasteurianum* was found to support the production of biohydrogen using acetate and butyrate pathway. Also, a 1.5-fold increase in biohydrogen yield was observed with lower substrate utilization in a continuous system as compared to the batch reaction [141]. After confirming the increased biohydrogen production in a continuous system, Liu *et al.* [142] worked on optimization of hydraulic retention time (HRT) of a continuously external circulating bioreactor, reporting that the highest hydrogen production rate was observed with an HRT of 4 h. The continuous production process also needs continuous organic loading. Therefore studies on OLR optimization demonstrated that biohydrogen production from RS increased, reaching maximum biohydrogen production of 2.6 L per day when the range of OLR was between 7.1 and 21.4 g COD/L per day [143].

Table 4. Biohydrogen production from rice wastes: main pretreatments, inocula and yields.

Feedstock	**Pretreatment**	**Type of inoculum**	**Best inoculum treatment**	**T (°C)**	**Best H_2 Yield^a**	**H_2^{a} (%)**	**Reference**
RH	Enzymatic	*Clostridium butyricum* CGS5	-	35	19.15 mmol/g reducing sugar	25	[139]
RS	Milling	ASS	100ºC, 60min	35	14.67 mL/g VS	70	[136]
RS	-	SS	100ºC, 15min	55	0.54 mmol /gVS added	42	[137]
		SS	-	55	0.74 mmol /g VS added	58	
RS	Milling	MWTPS	95ºC, 40min	55	24.80 mL/g TS added	-	[138]
DRB	Acid	*Clostridium acetobutylicum* YM1	-	35	117.24 mL/g consumed sugars	-	[140]
RBDW	-	SHS	100ºC, 60min	57	2.20 mol /mol substrate	42	[144]

^{a} -Highest values of hydrogen yield or percentage are reported (or calculated from available data) when available. ASS-Activated Sewage Sludge; DRB-Deoiled rice bran; RBDW- Rice Bran De-oiled wastewater; SHS- Slaughterhouse Sludge; SS- Sewage sludge; MWTPS- Municipal wastewater treatment plant sludge

4.4 Biodiesel

Biodiesel refers to fatty acid methyl ester (FAME) produced through the transesterification of oils, mainly obtained from specific energy crops such as rapeseed, RB, sunflower, palm and soy, but even from animal fats or waste oils [145,146]. In addition, specific oleaginous microorganisms have been selected and proposed for the sustainable production of lipids as already elegantly reviewed [147,148]. Oleaginous yeast, bacteria, and microalgae are defined as microorganisms with an intracellular lipid content exceeding 20% and reaching up to 70%. Lipids accumulation usually starts when a nitrogen source is limiting but in the presence of an excess of carbon, which will be converted into triacylglycerols [149]. In the perspective of reducing biodiesel costs, residues from rice could be profitable substrates for microbial biomass and lipids production. For this purpose, rice starchy or lignocellulosic wastes have been assessed as feedstocks by few research groups. Since the employed microorganisms are generally lacking specific hydrolytic enzymes, again lignocellulose or starch hydrolysis was found to be necessary as well as the optimization of fermentation conditions. RS and rice food waste were mostly adopted so far as feedstocks for lipids production (Table 5).

Azad *et al.*[78] optimized pH values of a fermentation broth containing H_2SO_4-hydrolysated RS as a carbon source for *Lipomyces starkeyi,* and found that the yeast accumulated microbial lipids up to 36.14% of cell dry weight (CDW). Diwan *et al*. [150] developed an effective H_2SO_4 based mild saccharification of RS and successfully employed the crude, non-detoxified hydrolysate for growth of the yeast *Mortierella alpina* MTCC-6344 that accumulated lipids up to 40% of CDW.

A different approach was pursued by using the amylolytic oleaginous yeast *Sporidiobolus pararoseus* KX709872 [151]. This strain produces α-amylase and amyloglucosidase, and was used to directly convert canteen rice residues into biolipids in both flasks and stirred tank bioreactor without previous starch hydrolysis. After broth optimization, lipids reached 56.61% of CDW. Moreover, the produced fatty acids contained high oleic content (60-62%) similar to those of vegetable oil, indicating that these lipids could be a promising alternative to plant fats.

Another methodology was tested by exploiting *Cryptococcus curvatus* ATCC 20509 ability to accumulate lipids from RS. Firstly, RS was treated with NaOH and anaerobically digested using sewage as inoculum. Resulting VFAs were then used by *C. curvatus* ATCC 20509 as building blocks for the synthesis of lipids (up to 26% CDW). The authors also assessed the techno-economical viability of their process, concluding that VFAs broth from anaerobic digestion of RS, compared to synthetic VFAs, appeared the most suitable carbon source for lipids production [149].

