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Review

Effect of Microplastics Pollution on Hydrogen Production from Biomass: A Comprehensive Review

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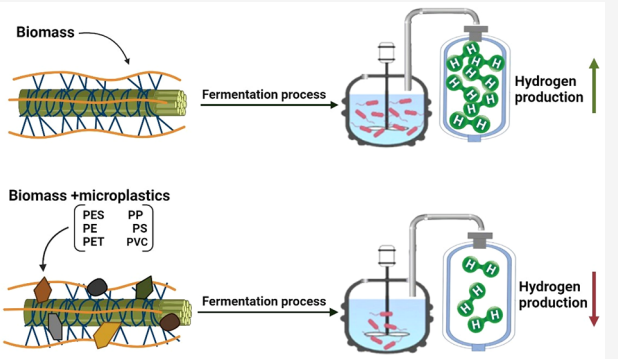
ABSTRACT: Hydrogen production from biomass and organic wastes is considered as a potential alternative energy source and is known as a clean and CO_2-free fuel energy carrier. H_2 is considered very promising among the other energy sources; therefore, the effective conversion of biomass and organic solid waste to this secondary energy source is urgently sought. As a result, it is of importance to assess the impact of the existence of microplastics (MPs) pollution in biomass during its fermentation. It was found that, besides the number of plastic particles present in biomass, the size of them plays the most important role in the yield of hydrogen. While plastics in nanoparticle sizes always suppress the production of H_2, depending on their concentration, MPs may increase or reduce it. For example, the presence of 60 particles/L of micrometer-sized polyethylene terephthalate reduces hydrogen production by 30%, while 200 particles of it yields 63.6% more hydrogen. This Review examines all available literature on the effects of the presence of various MPs on biohydrogen production.



1. INTRODUCTION

Due to the increased demand for hydrogen as a reliable energy source, in 2020 roughly 87 million tons of hydrogen was produced worldwide.[1] The combustion of hydrogen produces only water vapor, which makes it a pollutant-free, important form of energy supply. Hydrogen releases almost 3 times more energy than methane or natural gas.[2] Hydrogen also can be used for electricity generation in fuel cells. As a primary or secondary form of energy, hydrogen needs to be produced from different processes, including the electrolysis of water, hydrocarbons steam reforming, and partial oxidation of combustible fossil fuels.[3] These methods consume high amounts of energy and need elevated temperatures, which result in CO_2 and other pollutants being liberated as byproducts. As a promising method, biological hydrogen production from biomass seems to be one of the best alternative ways to replace traditional approaches. It can produce large quantities of hydrogen under ambient conditions for the long-term with almost no environmental pollution. Biomass is a renewable resource, and the cost of hydrogen production through its fermentation is relatively low as compared to that of the other methods, making it a more accessible option for large-scale hydrogen production. Moreover, the higher yield of hydrogen production from biomass fermentation makes it a more efficient way to produce hydrogen in comparison to the other biological processes. As a result, it has been considered as an ideal source in the steam reforming process to replace fossil fuels for hydrogen production, because organic wastewater can be used to produce hydrogen, which reduces the costs. Using wastewater provides dual environmental benefits in the direction of wastewater treatment along with hydrogen production. Hydrogen production from biomass can be achieved via synthesis gas (a mixture of hydrogen and carbon monoxide) production that typically forms first in all hydrogen production processes using carbon-containing substances as raw materials.[4] Almost all types of biomasses can be employed as a carbon source for the steam reforming process, including agricultural waste, municipal waste, and algae, making it a versatile option for hydrogen production. Hydrogen can be produced from biomass via either thermochemical or biological routes. Direct biophotolysis, indirect biophotolysis, photo fermentation, and dark fermentation are the most common biological production processes for H_2 production in this regard.[5] Both marine and land-based biomasses have been used for gasification; of them, the most common are edible crops (sugar beet, sugar cane, grains, and wheat), nonfood plant, seagrass, and seaweed. It has been observed that any microbial contamination can lead

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Table 1. Effect of MPs on Hydrogen Production from Biomass

