Towards hydrogen production from waste activated sludge: principles, challenges and perspectives

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Abstract: Hydrogen production from waste activated sludge (WAS) was widely considered and intensively investigated as a promising technology to recover energy from wastewater treatment plants. To date, no efforts have been made on either systematic summarization or critical thinking of the application niche of hydrogen production from WAS treatment. It is therefore time to evaluate whether and how to recover hydrogen in a future paradigm of WAS treatment. In this critical review, the principles and potentials, microorganisms, possible technologies, and process parameters of hydrogen generation were analyzed. Microbial electrolysis cell shows high theoretical hydrogen yield and could utilize a variety of organic compounds as substrates, which is regarded as a prospective technology for hydrogen production. However, the poor organics utilization and rapid consumptions of produced hydrogen hindered hydrogen recovery from WAS. Based on the analysis of the current state of the literatures, the opportunities and challenges of hydrogen production from WAS are rethought, the detailed knowledge gaps and perspective of hydrogen production from WAS were discussed, and the probable solutions of hydrogen recovery from WAS treatment are figured out. To guide the application and development of hydrogen recovery, a more promising avenue through rational integration of the available technologies to form a hybrid process is finally proposed. The integrated operational paradigm of WWTPs could achieve substantial technical, environmental and economic benefits. In addition, how this hybrid process works is illustrated, the challenges of this hybrid process and future efforts to be made in the future are put forward.

Keywords: Hydrogen; waste activated sludge; anaerobic fermentation; hybrid process

1. Introduction

With the development of society, people need more and more energy, which inevitably leads to the shortage of fossil fuels. In addition, the combustion of fossil fuels makes large amounts of greenhouse gas (i.e., carbon dioxide) release in the atmosphere, causing the climate change and global warming. The issues of energy shortage and global warming drive the efforts to seek clean, recyclable, and renewable energy. Compared with methane, hydrogen possesses higher energy yield (i.e., 142.35 kJ/g which is 2.75 folds than that of other hydrocarbons) and generates water rather than greenhouse gases while it is combusted [1]. Therefore, hydrogen is considered as the green energy and widely accepted to be the most promising alternative to fossil fuels

Hydrogen can be produced from valuable resources such as water and fossil fuels, and also from wastes such as sewage and food waste via a series of chemical-physical and biological methods. There are many publications that reported hydrogen production by utilising the chemical-physical methods and biological methods [2]. Currently, most of the hydrogen (>85%) is produced through the pyrolysis of fossil fuels, and the gasification of biomass [3]. Although these chemical-physical methods could obtain high hydrogen yield, they are not sustainable due to high energy consumption [4, 5]. By contrast, biological hydrogen production is a more cost-effective and environmental friendly method to produce hydrogen from varieties of organic wastes (e.g., waste activated sludge) due to the simple operational conditions, steady H_2 yield and low energy consumption [6, 7]. As for different substrates, using wastes to produce hydrogen is an environmentally favorable and economically sustainable way. With the growing energy crisis worldwide, this aspect is becoming more important and pushing forward new attempts employing more wastes.

Waste activated sludge (WAS), which is the main byproduct of municipal wastewater treatment plants (WWTPs), is generated with large amounts annually [8]. For instance, it was documented that 11.2 million metric tons of dry sludge were generated in China while 10 million tons were produced in EU countries [9]. On one hand, treatment and disposal of such massive amount of WAS are costly, accounting for up to 60% of the total operation cost of a WWTP [10]. On the other hand, WAS contains high levels (50~70%) of organic compounds such as protein, carbohydrate, and lipid [11, 12], which makes it an ideal renewable resource. For example, Jiang et al. investigate the physicochemical characteristics of WAS and found that when the volatile suspended solid (VSS) of WAS was 10.81 g/L, WAS contained 14.88 g/L total chemical oxygen demand (TCOD), 9.94 g COD/L total protein, 0.86 g COD/L total carbohydrate, and 0.17 g COD/L lipid and oil [13]. Similar WAS characteristics were also reported in other papers [14, 15]. In addition, it was reported that ~35% of carbon element, ~3.8% of nitrogen element, ~1.6% of phosphorus element, and other trace elements contained in WAS [16].