Microalgae have also been considered promising for biodiesel production due to their short cell cycle, ability to adapt to harsh environments, and high oil content (up to 80% CDW). Moreover, algae can be grown in fermentors without occupying cropped areas. Although algal biodiesel has still a price higher than conventional diesel which makes large-scale industrial applications not economically sustainable, attempts were made to reduce costs, such as using cheap carbon sources. For this purpose, Li *et al.* [152] used rice straw hydrolysate to support the fast-growing alga *Chlorella pyrenoidosa* MTCC-6344 which accumulated lipids up to 56.3% CDW. The following *in situ* transesterification obtained promising results with 95% biodiesel yield.

Table 5. Biolipids production from rice wastes: main pretreatments, microbes and yields.

Feedstock	Pretreatment			Microorganism	T	Lipids	Reference
	Physical	Chemical	Enzymatic/microbial		(°C)	(%CDW)	
RS	Microwave, Autoclaving	4.8% NaOH, 1.5% H_2SO_4	-	*Mortierella alpina* MTCC-6344	25	40	[150]
RS	-	1% Trifluoracetate at 95°C	Cellulsae	*Chlorella pyrenoidosa* MTCC-6344	25	56	[152]
RS	Autoclaving	2% NaOH	Synthesis of VFA by anaerobic digestion	*Cryptococcus curvatus* ATCC 20509	25	28	[149]
RS	Autoclaving	3.5% H_2SO_4	-	*Lipomyces starkeyi*	30	36	[78]
RS	Gamma ray irradiation	1% NaOH	Cellulase	*Chlorella protothecoides* strain 25	-	45	[85]
RRC	-	-	Gluco-amylase & α-amylase	*Sporidiobolus pararoseus* KX9872	22.4	57	[151]

RRC- Rice residues from canteen.

4.5 Biobutanol

Biobutanol is less popular among clean fuels although it represents a good alternative to fossil fuels, due to its unique features such as high energy content, improved heating value, and reduced corrosive action [153]. Moreover, it can be blended with gasoline with a proportion higher than ethanol. Butanol is largely used as an industrial intermediate, particularly for the manufacture of butyl acetate and other industrial chemicals, as a flavour in many food and beverage industries, or as an extractant for various manufactured chemicals and pharmaceuticals. Industrially, butanol is mainly produced via petrochemical synthesis (Oxo process) although biological synthesis is also possible and, for food safety reasons the butanol used in the food industry must be obtained only by microbial fermentation [154]. Biobutanol can be manufactured by the fermentation of glucose by anaerobic Clostridia performing the acetone, butanol, ethanol (ABE) metabolism. The ABE catabolism involves a first acetogenic step generating acetic and butyric acids, CO_2, and hydrogen, and a second step (solventogenic) in which acetone, butanol, and ethanol are produced from the acids [155].

Commentato [AM3]: There is no oxo process in figure 2

Butanol fermentation is much less efficient compared to ethanol fermentation. Therefore, great amounts of energy are necessary for product recovery from the diluted broth. This, together with the substrates cost, makes the entire process non-sustainable [156]. Thus, many efforts have been devoted to improve the efficiency of the process or decrease the costs of the raw material supporting microbial growth.

Rice wastes, especially RS, have a great potential to be efficiently used as a carbon source for butanol. Again, the use of such low-cost feedstock requires pretreatments, subsequent enzymatic hydrolysis to obtain fermentable sugars, and/or butanol-producing strains able to proficiently metabolize the released sugars, such as xylose together with glucose, into butanol (Table 6).

The sulphuric or phosphoric acids or alkali pretreatments of RS are reported as cheap and effective, and thus have been extensively evaluated [35,155–160]. Once obtained, the sugars are utilized by specific Clostridia to perform the ABE fermentation, with a yield of 2.0-18 g/L. Chen *et al*. [75] assessed a synthetic non-pretreated enzymatically hydrolysate from RS, under non-sterile conditions minimizing the contaminants interference by increasing the initial cell concentration of *C. sacchaperbutylacetonicum*. Such conditions ensured not only the biobutanol production in a non-sterile environment but demonstrated that the sterilization step of the agricultural wastes used as substrate can be avoided, thus reducing manufacturing cost.