MP	amount of MPs existed/added	size	hydrogen produced by	effect on hydrogen production	ref
polystyrene	1.428×10^2 to 1.428×10^7 P/cm^3	0.88 μm	hydrogen energy was photocatalytically reclaimed from natural water matrixes	slight improvements up to 1.428×10^7 P/cm^3 and significantly decrease for 1.428×10^7 P/cm^3	21
sea salts[a]	2 wt %	not applicable	steam gasification of eelgrass under different temperatures	26% reduction at 550 °C	3
polyethylene terephthalate	10, 30, and 60 particles/g-TS	25 μm	anaerobic fermentation of waste-activated sludge under alkaline condition	30% reduction for 60 particles/g-TS	23
polyvinyl chloride	10−200 particles/g TS	1 mm	anaerobic fermentation of waste-activated sludge	MPs at 10 particles/g TS increased methane production by 5.9%; whereas at 100−200 particles/g TS decreased methane production by 75.8−90.6%	24
polystyrene	0.2 g/L	54.8 nm	anaerobic fermentation of municipal wastewater treatment plants with *Acetobacteroides hydrogenigenes* microorganism	decreased methane production by 14.4% and 40.7% and H_2 production significantly	25
polyester	0−200000 PS particle/kg of activated sludge	200 μm	anaerobic fermentation of sludge of municipal	the methane production was reduced between 88.53% and 95.08% after digestion for 59 days	26
polyethylene	1−10044 particles/L	0.7−450 μm	anaerobic digestion of dairy wastes	the methane production was improved by 8.4% and 41.2% at 55 and 65 °C, respectively	28
polyethylene terephthalate	200 particles/L	30−100 μm	anaerobic (mesophilic and thermophilic conditions) fermentation of sludge was obtained from a wastewater treatment plant	21.6−63.6% more hydrogen production	29
polystyrene micro and nanoplastics	PS MPs with ≤0.25 g/L	80 nm and 5 μm	anaerobic fermentation of synthetic wastewater	PS MPs at ≤0.2 g/L did not significantly affect the cumulative methane production; level of 0.25 g/L reduced CH_4-production by 19.3%, while 5 μm PS decreased it by 17.9%	30
polystyrene and poly(methyl methacrylate)	0.5 and 50 mg/L	150 μm 250 μm	microalga *Phaeodactylum tricornutum* was exposed to water contaminated with MPs	the production of biohydrogen was not studied; only biochemical effects and potential industrial consequences were investigated	31
polyethylene	30 and 90 mg/L	200 μm 50 nm	anaerobic hydrogen-producing granular sludge derived from an up-flow anaerobic sludge blanket reactor using sucrose-rich wastewater	hydrogen yield downgraded to 79.9% and 63.0%	32
polyethylene, polyethylene terephthalate, and polyvinyl chloride	90 mg/L	100 μm	anaerobic hydrogen-producing granular sludge derived from an up-flow anaerobic sludge blanket reactor using sucrose-rich wastewater	hydrogen production downgraded to 82.0%, 72.3%, and 66.6% for polyethylene, polyethylene terephthalate, and polyvinyl chloride, respectively	33
the mixture of polyethylene terephthalate 36%, polypropylene 8%, polyethylene 42%, and polystyrene 15%	added into three groups of reactors at three levels of 0, 40, and 80 mg/L	150 μm	anaerobic hydrogen-producing granular sludge harvested from a laboratory up-flow anaerobic sludge blanket reactor	in the presence of 40 and 80 mg/L of MPs, hydrogen production decreased to 85.8% and 81.3%, respectively	35
polystyrene	0.16 g/L	MPs 50 μm nanoplastic 50 nm	anaerobic fermentation of sludge harvested from a biotechnology center	for MPs, hydrogen production increased by 34.0% and for nanoplastics decreased to 34.6%	36

[a]Impurity, not an MP.

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to a significant loss in biohydrogen generation.[6] As a result, to reduce the risk of generating interfering inhibitors, and to decrease the process time and cost for large-scale plants, most of the research has been focused on the removal of microorganism contamination, and less attention has been paid to the effect of other particles such as MPs, which may be in the biomass before its treatment. In this Review, we tried to demonstrate the importance of the presence of MPs in the biomass, which is employed for biohydrogen generation.

About 5 Gt of plastic waste is accumulated in the environment.[7] Generally, plastics are derived from oil or coal and consist of long carbon chains as their backbone with other elements, mainly hydrogen, chlorine, oxygen, nitrogen, and sulfur. Plastic has 30−40 types, which are sold under 25 000 trade names in 15 000 variants. Global plastic production is now estimated at 0.3 billion metric ton per year, one-half of which is for single-use items.[8] Polyethylene (PE), polyethylene (PP), polyvinyl chloride (PVC), polyethylene terephthalate (PET), polystyrene (PS), and polyester (PES) are light, low-priced, and water-resistant and are thermally and electrically insulating, so they are the most widely used plastics. These polymers account for ∼75% of plastics production and are used primarily in packaging and consumer goods, but they are also extensively applied in electronics, automotive manufacturing, and construction. From exposure to sunlight, weathering, and physical or biological degradation, they are fragmented into tiny particles, and partly into hazardous MPs. MPs are known as small plastic debris, which often have a range of several millimeters, mostly less than 5 mm.[9] MPs may belong to a wide range of polymers with aliphatic and aromatic structures and can have different compositions such as blends and copolymers. They may also contain various functional groups or additives with a range of sizes and variety in shapes, which cause excessive health issues. Because MPs are light and nonbiodegradable, they have been detected all around the world, from the air to deep sea sediments, from arctic sea ice to all ecosystems. An analysis of seabirds showed that 95% of southern North Sea fulmar stomachs contained an average of 35 pieces of plastic, while 58% of fulmars carried more than 0.1 g of plastic in their stomachs.[10] Most marine mammals also eat microplastics. Smaller plastic particles posed a higher risk of ingestion, and the number of animal species that ingested the particles was greater than the number that ingested food particles. MPs have also been found in planktonic animals and fish larvae. Consequently, they can easily enter the body of plankton-eating animals. MPs can enter animals and human beings via the food chain.[11] As a result of the SARS-CoV-2 pandemic, plastic wastes such as single-use face masks are increasing, which may release a great number of MPs.[12] MPs can be transported into wastewaters through domestic or industrial drainage systems before they eventually find their way to the freshwater or marine environments.[13] MPs also enter the environment via wind[14] and can be transported over great distances via ocean currents. As a result, they can easily enter the biomass, which is in use for hydrogen generation. It is proven that MPs are toxic and have a negative effect on the growth and metabolism of plants when entering their living environment. While one of the most attractive raw materials for biohydrogen production is wastewater sludge (due to its enormous volumetric generation), it has been shown that, even after treatment, about 73.8% of MPs can be accumulated in the sludge of the wastewater treatment plants,[15] which have a negative effect on the efficiency of the biohydrogen that is produced from this source of biomass. Obviously, the type, shape, and age of the MPs presented in sludge are not always the same, which greatly affects the amount and quality of the hydrogen obtained. Recently, several studies have confirmed that plastic debris at the submicrometric scale (<1 μm) can be absorbed by plant roots and translocated above ground to aerial tissues,[16,17] which can affect the biomass taken out from these plants used for the production of H_2. It is obvious that microorganisms cannot perform fermentation on these MPs. Similarly, they can remain in the different organisms of the terrestrial and sea plants,[18] which can then be used as biomass for hydrogen production. Moreover, because MPs have a large ratio of surface area to mass, they can adsorb and accumulate many contaminants, particularly hydrophobic organic contaminants[19] and heavy metals,[20] on their surface. These adsorbed materials have a great effect on the hydrogen production from such biomasses.

