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UTILIZATION OF AGRO-WASTES IN BIOHYDROGEN FERMENTATION BY VARIOUS MICROORGANISMS

KATALIN BÉLAFI-BAKÓ[1*], GÁBOR TÓTH[1], PÉTER BAKONYI[1] AND NÁNDOR NEMESTÓTHY[1]

1 Research Group on Bioengineering, Membrane Technology and Energetics, University of Pannonia, Egyetem u. 10, Veszprém, 8200, Hungary

Biohydrogen production based on agro-industrial wastes might be an attractive and effective technology for providing an energy source in the future. Dark fermentation is considered to be one of the most suitable biohydrogen formation processes. In this paper, various agro-industrial wastes as well as microorganisms applied for biohydrogen formation are reviewed.

Keywords: anaerobic process, dark fermentation, bioreactor

1. Introduction

Hydrogen produced biologically (biohydrogen) can be considered as a renewable source of energy [1]-[4]. Moreover, it is regarded as one of the most promising, environmentally-friendly “fuels” of the future due to its favourable characteristics, e.g., only water is formed by the combustion of H_2, no environmental pollution is produced, highest energy density of 143 kJ/g. Agro-industrial residues seem to be suitable feedstocks for the production of biohydrogen [5].

Large amounts of solid and liquid waste are formed in agriculture every year, moreover, the majority of it is unutilized. However, promising technologies are available whereby agro-industrial residues are applied to produce value-added products and green energy by biological methods [6]. Liquid wastes are usually found in the form of wastewater. Solid agro-wastes can be classified into four groups [7]:

(i) field residues
(ii) waste of processing
(iii) livestock waste
(iv) chemical waste

Field residues generally originate from crop production and consist of the remains of crops, e.g., leaves, stalks, stems, seeds, straw, husks, shells, pulp, roots, woodland waste, etc.

Industrial food processing results in solid wastes which are mainly by-products of and leftovers from various plants, e.g., bagasse, sugarcane molasses, wheat bran, rice bran, groundnut shells, apple pomace, fruit peels, de oiled rice bran cake and oil cakes.

Livestock waste consists of by-products of slaughterhouses or originates from processing, e.g., bones, feathers, shells of crustaceans, as well as bedding/litter, carcasses and damaged feeders.

Chemical waste includes plant protection compounds, e.g., pesticides, insecticides and herbicides, as well as their containers and bottles. However, since these materials cannot be utilized in biological methods, this study focuses on the first three groups of solid wastes.

Biological hydrogen production from agro-industrial wastes includes dark fermentation, photofermentation and microbial electrolysis cells (MEC) [5]. Photofermentation is a process whereby organic substrates are converted into hydrogen by photosynthetic organisms under anoxic conditions. H_2 production in MECs is a bioelectrochemical process, where electrochemical and biological techniques are combined [8]. Given that dark fermentation is one of the most common methods used for hydrogen production from agro-wastes due to its higher hydrogen production rate, it is the focus of this mini-review.

2. Biohydrogen production by dark fermentation

The composition and certain characteristics of agro-wastes are determining factors of biohydrogen production. Generally speaking, although these materials are rich in carbon sources, the majority of them require some sort of pretreatment. The lignin content in particular should be treated to increase the efficiency of

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*Correspondence: belafine.bako.katalin@mk.uni-pannon.hu

biodegradation and conversion processes [5]-[7]. Pretreatments of agro-industrial residues include:

- (i) physical,
- (ii) chemical,
- (iii) physicochemical and
- (iv) biological methods.

Physical methods involve ultrasonication, ozonization, thermal- and microwave-assisted techniques as well as size-reduction processes, e.g., milling, grinding, etc.

Chemical methods include acid or alkali treatments, moreover, more recently organic solvents have been applied.

In physicochemical methods, a combination of physical and chemical techniques is used, e.g., steam explosion, hydrothermal treatment and chemical treatment complemented with microwave irradiation.

Biological methods include enzymatic and microbial (whole cell) treatments, where mainly the degradation of biopolymers occurs.

Using these pretreatment methods, higher yields and more efficient hydrogen formation can be achieved.

Dark fermentative biohydrogen production can be carried out by facultative anaerobes and obligate microbes [1], [4]-[5]. In many cases, the suitable types of microorganisms are determined by the substrate applied and mixed microbial consortia are frequently used. Nevertheless, numerous microbes have still been used in dark fermentative biohydrogen production based on agro-wastes, examples of which are listed in *Table 1*:

Table 1: Examples of the utilization of various agro-wastes to produce biohydrogen