While various research groups focused on the optimization of pretreatment and hydrolysis, others concentrated on fermentation modes. Parameters, such as initial pH, temperature, age and size of the inoculum, and the agitation rate, were optimized for the butanol production from pre-optimized RS hydrolysate [161]. Gottumukkala and coworkers fine-tuned ABE fermentation parameters (i.e., pH, inoculum concentration and calcium carbonate concentration) resulting in enhanced biobutanol yields from a detoxified enzymatic hydrolysate of acid pretreated RS by *Clostridium sporogenes* BE01 [162]. Although not considered as efficient butanol producer in comparison with commercial strains such as *C. acetobutylicum*, *C. sporogenes* BE01 reached a maximum butanol concentration of 5.52 g/L in optimized conditions, one of the highest reported for this species. Moreover this strain produced ethanol and butanol without acetone in the final mixture which is considered an advantage in the industrial bioconversion of biomass to alcoholic fuels [163].

To decrease the cost of the enzymes and increase sugar utilization and biobutanol production, Chi *et al.* [164] proposed a staged acidogenic/solventogenic fermentation process. In this study, alkaline-pretreated RS was firstly fermented by a microbial consortium of *Clostridium thermocellum* and *Clostridium thermobutyricum* to both hydrolyze lignocellulose and enrich the system with butyric acid. The resulting supernatant was used for ABE fermentation by *Clostridium beijerinckii* NCIMB8052. This strategy resulted in higher butanol production when compared to a conventional SHF (Separated Hydrolysis and Fermentation) process involving the use of commercial cellulases in the lignocellulosic hydrolysis step followed by the fermentation.

Table 6. Biobutanol production from rice wastes: main pretreatments, microbes and yields.

Feedstock	**Pretreatment**			**Microorganism**	**T** (°C)	**Biobutanol Yield**^{b} (g/L)	**Reference**
	Physical	**Chemical**	**Enzymatic/microbial**				
RS	Autoclaving	4% H_2SO_4, Detoxification	Cellulase	*Clostridium. sporogenes* BE01	35	5.52	[162]
RS	Milling, Autoclaving	1% H_2SO_4	-	*Clostridium acetobutylicum* NCIM 2337	37	13.50	[155]
RS	Temperature	1% NaOH	Cellulase, *Clostridium thermocellulm* ATCC 27405, *Clostridium thermobutyricum* ATCC 49875	*Clostridium beijerinckii* NCIMB 8052	37^a & 55^a	15.90	[164]
DRB	Autoclaving	1% H_2SO_4, Detoxification	-	*Clostridium acetobutylicum* YM1	30	6.87	[158]
DRB	Autoclaving	1% HCl or H_2SO_4, Detoxification	Cellulase	*Clostridium saccharoperbutylacetonicum* N1-4	30	7.72	[157]
SRSH	-	-	-	*Clostridium saccharoperbutylacetonicum* N1-4	35	6.60	[166]

DRB- Deoilded rice bran, SRSH- Synthetic rice straw hydrolyzate. ^{a}-Temperature adopted for lignocellulosic hydrolysis by *Clostridium thermocellulm* ATCC 27405 and *Clostridium thermobutyricum* ATCC 49875, ^{b}- Best biobutanol yield.

4.6 Bioethanol

Although bioethanol is considered the most promising liquid biofuel potentially obtainable from rice waste streams (Table 1), its commercialization would be possible only if the cost of the entire process, from feedstock collection and treatment to the attainment of the final product, will be sustainable [167]. This would be possible by (i) firstly reducing the number of steps, i.e. by clubbing them together in a single vessel, (ii) by reducing as much as possible the use of extra reagents such as commercial enzymes, (iii) by shortening the processing time. In addition, fermentation efficiency represents another key factor directly linked to the available microorganisms used in the bioreactor. Further strategies are being applied in which organisms were genetically modified to produce enzymes for saccharification and fermentation, or consortium of different organisms or commercially available enzyme cocktails were used. In terms of fermentation effectiveness, *Saccharomyces cerevisiae* is the main candidate, even if several strains proved not capable of tolerating the inhibitors formed during pretreatments. Hence, detoxification of the resulting hydrolysates is needed or tolerant strains have to be developed [168,169]

This section reviews the following strategies available for the production of bioethanol from rice waste streams:

1. Separate Hydrolysis and Fermentation (SHF)
2. Simultaneous Saccharification and Fermentation (SSF)

Consolidated Bioprocessing(CBP)

4.6.1 SHF for Bioethanol

Through this method, enzymatic hydrolysis and fermentation are performed in sequence.

Positive aspects are (i) the different optimal temperatures required by the two steps of the process can be optimized separately, (ii) the use of enzyme cocktails demands for different pHs, (iii) the whole design of the equipment, including stirring, can be organized independently [170–172].