In this Review, an attempt has been made to collect and analyze chronologically all available research studies on the effect of MPs pollution on hydrogen production from biomass, either directly or indirectly. To the best of our knowledge, no review describing this subject has yet been published, and it is the first publication of this type summarizing research on this subject. Since the first article in this area appeared in 2018, the existing literature on the topic is limited, but we tried to cover all available resources.

2. EFFECT OF MPs ON HYDROGEN PRODUCTION

Table 1 presents a comprehensive list of research performed on the potential effect of the presence of MPs in biomass on the performance of biohydrogen production. The first effect of MPs on the production of biohydrogen was reported by Wang et al. in 2018.[21] While they were trying to remove tetracycline antibiotics to increase the photocatalytically reclame hydrogen energy by polymeric carbon nitride foam from natural water matrixes and wastewater, they noticed that PET, PE, PVC, PP, and PS are present in their samples; among them, PS was the most abundant. As a result, they were triggered to evaluate how MPs affect the photocatalytic removal of tetracycline. They selected PS as the MP model, and to study the impact of PS particles, they added ∼0.88 μm diameter PS MPs into the reaction system at different concentrations. Interestingly, they observed that the low amounts of the emerging PS slightly improved tetracycline removal, whereas amounts higher than 1.428×10^7 P/cm^3 restricted removal due to light absorption and the intrinsic adsorption interaction, which in turn reduces the amount of released hydrogen. This phenomenon can be related to the light absorption and the intrinsic adsorption interaction that were described previously in a report by Rochman et al.[22] They showed that the light absorption by PS, and interaction after that between tetracycline and PS, lead to a significant reduction of the degradation efficiency of tetracycline, which results in a decrease in hydrogen production.

A report on the effect of MPs in sludge on the production of hydrogen was presented by Wei et al.[23] The waste-activated sludge they used in their study was collected from the secondary sedimentation tank of a wastewater treatment plant. The collected sludge was concentrated by allowing it to settle for a day, and then it was stored at 4 °C. They detected MPs of PET, PVC, PE, PS, and PP in the collected waste-activated sludge. Among them, PET was determined to be dominant with a content of 2.1 ± 1.1 particles/g-TS (TS = total solids),

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so it was selected as the model MP to explore its influence on H_2 production from the wastewater during alkaline anaerobic fermentation. For their experiments, they used spherical PET MPs with an average size of 25 ± 2 mm and a density of 1.4 g/cm^3. They found that, due to sludge hydrolysis, acetogenesis, and acidogenesis involved in hydrogen generation, PET MPs content in waste-activated sludge (at three levels of concentration of 10, 30, and 60 particles/g-TS) repressed significantly the hydrogen production, although the homoacetogenesis and methanogenesis for hydrogen consumption were also inhibited under alkaline condition. They found that the hydrogen yield at 60 particles/g-TS for MPs contaminated is around 70.7 of the control. This was further confirmed by the microbial analysis, which clearly showed that the presence of PET MPs causes the microbial community to be shifted toward a direction that is against hydrolysis-acidification. Mechanism studies revealed that the existence of PET MPs has a negative influence mainly through leaching the toxic di-*n*-butyl phthalate. The reactive oxygen species (ROS) and live/dead staining examinations showed that PET MPs induce ROS increment, causing more cells to die, which further resulted in the decreased production of hydrogen. They discovered that the di-*n*-butyl phthalate leached and ROS induced by PET MPs were the two main factors to diminish hydrogen yield by causing the loss of viability and activity of the relevant anaerobic microorganisms.