Many publications showed that various wastes containing high organic substrates could be utilized to produce hydrogen by anaerobic fermentation process [17, 18], indicating that WAS is a potential substrate for hydrogen production. Although WAS is generally treated by the anaerobic digestion to produce methane, several hydrogen producers are found to be present in the digester such as *Clostridium pasteurianum* [19] and *Thermoanaerobacterium* [20, 21]. In fact, hydrogen is observed as an important intermediate in the

anaerobic digestion process [22]. Thus, hydrogen production from WAS attracted much attention in the past decades, by which fossil fuels are saved, greenhouse gas (e.g., CO_2) emission is reduced, WAS is reused and reduced, and sustainable clean energy H_2 and volatile fatty acids (VFAs) are also obtained. However, practical application of hydrogen production from WAS has not yet been achieved. On the contrary, some doubts and debates have been arisen recently about its technical and economic feasible in full-scale situations due to the low hydrogen yield. There are many challenges in reactor control, system development, and energy recovery. For example, hydrogen is an intermediate product in the anaerobic digestion, thus the produced hydrogen would be quickly consumed by hydrogen-utilizing methanogens to produce methane, homoacetogens to produce acetic acid, or sulfate-reducing bacteria to produce hydrogen sulfide. The highest hydrogen yield from WAS reported so far has been only 20.30 mg per gram volatile suspended solids [23] .

Several review papers were published on hydrogen production using the various wastes such as biodegradable municipal wastes [24], food waste [25], agriculture waste, wastewater [26], and lignocellulosic materials [27, 28]. These works were mainly to review the progress of hydrogen production in one aspect or several aspects. For example, Yang et al. provided a review on fermentative hydrogen production from sewage sludge but only focus on pretreatment methods and co-fermentation with other substrates [5]. Systematic summarization and critical thinking of the application niche of hydrogen production from WAS is still lacking. In addition, many endeavors were dedicated recently to improve hydrogen yield from WAS through enhanced the disintegration of WAS and suppressed or killed the competitive microorganisms (e.g., methanogens), which have made great progress [7, 29]. Hence, this review article aimed to comprehensively sum up the knowledge obtained in this field and critically think the prospect of biohydrogen production from WAS. To find out whether and how to recover hydrogen in a future paradigm of WAS treatment, the principles and advances of hydrogen production from WAS were needed to systematically review, its opportunities and challenges were required to critically re-examine, and the possible solutions were essential to carefully think about.

Based on reviewing more than 200 publications and critically analyzing the opportunities and challenges of hydrogen production from WAS, this review aims to offer useful information to remove key barriers that hinder hydrogen production from WAS to be applied in full-scale situations, and to stimulate more thinking and discussion of a probable application niche for hydrogen production from WAS. To guide the application

and development of hydrogen recovery, a more promising hybrid process through rational integration of the available technologies is proposed as an example, and how this hybrid process works is illustrate d and future efforts to be made in the future is discussed.

2. The pathways of hydrogen production from waste activated sludge

Several methods such as direct-biophotolysis, indirect-biophotolysis, photo-fermentation, dark-fermentation, and microbial electrolysis cell can be theoretically used for hydrogen production [30]. To date, however, only three approaches, i.e., dark-fermentation, photo-fermentation, and microbial eletrolysis cell, have been documented to produce hydrogen from WAS (Figure 1). Dark-fermentative hydrogen production is a process that uses organic matters in either soluble or solid state as electron donors and protons as electron acceptors to produce hydrogen by strict or facultative anaerobic bacteria. Photo-fermentative hydrogen production is a process that photo-fermentative bacteria (mainly purple nonsulfur bacteria) use light as energy and soluble organic matters (mainly small molecule organic acids) as electron donors to produce hydrogen. As for microbial electrolysis cell, exoelectrogenic microbes at the anode first oxidize organic matters into electrons and protons. The electrons produced are gathered at the anode and then transferred to the cathode, in which the electrons transferred are utilized to reduce protons for hydrogen production.

Figure. 1. Schematic diagram of hydrogen production from dark-fermentation (a) [31, 32], photo-fermentation (b) [33, 34] and microbial electrolysis cells (c) [35, 36].