Agro-waste	**Microorganism**	**H_2 production rate or yield**	**Ref.**
Sugar and rice mill wastewater	*Acinetobacter junii*	Rate 5.23 $mLH_2L^{-1}h^{-1}$	[9]
Rice mill wastewater	*Clostridium beijerinckii*	Yield 214.9 mLH_2L^{-1}	[10]
Cheese whey powder	*Enterobacter asburiae*	Yield 1.19 ± 0.01 $molH_2mol^{-1}$	[11]
Sugarcane molasses	*Eisenia fetida*	Yield 1571.81 mLH_2L^{-1}	[12]
Cashew apple bagasse	*Clostridium roseum*	Yield 15 $mmolH_2\,L^{-1}$ hydrolysate	[13]
Fruit and vegetable wastes + seawater	*Thermotoga maritima*	Rate 12.4 $mmolh^{-1}L^{-1}$	[14]
Fruit wastes (apple, banana, grape, melon, orange)	*consortia from biogas sludge*	Yield 523 mL/g VS (volatile solid)	[15]
Sweet sorghum bagasse	*Caldicellulosiruptor saccharolyticus*	Yield 73.6 mLH_2mmol^{-1} C6 sugars	[16]
Cornstalk	*Thermoanaerobacterium thermosaccharolyticum*	Yield 89.3 mLH_2g^{-1} DB (dry biomass)	[16]
Distillers grains	*Caldicellulosiruptor thermocellum*	Yield 29.2 mLH_2g^{-1} DB	[16]
Fruit and vegetable wastes as well as corn stover	*anaerobic sludge*	Yield 1.91 $molH_2mol^{-1}$ glucose	[17]
Beer lees	*consortia from cow dung compost*	Yield 68.6 mlH_2/g TVS	[18]
Corn straw	*Ethanoligenens harbinense*	Yield 9 mLH_2g^{-1}	[19]
Wheat straw	*consortia from cow dung compost*	Yield 68 mLH_2g^{-1}	[20]
Grass silage	*manure*	Yield 16 mLH_2g^{-1}	[19]
Extracts of sweet lime peel	*anaerobic mixed consortia*	Yield 76.4 ml/g COD	[21]
Palm oil mill effluent	*Thermoanaerobacterium-rich sludge*	Yield 84.4 mLH_2g^{-1}	[22]
Beet pulp	*mixed culture from anaerobic sludge*	Yield 115.6 mLH_2g^{-1} COD	[24]
Sunflower stalks	*Clostridium sp.*	Yield 2.04 $molH_2mol^{-1}$ eq. hexose	[25]
Miscanthus sinensis	*Thermotoga elfii*	Rate 23.99 mLH_2h^{-1}	[16]
Cassava pulp hydrolysate	*mixed culture*	Yield 342 $mlH_2g^{-1}\,COD_{reduced}$	[26]
Tequila vinasse	*Eisenia fetida*	Yield 1246.36 mLH_2L^{-1}	[12]
Sugarcane bagasse	*Eisenia fetida*	Yield 232.72 mLH_2L^{-1}	[12]

As can be seen, although several types of agro-wastes and microbes have been used for biohydrogen fermentation, the results regarding the yield or production rate of the processes are difficult to compare since their units vary and the data on hydrogen formation is diverse. Moreover, in this study, no information was collected about the treatment of the substrate (if any) nor which kind of bioreactor, set-up or mode of operation was used, making their comparison even more difficult. Therefore, only some special considerations can be discussed in this work.

3. Special considerations

In addition to single microorganisms, mixed consortia are often applied in these processes, which originate from various sources [19], e.g., sludge from biogas production; anaerobic sludges from municipal wastewater treatment plants and cow-dung composts; cattle or dairy residue composts; sludge from palm oil mill effluent; soil, rice and straw composts; fermented soybean meal, etc. Even though these sources usually contain the microbes which are able to form biohydrogen, they need to be acclimated which includes some sort of pretreatment, e.g., heat shock, starving for a couple days, etc., to enrich the suitable microorganisms [3], [28] or sometimes bioaugmentation [10].

In hydrogen-producing consortia, a wide range of species have been isolated [19], e.g., under mesophilic conditions, *Clostridium (C. pasteurianum, C. saccharobutylicum, C. butyricum), Enterobacter (E. aerogenes) and Bacillus*, while under thermophilic conditions, the genera *Thermoanaerobacterium (T. thermosaccharolyticum), Caldicellulosiruptor (C. saccharolyticus, C. thermocellum)* and *Bacillus thermozeamaize*.

Regarding the utilization of certain fruit wastes, some flavor compounds were found to exhibit an antimicrobial effect [15], e.g., a citrus flavor, since D-limonene is able to weaken the fermentation even at very low concentrations (0.01% w/v). Therefore, the undesired effect of these substrates can be diminished by special treatments, e.g., encapsulation of the microbes in a membrane or pretreatment of the feedstock.

4. Conclusions

In biohydrogen production, the application of agro-industrial residues has a great potential since they are renewable, huge amounts of them are formed year by year and have not been utilized. Various types of agro-wastes have been investigated with regard to biohydrogen production using a wide variety of microbes, namely single microorganisms or consortia. Although it seems that both can be effective in terms of fermentation, higher yields and/or production rates could be achieved when carbohydrates – which can be easily uptaken – are present in the initial substrate.

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