Wei et al.[24] found that PVC MPs can release toxic bisphenol A and consequently can prevent the anaerobic digestion process. In their work, the effects of PVC MPs (1 mm, 10−60 particles/g-TS) on anaerobic methane production from waste-activated sludge were investigated. This methane can be further converted into hydrogen. The presence of 10 particles/g-TS of PVC MPs increased methane production by 5.9%, but higher levels of MPs (20, 40, and 60 particles/g-TS) inhibited its production to 90.6%, 80.5%, and 75.8% of the control, respectively. Model-based analysis showed that the presence of PVC MPs at levels higher than 20 particles/g-TS decreases both methane potential and hydrolysis coefficient waste-activated sludge. The mechanistic studies showed that bisphenol A leaching from PVC MPs is the major reason for lower methane production, causing significant inhibitory effects on the hydrolysis-acidification process. The long-term explosion to PVC MPs revealed a shift of microbial community against hydrolysis-acidification and methanation. So, they concluded that PVC MP has a negative effect on waste-activated sludge anaerobic digestion through the bisphenol A leaching.

Fu et al.[25] already revealed the effects of PS nanoparticles (average size of 54.8 nm) in anaerobic digestion systems. According to their research, these nanoparticles can attach on the surface of cell membranes and inhibit the metabolism and growth of some of the microbes. They concluded that the different shapes and sizes, abundances, and composition of MPs should have different effects on the activated sludge anaerobic digestion process. Conditions under which the anaerobic digestion is operated can also affect the leaching of MP toxic compounds and accordingly affect the digestion process. In their study, the impacts of nanoplastic on the pure anaerobic digestion culture were investigated using *Acetobacteroides hydrogenigenes* as a model anaerobic microorganism. *Acetobacteroides hydrogenigenes* is a strictly anaerobic, mesophilic, carbohydrate-fermenting, hydrogen-producing gram-stain-negative, nonmotile rods (0.7−1.0 × 3.0−8.0 um) bacterium. The growth and metabolism of this microorganism were characterized by the amount of hydrogen yield, which is depicted in Figure 1. Because hydrogen is the main metabolite

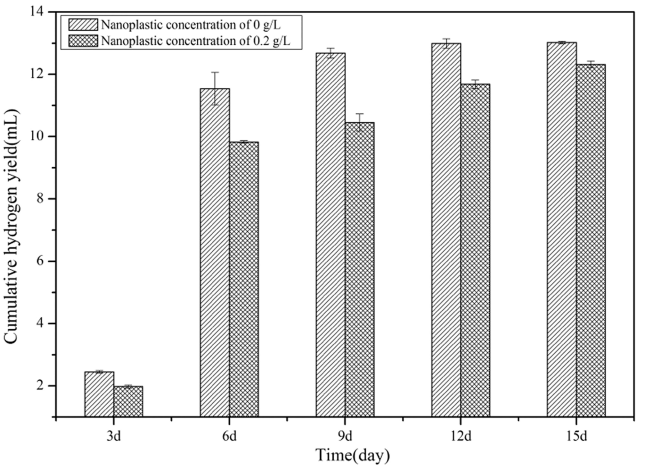

Figure 1. Decrement of hydrogen yield in the presence of 54.8 nm PS MP. Reproduced with permission from ref 25. Copyright 2018 Elsevier.

of *Acetobacteroides hydrogenigenes*, in the pure anaerobic digestion system, a lower yield of hydrogen means the lower growth and metabolism of this bacterium, which occurred at a nanoplastic concentration of 0.2 g/L. Moreover, *t* test results showed that hydrogen production from pure culture in the presence or absence of nanoplastic has a large difference, and hydrogen production without nanoplastic presence is significantly higher. They found that, in the mixed anaerobic digestion system, the methane production performance was also inhibited by the MPs existing in the system. The hydrogen yield increased rapidly during the third and sixth days of the experiment, which implies the rapid growth of the bacterium. Cumulative hydrogen yields with nanoplastic concentrations of 0 and 0.2 g/L on the sixth day were 11.5 and 9.8 mL, respectively, and the final cumulative hydrogen yields with nanoplastic concentrations of 0 and 0.2 g/L were 13.0 and 12.3 mL, respectively. The interaction between nanoplastic and *Acetobacteroides hydrogenigenes* was analyzed by SEM, which is shown in Figure 2. As can be seen, many nanoplastics are attached to the cell membrane of *Acetobacteroides hydrogenigenes*. In addition, many nanosize pores appeared on the cell membrane of this bacterium, which might be caused by the diapirism of nanoplastic particles.

In research performed by Li et al.,[26] the effect of PES MPs on the anaerobic digestion of waste-activated sludge was investigated. It is known that more than 90% of MPs, which enter the wastewater treatment plants, remain in the sludge after removal from wastewater.[27] This study investigated the effect of PES MPs concentration on the anaerobic digestion for the production of methane, which would then be used for hydrogen production. The results indicated that the methane production in the presence of MPs at abundances of 0−200 000 particles/kg-TS was inhibited in comparison with that of the control. During anaerobic digestion with different levels of MPs (0, 1000, 3000, 6000, 10 000, 30 000, 60 000, 100 000, and 200 000 polyester particle/kg activated sludge), the methane production was reduced to 88.53%, 90.09%, 89.95%, 95.08%, 90.29%, 93.16%, 92.92%, and 92.72% after