Beyond several positive aspects, there are also some negative sides such as (i) this process requires considerable capital investments as more than one vessel must be involved, (ii) it is generally more time-consuming as the two steps are done separately, (iii) the increasing sugar concentration produced by cellulases activity leads to inhibit the enzyme action itself, (iv) in the pretreated biomass slurry several inhibitors are generally present, which may hinder the cellulases. These aspects will increase the final cost of the process [170–172].

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Taken together, the above considerations gave rise to the limited number of SHF applications in the last decade, even if some interesting reports are available on several rice waste substrates (Table 7). For instance, some SHF approaches used enzymatic cocktails containing xylanase and pectinase on pretreated RS using ammonia fiber expansion (AFEX). The combination with *S. cerevisiae* in separate fermentation produced more than 175 g EtOH/kg treated RS. Interestingly, this ethanol yield was achieved even though pretreated biomass was not washed, detoxified, and added with supplemental nutrients. Fermentation of such hydrolysate with two *P. stipitis* strains also gave appreciable results in terms of g ethanol/L [173].

Abedinifar *et al.* [59] after investigating on optimal pH and temperature for commercial cellulase and β-glucosidase, reported that SHF could be efficiently adopted by using diluted acid pretreated RS. They also reported that the filamentous fungus *M. indicus* can perform at the same level as *S. cerevisiae* in terms of growth and ethanol yield. Moreover, filamentous fungus can convert pentoses into ethanol and produce chitosan, an interesting byproduct.

Saha *et al.* [174] worked with rice hull (RH) pretreated with alkaline peroxide and hydrolysed with a three enzyme cocktail containing cellulase, β-glucosidase and xylanase. This procedure resulted in a sugar yield of 90%, without the release of any furfural and hydroxymethylfurfural into the medium, increasing up to 96% by separately saccharifying the liquid and solid fractions. In that case, the fermentation step was performed using a recombinant strain of *E. coli* with noticeable ethanol production (Table 7). Biological pretreatments were proposed as promising alternatives to severe thermo-chemical applications on paddy straw by the use of a white-rot fungus coupled to steam at 121°C [86]. The saccharification efficiencies between the two approaches resulted to be very similar, but in the case of thermo-chemical strategies, the following *S. cerevisiae* fermentation resulted in low ethanol production, thus indicating the presence of inhibitory compounds within the hydrolysates that need to be detoxified.

When the complete process of fermentation is taken into consideration along with all the parameters involved (Table7), detoxification of pretreated biomass resulted in a significant increase in bioethanol production.

The ethanol production from lime-pretreated and enzyme-hydrolysed RH was reported by Saha *et al.* [67]. These Authors used a recombinant *E. coli* FBR5 strain for both SHF and SSF and found that the total time to obtain the final product was shorter for SSF as saccharification and fermentation was simultaneous, while the SHF approach worked better in terms of fermentation time as saccharification was already done in the step before fermentation. However, one of the main benefits deriving by the use of lime could be the avoiding of inhibitors, completely absent in the resulting fermentation substrate. Unfortunately, the reported conversion yield seems to be still too low.

Table 7: Bioethanol production from rice waste streams using SHF technology.

Feedstock	Pretreatment			Organism	Fermentation time	Concentration[a]	Reference
	Physical	**Chemical**	**Enzymatic/microbial**		(h)	g/L	
RH	-	Alkali	Cellulase β-glucosidase Xylanase	*Escherichia coli* FBR5[c]	19	9.8	[67]
RH	-	Alkali peroxide	Cellulase β-glucosidase Xylanase	*Escherichia coli* FBR5[c]	24	8.2	[174]
RS	Milling, Autoclaving	Acid	Cellulase β-glucosidase	*Saccharomyces cerevisiae*	25	37	[59]
RS	-	Alkali	Cellulase β-glucosidase	*Clostridium acetobutilicum* NRRL B-591	80	2	[159]
RS	Ultrasound	Acid	*Trichoderma reesei*	*Saccharomyces cerevisiae*	168	11.0	[175]
RS	Milling, Autoclaving	-	Cellulase *Trametes hirsuta*	*Saccharomyces cerevisiae* LN	48	1.1	[86]
RS	Autoclaving	Alkali	Xylanase Pectinase Cellulase	*Saccharomyces cerevisiae 424A(LNH- ST)*	144	37.0	[173]

[c] – GMO, [a]- Highest values of bioethanol are reported (or calculated from available data) when available

4.6.2 SSF for Bioethanol

As also reported in 4.6.1, the SHF has evolved and later compared to the SSF approach as an alternative procedure that is generally more effective [172,176]. In SSF, the same vessel is used for both saccharification and fermentation with the original objective to reduce both the equipment costs and the possible contamination of the cell suspension. The two steps are indeed occurring simultaneously and, as a further resulting advantage, the process time is reduced. In addition, the possibility to select enzymes usually working at room temperature can reduce or completely eliminate heating and cooling costs. Together with the removal of end-product inhibition of the saccharification process, these are the main reasons leading to devote more and more attention to SSF. Table 8 summarise the organisms, the conditions and the yield obtained by using SSF technology. Overall, substrate loading is a pivotal parameter in SSF setting, with the highest substrate loadings supporting the highest ethanol concentrations.