2.1. Mechanism of hydrogen production from WAS dark fermentation

The main compositions of WAS are protein, carbohydrate, and lipids, and they usually locate in either extracellular polymeric substances or intracellular cells. Due to this characteristic, WAS must undergo disintegration and hydrolysis processes before the organics in WAS can be further degraded for hydrogen production. Dark-fermentation is the most intensively studied and widely used technique for hydrogen production from raw WAS. It is generally thought that the anaerobic digestion of WAS goes through five successive steps: disintegration, hydrolysis, acidogenesis, acetogenesis and methanogenesis (Figure 1a). As biosolids, sludge cells need to be disrupted in the disintegration step before the organics in sludge cells are utilized, and sludge disintegration is ordinarily considered the major rate-limiting step in anaerobic digestion [11, 22]. The released organics with large molecule weight such as protein and carbohydrate will be subjected

to hydrolysis process, in which soluble organics with large molecule weight are degraded to small molecule weight organics such as amino acids and glucose [37, 38]. Several enzymes including protease and α-glucosidase are involved in this step [39, 40]. After disintegration and hydrolysis, there are several pathways involved in acidogenesis and acetogenesis steps that can directly or indirectly produce hydrogen by a series of hydrogen producers such as *Enterobacter* sp. and *Clostridium* sp. The detailed pathways are summarized in Figure 2.

Figure. 2. The pathways of hydrogen production from sludge dark fermentation [41-46].

It can be seen that all the major compositions of WAS (i.e., protein, carbohydrate, and lipids) could be used as substrates for hydrogen production from dark-fermentation. Hydrogen could be directly produced via deamination of amino acids and β-oxidation of long-chain organic acids [47]. Hydrogen could be also generated via two different pathways from the degradation of pyruvate, an important intermediate produced from glycolysis of carbohydrate and deamination of amino acids. Pyruvate is easily degraded to acetyl-CoA via decarboxylate with reduced ferredoxin produced, which gives electrons to protons to generate hydrogen. This is the dominant pathway for hydrogen production by *Clostridium* sp. [48]. The other pathway is formate cleavage, which is generally dominated by facultative anaerobes, such as *Enterobacter* and *Klebsiella* [49, 50]. In the process of glucose glycolysis, a large number of NADH would be produced due to the shortage of electron transport chain in fermentative bacteria. NADH and H^+ are usually oxidized into NAD^+ in the stage of acidogenesis to maintain the proper ratio of NADH/NAD^+. However, when the oxidation of NADH is lower than the production of NADH, excess NADH and H^+ would inevitably existed. To maintain the normal metabolic activity, fermentative bacteria in this case would produce hydrogen from the oxidation of excess NADH [51]. Besides, in the stage of H_2-producing acetogenesis, some H_2-producing acidogens such as *Syntrophomonas wolfei*, and *Sytrophbacter wolinii* could convert propionic acid, butyric acid, ethanol and other organics into acetic acid and hydrogen [52-55].

2.2. Mechanism of hydrogen production from photo fermentation system

After dark fermentation, there are a large number of organic compounds such as fatty acids and alcohols left in the dark fermentation liquid, which has been demonstrated to be further bio-converted to hydrogen by photosynthetic bacteria [56]. To recover more hydrogen from WAS, combining dark fermentation with

photo fermentation is therefore recommended by researchers [56]. The purple nonsulfur bacteria, which can acquire electrons from volatile fatty acids to produce hydrogen, are the most extensively studied photosynthetic bacteria in photo fermentation systems [33]. Organics in such systems are first oxidized and the electrons generated are transported to the photosystem by ubiquinone. Then electrons are energized by light and cycled in the photosynthetic electron transport chain, which would produce a proton gradient. The energized electrons are further transferred to ferredoxin via oxidoreductase and utilized to produce hydrogen by nitrogenase, with energy and ATP being derived from the proton gradient (Figure 1b) [33, 57].

2.3. Mechanism of hydrogen production from microbial electrolysis cell

Microbial electrolysis cells, which directly utilizes electrons generated from substrate degradations by exoelectrogens to reduce proton for hydrogen production, has also received much attention recently. In such systems, exoelectrogens at the anode first oxidize substrates to produce electrons, protons (H^+), and carbon dioxide. The electrons produced are gathered at the anode and then transferred to the cathode through an external circuit with an applied potential, while the protons move to cathode by diffusing directly through the electrolyte (single chamber) or membrane (double chamber). The electrons are finally used to reduce protons to produce hydrogen at cathode [35, 58] (Figure 1c).