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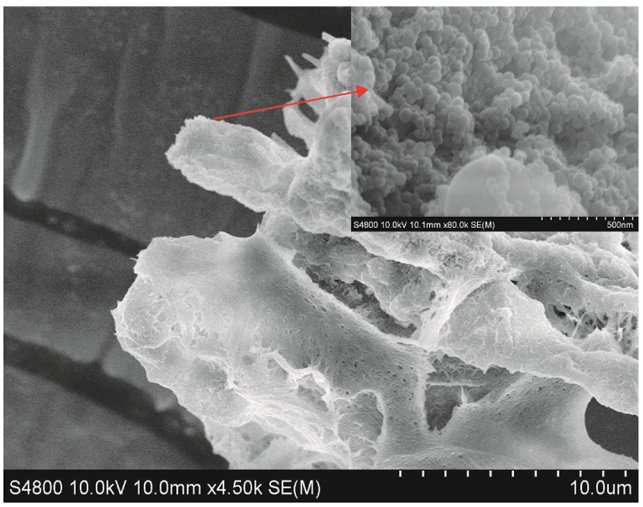

Figure 2. SEM image of *Acetobacteroides hydrogenigenes* exposed to PS MPs. Reproduced with permission from ref 25. Copyright 2018 Elsevier.

59 days of digestion. The one-substrate model indicated that in the presence of MPs the hydrolysis rate and biochemical methane potential decreased, but no linear correlation was found among these two parameters and MP concentration. The digestate supernatant character results showed that MPs can cause incomplete digestion, which prevents the methane production and causes higher amounts of retained organic matters and nutrients, but a slight improvement in dewaterability. However, the addition of MPs showed no significant effect on the microbial population structure.

In 2021, Zhang et al. published a paper[28] in that the effects of PE MPs on the digestion performance and the fate of tetracycline antibiotic resistance genes and microbial communities in thermophilic anaerobic digestion (55 °C) and hyperthermophilic anaerobic digestion (65 °C) of dairy wastes were investigated. The results showed that the effects of PE MPs were similar at different temperatures. At 55 and 65 °C, the presence of PE MPs improved methane production by 8.4% and 41.2% and enhanced chemical oxygen command (COD) removal rates to 52.8% and 52.4%, respectively, mainly by increasing the abundances of microorganisms involved in hydrolysis, acidogenesis, and acid utilization (e.g., *Clostridium*, *Dechloromonas*, and *Caldicoprobacter*). PE MPs significantly increased the fold changes in the abundances of nine typical tetracycline resistance genes from 0.71−12.63 to 1.15−28.62, which means the PE MPs shifted the microbial communities in the direction of hydrolysis, acidogenesis, and acid utilization, thus affecting the digestion performance, which in turn promoted the proliferation of tetracycline antibiotics by shifting their host bacteria profiles and abundance. According to the redundancy analysis and correlation analysis, free ammonia was the compound that had the highest contribution (51% of the total variation) to the fate of antibiotic resistance genes in different anaerobic digestion systems. The dairy wastes used in this study were collected from a large-scale dairy farm and had a TS of 2.05%, a COD of 5841.1 mg/L, a pH of 7.99, a total ammonium nitrogen content of 515.2 mg/L, a total phosphorus content of 11.0 mg/L, and a total alkalinity of 3461.5 mg/L. While different MPs were present in the dairy wastes that were selected, the specific concentration was not measured. PE MPs added were spherical particles with an average diameter of <400 μm and a density of 0.92 g/cm^3. The results showed that the effects of PE MPs were similar at different temperatures, which is opposite to the results obtained by Wei et al.[24] This can be attributed to the difference in the dose of PE MPs. Wei et al. have shown that MPs induced reactive oxygen species and released acetyl tri-*n*-butyl citrate, but whether the leach products were different at various temperatures needs more study.

Ren and his colleagues[29] investigated the influences of PET MPs on the semicontinuous hydrogen production and microbial community under mesophilic and thermophilic fermentation. The results indicated that PET MPs can enhance this H_2 production performance. The highest hydrogen production rates in mesophilic and thermophilic MPs supplemented groups were 4.85 and 3.6 L/L·d, which were 21.6% and 63.6% higher than those of the control groups, respectively. Moreover, it was found that PET MPs could favor the growth of H_2-producing bacteria and optimize the structure of the microbial community. In mesophilic MPs supplemented groups, the abundance of *Lactococcus* and *Ethanoligenens* increased from 20.77% and 6.7% to 38.69% and 10.78%, respectively. The abundance of the *Thermoanaerobacterium* became the dominant bacteria in thermophilic fermentation, and its abundance increased from 9.02% to 69.38%. This work provides novel insights into the function of MPs in the fermentative H_2 production system. The average size of PET MPs used in this work was 30−100 μm, of which 200 particles per liter were added to the fresh hydrogen production medium in MPs supplemented groups.