Some studies indicated that inhibitor-free hydrolysates could be obtained from rice waste streams under specific conditions. For instance, Diwan *et al.* [150] optimized the hydrolysis process of RS by an experimental design with variable factors (duration, acid concentration, solid loading percentage, temperature) and found that the non-detoxified hydrolysate did not contain any furfural and hydroxymethylfurfural, thus supporting the growth and the metabolic activities of *M. alpina* much better than the detoxified hydrolysate (Table 5). Although the original objective of this work was the production of lipids, this hydrolysate could be efficiently used for alcoholic fermentation. Another efficient strategy to produce a sugar-rich hydrolysate that does not require a detoxification step, and hence simultaneously suitable as a fermentation medium, has been reported by Castro *et al.* [68] for RS processing, through SSF by *Kluyveromyces marxianus* NRRL Y-6860. In this case, a dilute acid pretreatment was preceded by biomass deacetylation, with the result to improve the recovery of both pentose and hexose sugars and the consequent ethanol production.

Another interesting attainment, carried out at 38°C for 48h, was described for RS by Poornejad *et al.* [73]. The ethanol production yield was improved if the straw was treated with NMMO and 1-butyl-3-methylimidazolium acetate ([BMIM][OAc]), respectively. The reduction of crystallinity by these two solvents was the main reason since glucan conversion yield increased from 28% of the untreated straw to 96 and 100%, respectively.

Zhu *et al.* [70] optimised SSF to ethanol for RS pretreated with 1% NaOH or a combination of microwave and 1% NaOH by using cellulases from *T. reesei* and *S. cerevisiae* YC-097 as fermenting yeast. They demonstrated that the microwave application improved the conventional alkali pretreatment.

The reduction of high heating energy costs for liquefaction and saccharification was also proposed [177]. They used rice wine cake as feedstock for SSF without cooking and raw-starch-digesting enzyme prepared from *Rhizopus* sp. SSF conditions were optimized for *S. cerevisiae* in terms of incubation temperature, pH, fermentation time, inoculum size. The effect of several additives such as nitrogen sources, surfactants and metal salts were also studied. The selected optimal SSF conditions resulted in ethanol production improvement within 90 hours of fermentation at 30°C.

A comparison between two filamentous fungi (*Rhizopus oryzae* and *M. indicus*) and a thermotolerant yeast strain of *S. cerevisiae*, was performed in terms of ethanol production in a SSF of RS [178]. The advantages of using the filamentous fungi are that they can grow at higher temperatures than *S. cerevisiae*, thus approaching the optimum for SSF process, and finally resulting in higher ethanol yield.

By quantitative NMR screening methods, Wu *et al.* [179] investigated the different compositions of the pretreatment liquors deriving from RS and RH, and their consequences on SSF. High-pressure microwave processing was applied in combination with a range of severities, and among a number of different compounds, they found that while fermentation inhibitors, such as hydroxymethylfurfural and furfural, were more present in husk liquor, formic acid was higher in straw liquor.

The ethanol production from alkali-treated (NaOH) RS in a SSF process was reported by Oberoi *et al.* [180]. They used for the first time the recombinant *Pichia kudriavzevii* HOP-1 thermotolerant strain, producing ethanol at amounts comparable to those produced by *S. cerevisiae*. Further interesting investigations by coupling alkali pretreatment of RH with the use of zygomycetes fungi (*M. hiemalis*) for the production of ethanol, was performed [181]. The alkali pretreatment enables to increase the low ethanol yield generally obtainable (around 15%) to more than 85%, as a consequence of lignin removal and cellulose crystallinity decrease. On the other hand, the use of M. *hiemalis* resulted in ethanol yield higher than *S. cerevisiae,* probably due to its high resistance against the inhibitors and to the utilization of pentoses, and also resulted in the production of other value-added proteins and lipids. The same filamentous zygomycetes M. *hiemalis* was used by SSF in combination with sodium carbonate pretreatment [182]. The use of this chemical enabled to remove the high silica content from RS and consequently to enhance enzymatic hydrolysis and ethanol production by the fungus, that proved once more to perform better than *S. cerevisiae*.