There are lots of organic matters that could be used as substrates to produce hydrogen by exoelectrogens, such as raw sludge [59], protein, organic acids [60], and cellulose [61, 62]. Like dark fermentation, raw sludge also requires to be disintegrated in the disintegration step before organic matters in sludge cells could be used by exoelectrogens. Generally, hydrolytic microbes, fermentative acidogenic bacteria, and exoelectrogens are co-existed in microbial electrolysis cells. On one hand, exoelectrogens could utilize the products of hydrolytic microbes and fermentative acidogenic bacteria at anode to generate electrons, thereby prompting the cascade utilization of organics in WAS efficiently through syntrophic interactions [63]. On the other hand, exoelectrogens would also compete with hydrolytic microbes and fermentative acidogenic bacteria for the available substrates such as soluble carbohydrate. It is noted that the electrons from oxidation of organics in dark-fermentation are given to the intermediate products. However, the electrons produced from exoelectrogens are gathered at the anode and transferred to the cathode, which would make the oxidation of organics completely to electrons, CO_2 and protons. If all the electrons transferred to the cathode are used to reduce protons, the hydrogen yield from microbial electrolysis cells

would be greater than that from dark fermentation. The high theoretical hydrogen yield and the ability to utilize a variety of organic compounds (e.g., volatile fatty acids, sludge fermented liquid and WAS) make microbial electrolysis cell a prospective technology for hydrogen production.

Sludge disintegration is also considered as the rate-limiting step in microbial electrolysis cells, thus some pretreatment methods of sludge were used to prompt the disruption of extracellular polymeric substances and cell envelops before the sludge entering into the microbial electrolysis cells. It was found that pretreatment methods could increase largely the yield and rate of hydrogen production from microbial electrolysis cells. Besides, adding fermentative bacteria into microbial electrolysis cells was also demonstrated to be an effective strategy to enhance hydrogen production. Wang et al. reported that combined sodium dodecyl sulfate pretreatment and microbial electrolysis cell could get the higher hydrogen yield of 8.5 mg H_2/g VSS, which was ~3.4-fold higher than using microbial electrolysis alone [60].

3. The bottlenecks of hydrogen production from WAS

3.1. The potential of hydrogen production from WAS

WAS shows huge theoretical potential in hydrogen production. The theoretical methane yield of 354 mg-CH_4 can be produced from WAS anaerobic digestion if 1 gram sludge cells (expressed as $C_5H_7NO_2$) are completely digested [64]. It is reported that 28% methane is produced from the hydrogenotrophic methonogenesis pathway [65], this indicates ~50 mg-H_2 would be consumed theoretically in this process. Besides, two other processes, i.e., homoacetogenesis and sulfate-reducing processes, are known to consume H_2 largely. The theoretical hydrogen yield, therefore, is much greater than 50 mg-H_2 if 1 gram of sludge cells is completely degraded in dark-fermentation process. When 1 gram acetate is used to produce

As mentioned above, however, hydrogen yield reported is always at very low levels even at bench-scale, and this is considered the bottleneck that hinders hydrogen recovery being implemented in field situations. It is documented that the highest hydrogen yields produced from WAS by the anaerobic fermentation or microbial electrolysis cells are only 20.3 mg-H_2/g-VSS[23]. Thus, why is hydrogen yield often at low levels?

3.2. Key factors affecting hydrogen production from WAS

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hydrogen in photo-fermentation .or microbial electrolysis cells, 133.3 mg-H_2 would be produced in theory [66].

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3.2. Key factors affecting hydrogen production from WAS

There are several factors restricting the production of hydrogen from dark fermentation. First of all, organic matters (e.g., protein, carbohydrate, and lipids) contained in sludge are much underutilized due to the rigid structure of microbial walls.[37, 67] According to previous report [9], less than 40% of VSS reduction could be achieved in sludge anaerobic fermentation systems. The released organics are not completely utilized to produce hydrogen. Apart from a small amount of soluble substrates being used for the growth and maintenance of anaerobes[68], a large number of soluble organics such as protein, humic substances, volatile fatty acids, and alcohol are still remained in fermentation liquid. Secondly, as an intermediate product in the anaerobic digestion, the hydrogen produced is readily consumed by (1) hydrogen-utilizing methanogens to produce methane [69], (2) acetobacteria to produce acetic acid [70], and (3) sulfate-reducing bacteria to produce hydrogen sulfide [71], which reduce the final hydrogen yield directly.