In 2020, Zhang and her colleagues discussed the impact of PS micro and nanoplastics on the methane and hydrogen generation by anaerobic digestion.[30] They reported the impacts of different sizes of MP and nanoplastic on the anaerobic digestion process. The 80 nm and 5 μm PS microplastics at 0.25 g/L showed the most negative effect on the cumulative methane production, which were 442.4 and 449.8 mL, representing a significant decrease of 19.3% and 17.9% in comparison to the control, respectively. These sizes of MPs with a concentration of 0.25 g/L could inhibit the acidification and methanogenesis, while MPs with the concentrations of 0.2 g/L or lower showed little or no impact on the anaerobic digestion process. The inhibitory effects of nanoplastics on the anaerobic digestion process were slightly more significant than those of MPs. The methanization stage was more sensitive to the toxicity of PS MPs than was the acidification stage.

Cunha et al.[31] performed a biochemical study on the effect of MPs pollution in microalgal biomass production; however, they did not study the effect of MPs contamination on biohydrogen production. Microalga *Phaeodactylum tricornutum*, which can be used as a biomass for H_2 production and can be converted to biodiesel and bioethanol, was exposed to water contaminated with 0.5 and 50 mg/L of PS and/or PMMA over its full growth cycle. The results showed that the microalgal cultures exposed to lower concentrations of PS displayed a growth enhancement of up to 73% in the first stage (days 3−9) of the exponential growth phase. Surprisingly, and despite the fact that long-term exposure to MPs contamination did not impair microalgal growth, a severe decrease in biomass yield was observed (up to 82%), even in low environmental contamination levels. The production of photosynthetic pigments of chlorophyll-a and carotenoids was shown to be pH-correlated during the full growth cycle, but was cell

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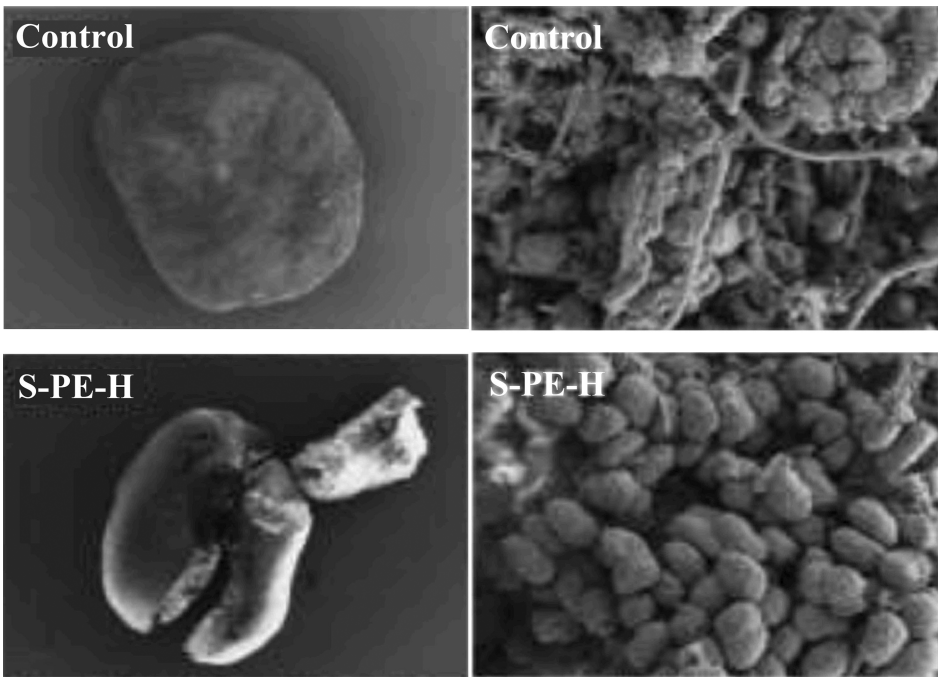

Figure 3. SEM images of HPG derived from the control group and S-PE-H group. The granule under stress of nanosize PE-MPs had deep cracks in the surface and was clearly disintegrated. In contrast, the granule from the control reactor without PE-MPs addition was compact and intact. The 30 mg/L, 200 μm PE-MPs; 90 mg/L, 200 μm PE-MPs; and 90 mg/L, 50 nm PE-NPs are marked as L-PE-L, L-PE-H, and S-PE-H, respectively. Reproduced with permission from ref 32. Copyright 2022 Elsevier.

density-independent in later stages of culturing. The extracellular carbohydrates production exhibited a major decrease during long-term exposure. Still, the production of extracellular proteins was not affected by the presence of MPs. This pilot laboratory-scale study addresses the potential effects of the use of water contaminated with MPs in microalgal-biomass biotechnology and ultimately biohydrogen production from such biomass resources.