On broken rice, Gronchi *et al.* [93] found a great potential as ethanol producers by newly isolated yeast strains, performing better in a SSF than other well-known benchmark strains. This approach can be followed even with the objective to find superior outperforming phenotypes to be further selected at bioreactor scale for specific feedstocks and also in view of the construction of a recombinant strain for consolidated bioprocessing (CBP).

Table 8. Bioethanol production from rice waste streams using SSF technology.

Feedstock	**Chemical Pretreatment**	**Substrate Loading**[a]	**Enzymatic/Microbial Saccharification**	**Organism**	**Concentration**[c] g/L	**Reference**
RH	Alkali	5% (w/w)	Cellulase	*Mucor hiemalis* CCUG 16148	9	[181]
			β-glucosidase	*Saccharomyces cerevisiae* Thermosacc®	6	
RS	Acid	15% (w/v)	Cellulase	*Rhizopus oryzae*	12	[178]
				Saccharomyces cerevisiae	10	
				Mucor indicus	16	
RH	Acid	5% (w/w)	Cellulase	*Saccharomyces cerevisiae* NCYC2826	4	[179]
RS	Acid	5% (w/w)	Cellulase	*Saccharomyces cerevisiae* NCYC2826	7	[179]
RS	dAT	10% (w/v)	Cellulase	*Kluyveromyces marxianus* NRRL Y-6860	20	[68]
RS	Alkali	5% (w/v)	Cellulase β-glucosidase	*Mucor hiemalis*	13	[182]
RS	NMMO	5% (w/w)	Cellulase β-glucosidase	*Saccharomyces cerevisiae* CCUG 53310	14	[73]
RS	Alkali	10% (w/v)	Cellulase β-glucosidase Pectinase	*Pichia kudriavzevii* HOP-1[b]	24	[180]
RS	Alkali	60% (w/v)	Cellulase	*Saccharomyces cerevisiae* YC-097	18	[70]
BR	-	20% (w/v)	α-amylase glucoamylase	*Saccharomyces cerevisiae* L20	107	[93]
RWC	-	77% (w/w)	*Rhizopus sp.*	*Saccharomyces cerevisiae* KV25	133	[177]

Nr- Not reported; dAT- deacetylationAcid pretreatment; NMMO- N-methyl morpholine N-oxide; [a]- for pretreatment, [b]- GMO, RWC- Rice waste cake, [c]- Highest values of bioethanol reported (or calculated from available data)

4.6.3 CBP for bioethanol

The CBP of biomass into bioethanol is gaining increasing recognition as a potential breakthrough for low-cost biomass processing [183–185]. A four-fold reduction in the cost of biological processing and a two-fold reduction in the overall production cost is projected when a mature CBP yeast will be available [168,184,186].

A CBP approach was proposed also from cellulosic- and starch-rich rice streams (Table 9), using engineered *S. cerevisiae* strain specifically developed for co-expression of efficient cellulases or amylases. Specific efforts were focused on RS, once pretreated with hot water (80°C, 16 h), which was converted into ethanol by the *S. cerevisiae* strain MNII/cocδBEC3 co-producing β-glucosidase, endoglucanase and cellobiohydrolase tethered to the cell surface [187]. Although the enzymatic activities of the CPB strain were promising, the ethanol levels obtained from 100 g/L HWP RS were low (with 33% of the theoretical yield), pointing out that both substrate loading optimization and harsher pre-treatment conditions were the most important drivers towards higher ethanol yields. The same group indeed applied heavier pre-treatment on RS (Liquid Hot Water method, 130-300 °C under the pressure of less than 10 Mpa). The resulting hydrolysate was converted into ethanol by the CBP *S. cerevisiae* strain MN8140/XBXX able to hydrolyzed hemicellulose by co-displaying the endoxylanase from *T. reesei*, the β-xylosidase from *R. oryzae* and the β-glucosidase from *Aspergillus aculeatus* and to assimilate the released xylose through the expression of *P. stipites* xylose reductase and *S. cerevisiae* xylitol dehydrogenase. The ethanol concentration reached was 8.2 g/L after 72 h fermentation, with an ethanol yield close to 82% of the theoretical [188].