Although photo fermentation can in principle degrade soluble organics with small molecule weight (e.g. volatile fatty acids and alcohols) completely, there still exist some restrictions in practice. To ensure reaction efficiency, continuous illumination is required due to the low light conversion of purple nonsurful bacteria. However, solar illumination is easily influenced by outdoor conditions while artificial illumination is expensive [72, 73]. The design and construction of photo-bioreactors are difficult and costly due to several limitations such as big surface-volume ratio and highly transparent [74]. Furthermore, the effluent directly from dark-fermentation is opaque and contains several toxic matters which would hinder light penetration and inhibit the activity of nitrogenase [75, 76].

Microbial electrolysis cell is not mature enough to be applied in real world. When WAS is directly used as substrate, the microbial communities of anodophilic biofilms have not yet been known clearly. Since exoelectrogens and fermentative microbes such as homoacetogens and methanogens co-existed in microbial electrolysis cells[77-79], most of the organics released from WAS are metabolized by fermentative microbes rather than exoelectrogens, which could achieve degradation of complex organics but then lead to the low coulombic efficiency at the same time [59]. Carbon-based materials (e.g., carbon felt, graphite fibres, and graphite plates) are usually used as anode but they are conductivity-poor[80]. Noble metals such as platinum and palladium were also tried to use as cathode due to their outstanding catalytic activity but they are also costly. It remains a challenge to find proper materials to make anode and cathode [81, 82]. There are two

kinds of reactor configurations (i.e., single chamber and double chambers) usually used in microbial electrolysis cells [83, 84]. The double chambers use membrane to separate cathode and anode, leading to the high internal resistance. Although single chamber is without membrane, it makes hydrogen produced at cathode easily consumed by anodophilic microbial communities [85, 86].

4. Recent efforts to improve hydrogen production from waste activated sludge

4.1. Recent progress in hydrogen production from WAS dark fermentation

In the past years, many endeavors were dedicated to improving hydrogen yield from WAS (Table 1). In order to enhance the disintegration of WAS and to suppress or kill the competitive microorganisms (e.g., methanogens) in dark fermentative systems, many WAS pretreatment methods such as heating [87], acid [88], alkaline, and ultrasonic [89, 90] pretreatments were tested. Xiao et al.[91] found that compared with the control, thermal pretreatment at 121℃ for 30 min enhanced soluble chemical oxygen demand from 113.7 to 2442.1 mg/L and reduced the dry solid from 8.96 to 7.55 g/L due to the acceleration of disruption of floc structure and microbial cells. As a result, hydrogen yield was improved from 1.46 mL H_2/g VSS to 8.62 mL H_2/g VSS. Cai et al. [6] found that alkaline pretreatment not only facilitated the disruption of WAS but also suppressed the activities of hydrogen-consuming microorganisms, which improved hydrogen yield from 9.1 mL H_2/g DS to 16.6 mLH_2/g DS. The summarization of these pretreatments was detailed in a recent review [92]. Hydrogen yields from anaerobic fermentation of WAS were expressed by a variety of indexes and units such as mL/g VSS, mL/g-total solids (TS), and mL H_2/g DS, which was reviewed in a recent paper[5].

Manipulating the fermenters was also documented to be effective. Zhao et al.[93] found that compared with alkaline pretreatment (i.e., initial pH 10) alone, controlling pH in the fermenter at constant pH 10 caused 47.8% higher in hydrogen yield (18.2 versus 26.9 mL H_2/g VSS). It was reported that shortening hydraulic retention time from 8 h to 6 h benefited to wash out H_2-consuming bacteria (e.g., propionic acid bacteria), as their growth rate is slower than H_2 producers'[94]. A relative high fermentative temperature facilitated the hydrolysis of substrates and simplified microbial diversity, which thereby profited for hydrogen accumulation. Yokoyama et al. observed the activity of H_2-consuming microbes was completely suppressed when the fermenter was controlled at 75 ℃for the treatment of cow waste slurry[95].