In a recent paper published by Zhang et al.,[32] the authors demonstrated that the PE-MPs inhibitory effect on hydrogen production by hydrogen-producing granular sludge is dependent on both the concentration and the size of the MPs. In this work, PE particles at 200 μm and 50 nm were employed, and for hydrogen production, the inoculated anaerobic hydrogen-producing granular sludge was derived from an up-flow anaerobic sludge blanket reactor using sucrose-rich wastewater. The proportion of volatile suspended solid to TSS of the hydrogen-producing granular sludge was 88.2%. The organic loading rate of the hydrogen-producing granular sludge was 7.1 g COD/L/d with a hydraulic retention time of 18 h. Two levels of 30 and 90 mg/L concentration of PE plastics were applied in this research for both sizes selected for the PE particles. They found that the increasing of PE-MPs concentration and decreasing size of PE significantly decreased the amount of hydrogen generated. The higher levels of PE also suppressed the extracellular polymeric substances generation more strictly. PE-MPs exposure also led to the disruption of granular sludge metabolism including extracellular polymeric substances secretion and biological reactions, resulting in the elevation of dead cells in hydrogen-producing granular sludge and the progressive disintegration of the granules. The infiltration of PE in the size of nano from outer sphere to inner layer of granule increased the possibility of cells in hydrogen-producing granular sludge directly exposed to the toxic environments, which were favored by the toxic contributors of excessive ROS generation and release of leachates from the plastic particles. SEM was used to photograph the overall appearances of hydrogen-producing granular sludge from the control group and experimental group with nanosize PE-MPs exposure. Microphotographs are displayed in Figure 3. As can be seen, the granules under stress of nanosize PE-MPs have deep cracks in their surfaces and are clearly disintegrated. In contrast, the granules from the control reactor without PE-MPs addition are compact and intact.

The same group in the same year studied mitigating the distinctive stresses induced by diverse MPs on anaerobic hydrogen-producing granular sludge.[33] They studied the different stress responses of anaerobic hydrogen-producing granular sludge to PE, PET, and PVC MPs with a size of 0.1 mm in wastewater. Again, the hydrogen-producing granular sludge they applied was derived from up-flow anaerobic sludge blanket reactor treated sewage containing 5.3 g-COD/L sucrose with a hydraulic retention time of 18 h. The proportion of volatile suspended solid to TSS in sludge was 88.2%. The waste corn cob was used to make biochar for the experiments. Eight groups of identical glass-made hydrogen-producing granular sludge reactors were built for experiments to explore the responses of it to the exposures of different MPs and the toxicity mitigation role of biochar on MPs in biological hydrogen-producing systems. They selected 90 mg/L for their experiments to compare the mitigation effects of biochar on different types of MPs. Other researchers already used the same concentration of MPs for their in-field research.[34] They observed that different MPs differentially damage the hydrogen-producing granular sludge and have different effects on the performance of hydrogen production and granule morphology. The effectiveness of biochar to mitigate the adverse impacts on

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hydrogen-producing granular sludge caused by MPs was also confirmed. Biochar reduced the adhesion of MPs on the surface of hydrogen-producing granular sludge through adsorption, resulting in attenuated oxidative damage caused by excess ROS and a restored defensive function of extracellular polymeric substances. In addition, the best mitigation effectiveness in hydrogen-producing granular sludge contaminated by PVC-MPs was due to the adsorption of PVC leachates by biochar and the stronger affinity of biochar to PVC-MPs.

The response of anaerobic hydrogen-producing granules to acute MPs exposure during biohydrogen production from wastewater was the research topic of Wei et al.[35] By investigating the toxic mechanisms of MPs toward granules, they concluded that MPs cause cell death and decrease H_2 production in the granules. Their study provided insight into the acute exposure−response relationship between multiple coexisting MPs and the anaerobic hydrogen-producing granule. In the presence of a 40 mg/L MPs mixture consisting of 36% PET, 8% PP, 42% PE, and 15% PS MPs (particle sizes ∼150 μm), hydrogen production of the anaerobic hydrogen-producing granule decreased to 85.8% of the control, and it decreased to 81.3% when the anaerobic hydrogen-producing granule was exposed to 80 mg/L of MPs. Its variation was in line with the physiological activity of the anaerobic hydrogen-producing granule measured in the system. The acute exposure experiments were assayed with three groups of serum bottles. The anaerobic hydrogen-producing granule of 2 g-TSS/L was inoculated in each serum bottle (COD 8000) and cultured with the synthetic wastewater using glucose as the carbon source. One group without exposure to MPs was used as the control. During the experiments, the pH in each bottle was constantly stabilized at 4.5 to prevent methanogens activity. The amounts of generated H_2 were monitored for 15 days until hydrogen production dropped to <1%. SEM images showed that the anaerobic hydrogen-producing granule in the control reactor exhibited a smoother and more solid entire shape, but the shock load of the MPs resulted in narrow cracks and broken surfaces. Moreover, the anaerobic hydrogen-producing granules were dispersed as densely packed aggregate granules, but granules exposed to the MPs were loose. In the images provided in this Review, MPs themselves were not visible, probably because they were washed out during pretreatment for SEM preparation. They concluded that the extracellular polymeric substances secretion was suppressed when the anaerobic hydrogen-producing granule was acutely exposed to MPs, losing protection to the microorganisms in granules. Also, MPs exposure caused the death of cells inside and outside of the granule, resulting in the decreased hydrogen production ability of the anaerobic hydrogen-producing granule. The toxicity of the microplastics chiefly stems from the excessive oxidative stress and the leachates released. The percentage of dead cells in total cells was 5.6% without MPs exposure, while its fraction reached 14.7% in the presence of MPs.