CBP applications were found to be very efficient in the case of starchy rice by-products such as rice bran, broken rice, unripe rice and discolored rice (Table 9). Two yeast strains, M2n[TLG1-SFA1] and MEL2 [TLG1-SFA1] co-expressing the glucoamylase TLG1 from *Thermomyces lanuginosus* and the α-amylase SFA1 from *Saccharomycopsis fibuligera,* previously reported for their promise as raw starch converting microbes [51] were effectively adopted to achieve high ethanol levels (Table 9). The higher the starch content (rice bran>unripe rice>broken rice and discolored rice), the higher ethanol concentrations were produced. Noteworthy, even higher ethanol levels were recently obtained by applying efficient amylolytic CBP strains on broken rice (20% w/v). Two strains *S. cerevisiae* ER T12 and *S. cerevisiae* M2n T1, simultaneously secreting an α-amylase and glucoamylase originating from *Talaromyces emersonii*, were adopted in a CBP setting [49]. No substrate pre-treatment was needed, and the final alcohol titers (100 g/L) indicated that this process can be industrially viable.

Table 9. Bioethanol production from rice waste streams using CBP technology.

Feedstock	**Physical pretreatment**	**Substrate loading** % (w/v)	***Saccharomyces. cerevisiae* strain**	**Fermentation time** (h)	**Concentration** (g/L)	**Reference**
RS	Milling,Thermal	100	MNII/cocδBEC3	72	8	[187]
RS	Autoclaving	80	MN8140/XBXX	72	8	[188]
BR	Milling	20	ER T12	168	101	[49]
			M2n T1		100	
BR	Milling	20	M2n[TLG1-SFA1	144	75	[57]
			MEL2[TLG1-SFA1]		68	
DR	Milling	20	M2n[TLG1-SFA1	144	79	[57]
			MEL2[TLG1-SFA1]		42	
RB	Milling	20	M2n[TLG1-SFA1	144	39	[57]
			MEL2[TLG1-SFA1]		68	
UR	Milling	20	M2n[TLG1-SFA1	144	66	[57]
			MEL2[TLG1-SFA1]		61	

4.7 Microbial Fuel Cell

Electricity is one of the most important energy forms that support most of the human activities. Recently, a new, future-promising segment has been added, i.e. electrical vehicles. Many personal cars and public transports are shifting to electricity run vehicles as they are more economical and less polluting. However, the current electricity supply is mostly based on thermal power, generated by coal burning, which unfortunately contributes to environmental pollution. To cope with this excessive demand, it is essential to find a renewable and non-polluting electricity source. Current studies indicate microbial fuel cell (MFC), as a possible future contribution. It is a strategy exploiting bacterial metabolism to generate electricity from a range of bio-wastes. The interest in this technology raised when the possible future use of the high producing bacterial strain *Geobacter sulfurreducens* KN400 was reported in 2009 by Time Magazine as one of the top 50 most important inventions [189].

MFC could be considered as a bioreactor with two chambers, an anode and a cathode separated by a proton exchange membrane (PEM). Electrons, generated at the anode, move to the cathode through an external circuit and protons travel to cathode through PEM, where they combine with oxygen and electrons to form water molecules [190].

Few experiences on MFC exploiting rice by-products are available in the literature (Table 10). The PEMs used in MFC are generally polymeric membranes like Nafion, expensive and susceptible to fouling after repeated usage. Ceramic are an affordable alternative to polymeric membrane. Studies showed that blending of 10% RH ash with soil to fabricate ceramic PEM gave higher volumetric power density as compared to that of control when rice mill wastewater was used as substrate and anaerobic sludge collected from the sediment of a pond was used as inoculum [76].

RH charcoal was also used as anode and cathode electrodes for MFC, showing the potential of RH to be used not only as a carbon source for microbes but also in the construction of MFC [192]. Jiao *et al.* [193] indicated that the power density is influenced by the surface area of the carbon electrode, i.e. porosity, used in MFC.

Rezaei *et al.* [194] demonstrated for the first time that it was possible to generate electricity using MFC with cellulose as a carbon source and a single strain of *Enterobacter cloacae*. On the other hand, single strain, i.e pure culture of *S. cerevisiae*, did not give promising results if maximum power density is compared with mixed cultures and consortium [195].

When non-pretreated RS was used as a substrate and the mixed culture of cellulose-degrading bacteria as inoculum, MFC could generate power density up to 145 mW/m^2. When the same MFCs were connected in series, the power density increased more than three times. After an initial lag period of 110 h, the stable power density was maintained for 10 days. The refuelling of the cell was done three times with a medium containing 1 g/L of RS and no lag period was observed, indicating that such MFCs can utilize RS for the production of energy [196].