The characteristics of fermented WAS (e.g., the ratio of carbon to nitrogen and the level of polyhydroxyalkanoates) affected hydrogen yield as well. It was documented that pertinent increases of carbon/nitrogen ratios benefited the conversion of protein while an increase of sludge polyhydroxyalkanoates enhanced the cell solubilization, the hydrolysis process of solubilized substrates, and the soluble protein conversion of non-polyhydroxyalkanoates biomass, thereby promoting the production of hydrogen [96]. Based on these findings, optimizing the composition of the fermented WAS via either promoting the content of intracellular polyhydroxyalkanoates in WAS or co-fermenting WAS with other carbon-rich substrates such as food waste and molasses was therefore recommended [97-99]. Wang et al.[22] found that when the content of sludge polyhydroxyalkanoates increased from 25 to 178 mg/g VSS, hydrogen yield from alkaline fermentation (i.e., pH 10) enhanced from 26.5 to 58.7 mL/g VSS. In addition, when co-digestion of food waste and WAS was at a C/N ratio of 33.14, hydrogen yield reached 102.63 mL H_2/g VSS, which was much more than hydrogen production from WAS alone[100].

Manipulating hydrogen-producers was also helpful to improve hydrogen yields, such as inoculating some specific microbes and using metabolic engineering to modify hydrogen-producers in fermenters. Kotay et al.[101] constructed a microbial consortium by inoculating with three established bacteria (i.e., Enterobacter cloacae IIT-BT 08, Citrobacter freundii IIT-BT L139 and Bacillus coagulans IIT-BT S1) and found that hydrogen yield could be improved 1.5-4 times with different ratio of these bacteria. Among these, 1:1:1 v/v ratio of these three bacteria was found to achieve the maximal hydrogen yield of 41.23 ml H_2/g $COD_{reduced}$. Kim et al. engineered the pentose phosphate pathway to enhance hydrogen production in recombinant *Escherichia coli* and found that hydrogen yield increased by 3.5-fold, i.e., 21.665 mmol H_2/mol glucose [102-105].

WAS, as the byproduct of wastewater biological treatment, adsorbs and concentrates lots of exogenous pollutants such as heavy metal, nanoparticles and micropollutants [106, 107]. Recently, some studies have investigated the effects of exogenous pollutants on hydrogen production from WAS anaerobic fermentation. Wu et al. found that 20 mg/g TSS poly aluminum chloride (a widely used inorganic coagulant) increased hydrogen accumulation from 20.9 mL to 27.4 mL/VSS. Mechanism analysis shows poly aluminum chloride inhibits the activities of all the microbes related to anaerobic fermentation, and its inhibition to

hydrogen-consuming microorganism was much severer than that to hydrogen producer [108]. Wang et al. reported that 1403 ± 150 mg/kg TSS triclocarban (an antimicrobial agent) enhanced the activities of acetate kinase and [FeFe] hydrogenase and restrained the activities of carbon monoxide dehydrogenase and Coenzyme F_{420}, thus improved hydrogen production from 10.1 to 14.2 mL/g VSS during WAS anaerobic fermentation [109]. Wei et al. found that polyethylene terephthalate microplastics inhibited hydrolysis, acidogenesis and acetogenesis processes, thus reducing hydrogen production from WAS anaerobic fermentation [14]. Many exogenous pollutants show dose-dependent impacts on WAS anaerobic fermentation [110]. The results exhibit that most of the pollutants at low dose could improve the hydrolysis, acidification and methanogenesis processes while high dose show negative influence due to their toxicity [111, 112]. Ordinarily, the activities of methanogens are more easily inhibited than those of hydrolytic and acidogenic bacteria due to the less tolerance of methanogens [113, 114]. However, there are still few studies to investigate the effect of exogenous pollutants on hydrogen production. More efforts are required to unveil and regulate their influences on hydrogen production from WAS anaerobic fermentation.

Additionally, to further utilize the organics left in the dark fermentation liquid such as fatty acids and alcohols, two-stage systems (e.g., dark fermentation-photo fermentation or dark fermentation-microbial electrolysis cells) were also proposed to improve hydrogen yield [63, 115-117]. For example, it was reported that a combined process (i.e., dark fermentation-microbial electrolysis cell) was used to recover bioenergy from WAS and acquired 20.30 mg H_2/g VSS of hydrogen yield while only 1.39 mg H_2/g VSS of hydrogen production from WAS anaerobic fermentation alone [7, 23]. The combined of dark fermentation and microbial electrolysis cell obtained the better performances of sludge reduction and hydrogen recovery.