Research performed by Wang et al.[36] revealed that PS MPs and nanoplastics distinctively affect anaerobic sludge treatment for hydrogen and methane production. Microbial activities and compositions were correlated with the PS plastic size. PS MPs stimulated hydrogen accumulation by increasing the solubilization of waste-activated sludge. They applied 50 μm and 50 nm PS particles at 0.16 g/L. Waste-activated sludge and anaerobically digested sludge were collected from a secondary settler. They observed that, except for the elevating hydrogen production with PS-MPs, plastics have an inhibition effect on gas accumulation. Process modeling indicated that PS regulated gas generation mainly through modifying the gas production potential. Overall, waste-activated sludge fermentation reactors affected by PS-MPs exhibited favorable parameters, while the performances of systems exposed to nanoparticles of PS were suppressed. To describe the reason, the authors of that article explained that PS-MPs had no obvious effect on microbial compositions for hydrolysis production but decreased the abundances of methane production-related microorganisms. However, PS-nanoparticles deteriorated microbial communities for hydrogen and methane generation, together with increasing ROS production. Enhancing the solubilization was the driving force for increasing hydrogen production with PS-MPs, which was otherwise trivial as compared to inhibition of other biochemical transformation steps in other cases. In fact, the extraordinary effects that plastic particles pose on sludge solubilization had been verified previously[37] and were also applicable in methane production. In MPs reactors, the maximum hydrogen production was changed from 18.8 mL/g in the control to 23.3 mL/g, but it was decreased to 12.8 mL/g in the nanoparticle reactors. Although nanoparticles also can enhance the solubility, the resulting excessive oxidative stress causes inhibition of microbial activities as well as the deterioration of microbial communities, which subsequently decreases hydrogen production. The detailed mechanisms with PS particle exposure were not exactly the same, with PS-MPs more strongly affecting methanation, and the nanoparticles were responsible for the higher extent of suppression in acidification, which was in accordance with the variations of microbial community compositions of the MPs and nanoparticles samples in opposite directions. More hydrogen production with PS-MPs can be related to an increase in solubilization. It is likely that, as the plastic concentration increases, PS-MPs release more toxic compounds to microbes in the sludge systems, thereby preventing their activity; at the same time, PS MPs increase hydrogen accumulation by increasing waste-activated sludge solubilization. They demonstrated that the inhibition of PS nanoparticles to hydrogen production was derived from the excessively inhibitory hydrolysis despite improving solubilization. Excessive oxidative stress would be generated in the presence of PS-MPs or PS nanoparticles, deteriorating the microbial activities and richness of species responsible for hydrogen or methane production.

3. CONCLUSION

MPs are widely spread all over the world, in the air, oceans, drylands, and over the entire ecosystem. They even exist inside the bodies of living things, including animals and a range of plants from higher to phytoplankton. As a result, MPs are present in all resources that are in use for (bio)hydrogen production, particularly in the biomass. In this Review, we tried to review all articles published to date on the effect of MPs pollution on hydrogen production from biomass. The polymers studied so far are PS, PET, PVC, PE, PES, and PMMA with different sizes and concentrations in different biomasses, mainly granular sludge applied for anaerobic fermentation. It was found that the size of the plastic particles is the most important parameter on hydrogen production. Plastic nanoparticles always suppress the production of H_2,

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while for MPs diverse effects were observed. However, the addition of MPs from a certain concentration decreases the hydrogen generation yield. Exposure of sludge to both MPs and plastic nanoparticles causes the death of the microbial community; however, because MPs can lose the granules, in lower concentrations, MPs may increase the production of hydrogen by enhancing the growth of H_2-producing bacteria and optimizing the microbial community structure. While most investigations have been performed on the effects of microplastics in sludge on the anaerobic digestion process, similar research on the other resources with more realistic MPs is necessary. MPs used in all of the research so far were new, regular, spherical particles, but, in reality, they may have changed in shape and structure because of aging. Although no dependence of the hydrogen production on the type of MPs was observed, the number of polymers studied was limited to six. Moreover, no research was reported on the influence of MPs on the fermentation of broth. However, many copolymers are in use today, which may behave differently from polymers with a single monomer. As a result, the underlying effects of micro(nano)plastics with different concentrations and sizes on the anaerobic hydrogen-producing system are still unclear and need more attention, because it is an important parameter for gaining the maximum amount of hydrogen from biomass. An important area that can be considered in future research on the effect of MPs for biohydrogen production is the presence or use of microorganisms that target the microplastics. For example, the bacterium *Ideonella sakaiensis* has been shown to be able to degrade polyethylene into smaller, less harmful components.[38] Another approach involves using bacteria to generate biofilms on the surface of microplastics, which can help to reduce their negative impacts. Biofilms are communities of microorganisms that adhere to surfaces and produce a sticky, protective matrix.[39]

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M.K. performed the conceptualization, writing−original draft, writing−revision and editing, visualization, and supervision; Z.H. performed the writing−review and editing; and J.G. performed the writing−review and editing.

Notes

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ABBREVIATIONS

MPs = microplastics
PE = polyethylene
PP = polypropylene
PVC = polyvinyl chloride
PS = polystyrene
PES = polyester
PET = polyethylene terephthalate
TS = total solids
ROS = reactive oxygen species
SEM = scanning electron microscopy
COD = chemical oxygen command
TSS = total suspended solid

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