RB was also used as a carbon source in single-chambered MFC inoculated with paddy field soil. The power density increased drastically when a mineral solution was used as liquid phase instead of pure water along with RB. The amplicon-sequencing showed the presence of *Geobacter* spp. at anode biofilm. The same MFC was continuously adopted for 130 days supplementing the system with RB after 10-20 days [197]. Phylogenetic analysis reveals the presence of a mutualistic behaviour between *Bacteroides, Clostridium* spp. and *Geobacter* spp. in the anode biofilm [198]. On the other hand, when pond bottom sludge was exposed to air, it gave higher volumetric power density as the methanogenesis was affected due to aeration. Schievano *et al.* [199] highlighted that rice waste streams can be usefully exploited in MFC applications. This is of great importance considering that the electricity can be obtained from MFC adopting the biorefinery approach after production of gaseous biofuels, such as biohydrogen and biomethane, from organic waste.

Table 10. Production of electricity using microbial fuel cell from rice waste streams.

Feedstock	**Pretreatment**		**Inoculum**	**Resistance applied**	**Power Density**	**Reference**
	Physical	**Chemical**		**Ω**		
RH	-	Acid, Alkali	AS	1000	318 mW/m^2	[193]
RS	-	-	Consortium	1000	145 mW/m^2	[196]
RS	Milling	-	CDSM	1000	190 mW/m^2	[200]
RB	-	-	PFS	10000	520 mW/m^2	[197]
RB		HC	PB Mud	510	17 mW/m^2	[198]
RB	-	-	SM	500	477 mW/m^2	[199]
Rice washing water	-	-	*Saccharomyces cerevisiae*	320	1 mW/m^2	[195]
Rice mill wastewater	-	-	PB sludge	100	656 $mW/m^{3\ V}$	[201]

AS- Anaerobic sludge, PFS- Paddy field soil, PB- Pond bottom , SM- Swine Manure, HC- Hydrodynamic cavitation, CDSM- Cellulose degrading soil microflora, ^{V}- Volumetric power density.

6. Biorefining of rice waste streams into added-value products

To ensure the cost-effective exploitation of rice waste streams, it is essential to recover all the potential co-products together with lower-value products such as bioethanol. As such, the overall process economics will be greatly improved.

Once the cellulosic or starchy rice residues are hydrolyzed to monomers (ie, sugars, amino acids, fatty acids, etc.), the latter can serve as a feedstock for biological fermentation or chemical processing to various chemical building blocks. Besides biofuels, potential fermentation products from rice waste could be enzymes [202,203], biopolymers [204], organic acids [205–207] and vitamins [208].

Nevertheless, it is hallmark to integrate processes for a mixture of products in a biorefinery setting to ensure the economic viability of a specific by-product [209,210]. For example, techno-economic modelling for the integrated waste streams-to-biofuels routes developed by IEA (International Energy Agency) demonstrated a positive outcome when 80% of the hexose sugars were processed to bioethanol and 20% to lactic acid [211]. Furthermore, the efficient integration of biorefineries into existing industrial plants can considerably contribute towards a sustainable bioeconomy [212]. This is particularly true in the case of rice milling residues which could be valorized into biofuels and higher values products nearby the paddy rice processing, thus reducing cost and greenhouse gas emission related to their transport [50,184].

Few research initiatives, mostly on RS [213], already explored this perspective paving the way for additional and more in-depth research and development efforts. For instance, Zahed *et al.* [214] developed a continuous co-production of ethanol and xylitol from RS using a membrane reactor. Lignin can be recovered from rice residues and utilized for the production of phenolic compounds which are categories of fragrances. Lignin recovery was indeed successfully pursued from the solid waste of RS after producing relevant quantities of bioethanol in a pilot biorefinery plant [215]. Zheng *et al.* [216] produced vanillin from ferulic acid present in waste residue of rice bran oil using fungi.

The few experiences of biorefining approaches from RS and rice bran indicated the promise of such substrates in a circular economy landscape relying on microbes as outstanding cell factories. Nevertheless, further research efforts are needed before large scale biorefinery plants can be installed from rice waste. Processes integration, implementation of new hybrid technologies (i.e. thermo, chemical and biotechnological routes) and life cycle analysis will be useful.

7. Conclusions

Rice waste streams have great potential to be converted into energy in order to meet the countries' energy demands. Biotechnological approaches were deeply adopted to convert rice waste into biofuels. Ethanol is one of the most important applications with biogas and biohydrogen, the most promising gaseous fuels. Moreover, rice by-products can be co-converted into a cluster of valuable compounds (i.e., organic acids, enzymes, pharmaceutical molecules, biopolymers) towards their full exploitation.

Despite all these great promises, further research is still required on up-scale and industrial commercialisation of the technologies so far developed. Moreover, future procecess integrations are needed towards biorefinery schemes where rice waste streams can be converted into biofuels and several other added value products.

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