4.2. Recent progress in hydrogen production from photo fermentation

Previous investigations made on photo-fermentative hydrogen production mainly focused on optimization of the fermenter construction and operation, metabolic engineering of hydrogen producers, or wavelength and intensity of light. Glass and polymethyl methacrylate are considered the most suitable materials for constructing photobioreactor[74]. Nitai et al.[118] reported that the suitable pH and temperature ranges for photo-fermentative hydrogen production were 6.8-7.5 and 31-36 ℃, respectively. Several metabolic engineering methods were used to manipulate H_2-producer, such as strain improvement by

modifying light-harvesting antenna complexes and replacement of nitrogenase with hydrogenase[119]. Kondo et al.[120] found that strains with a 41%-49% decrease in LH2 (LH: light-harvesting) content by UV irradiation produced 50% more hydrogen than untreated strains. Different light wavelengths and intensities also affected hydrogen yield. It was reported[121] that the suitable light wavelength and intensity were in the range of 400-1000 nm and 6-10 klux, respectively. The details about the impact of light wavelength and intensity on hydrogen production could be found in a recent review[118].

In the dark–photo fermentation combination system, however, some pretreatments of the effluent from dark fermentation (e.g., dilution, ammonium stripping, and centrifugation) are necessary before the effluent is used for hydrogen production through photo-fermentation [122, 123]. Yetis and coworkers[124] reported that when the concentration of fermentation medium was at 30% dilution of pretreated sugar refinery wastewater, hydrogen production would be stopped, but a small amounts of hydrogen would be produced (i.e., 130 mL H_2/L) at 20% dilution.

4.3. Recent progress in hydrogen production from microbial electrolysis cell

Efforts made on microbial electrolysis cell mainly focused on optimizations of the three aspects: improvement of electrode materials, reactor configuration, and manipulation of microbial community. Electrode is one of the most significant part of microbial electrolysis cell. While bacterial activities mainly occur at anode, hydrogen is produced at cathode, so in microbial electrolysis cell, it is important to find the highly efficient and low cost electrode materials (included anode and cathode) to achieve microbial electrolysis cell practical implication. Based on these principles, there are many new, cheap and effective anode materials (e.g., carbon nanotubes and graphene[125, 126]) and cathode materials (e.g., carbon nanotube and biocathode [127, 128]) developed, which are helpful to reduce the cost and improve hydrogen yields. Xie et al.[129] claimed that graphene-sponge was cheap and easy to fabricate which 1 m^2 2 mm-thick graphene-sponge would only cost \$4 compared to 1 m carbon cloth cost \$1000[130]. Rozendal et al.[131] reported that 0.63 m^3-H_2 per 1 m^3 cathode liquid volume would be produced from the microbial biocathode compared to 0.08 m^3-H_2 produced from the control electrode. In addition, anode surface characteristic is one of the most important factors to affect microbial attachment and electron transfer, so many treatments such as heat treatment and acid treatment[132] on anode materials surface have been used to improve the properties of

anode surface. It was reported that carbon mesh heated at 450 ℃ for 30 min could produce the maximal power density of 46 W/m^3, which produce 3% more power than that using cleaned carbon mesh treated with acetone in microbial fuel cell[133].

Moreover, microbial electrolysis cell reactor configuration, which is closely relevant to hydrogen production rate, has made great progress in both the traditional type (i.e., incorporated membranes) and membrane-free designs. More details about reactor configuration were reviewed elsewhere[134]. In microbial electrolysis cell, except exoelectrogens, there also exits methanogens which would consume hydrogen, so inhibiting the growth and activity of methanogens such as adding methanogen inhibitors [135], using low pH [136] and temperature [137] are helpful to improve hydrogen yields. Chae et al. [135] reported that adding 2-bromoethanesulfonate (286 μM) in microbial electrolysis cell could decrease methanogenic electron losses from 36.4±4.4 to 2.5±0.3%, i.e., CH_4 yield reduced from 145.8±17.4 μmol to 10.2±1.2 μmol, at the same time, hydrogen efficiency would be improved from 56.1±5.7 to 80.1±6.5%. In addition, harvesting hydrogen rapidly (e.g., use more efficient materials) is also helpful to enhance hydrogen yields.

Table.1. Endeavors that have been done to improve hydrogen yields from WAS.

Similarly to dark-photo fermentation combination system, the dark fermentation- microbial electrolysis cell two stages system is also constructed to improve hydrogen yields. In this system, scientist have found that acetate has the best conversion performance in microbial electrolysis cell compared to other dark fermentation products, such as propionic and butyric, so the ethanol type fermentation could be a more proper pathway in dark fermentation if this two-stages system is taken[138].

5. Research gap and perspective

Although numerous efforts have been performed, hydrogen production from WAS is still far from achieving at practical scales due to low hydrogen yield, high energy (chemical) input in WAS pretreatment and fermenter control, or cost-prohibitive materials in photo-bioreactors (or anodes). In view of the state of the art relate to hydrogen bioproduction from WAS, further research on the following perspectives is urgently needed:

(1) Many pretreatments were developed to promote sludge disintegration, but the utilization of sludge organics was still low due to WAS contains lots of refractory organics. Thus, more efforts are required to improve the biodegradability of WAS.

(2) In addition, the low activities and growth rate of hydrogen-production microorganism limited hydrogen generate rate and yield. More endeavors are essential to comprehensively study hydrogen-production microorganisms and improve their activities.

(3) Moreover, although all methods were used to improve hydrogen production, the hydrogen yield from WAS dark fermentation was still low due to the low energy conversion efficiency. Hence, more efforts are need to develop two-stage process (e.g., dark fermentation and MECs) to improve hydrogen recovery.

While continuing improvements in those aspects can be expected with the ongoing researches, it seems some of the challenges such as unstable performances and relatively high ratio of energy (or capital) input to energy output might also remain, making hydrogen production from WAS competitively unfavorable. According to the analyses, one major reason for the current situation is that previous studies mainly focus on the advancement of hydrogen production technology alone and lack rational integration of some available technologies. For example, Guo et al.[139] compared different pretreatment methods (e.g., multi-enzyme, heat and microwave pretreatments) and tried to find the best pretreatment method (heating pretreatment achieved the maximum hydrogen yield of 15.3 ml H_2/g VSS) but ignored other factors, such as fermenter control and WAS characteristic. Zhao et al.[93] investigated the effect of fermentation pH on hydrogen production and found the optimal constant pH of 10 (In this scenario, the maximal H_2 yield of 26.9 mL H_2/ g VSS could be achieved). However, other potential strategies were not considered as well. To further enhance hydrogen yield in a sustainable way, more appropriate strategies other than advancing hydrogen production technology alone should be therefore sought. Rational integration of the technologies available in both wastewater treatment and WAS treatment might be a more feasible avenue.

6. A future paradigm to improve hydrogen production

6.1. The hybrid process for the operation of WWTPs

It is widely acceptable that WWTPs are not only the places to purify sewage but also the facilities to

recovery energy and resource. The yield of each useful product (e.g., hydrogen) from WWTPs should be maximized, and meanwhile the operation input should be minimized as far as possible. In addition, the investment of WWTPs to recovery resource should be controlled at a reasonable level. Based on these principles, the combining dark fermentation technology with other available technologies including high-rate activated sludge process, mainstream deammonification, phosphate recovery, free ammonia (FA)-based sludge pretreatment and microbial electrolysis cells partially or completely might be a solution to accomplish desirable hydrogen production in sustainable ways. The schematic process of such combined strategy proposed is shown in Figure 3.

Figure.3. The conceptual operation of WWTPs with hybrid technologies for hydrogen and other energy recovery [140, 141].

In such WWTPs, wastewater is proposed to be cleaned by the adsorption-biodegradation (A/B) process, which is widely accepted as the most promising process that could achieve desirable nutrient removal and maximum energy recovery concurrently. Generally, most organic carbon is first adsorbed in the A-stage while the effluent with a low C/N ratio is further biodegraded in B-stage. In this hybrid system, wastewater is first fed to by a high-rate activated sludge reactor (called A-stage), where most organics are captured through various mechanisms such as bio-sorption and storage. Up to now, many methods including chemical, biological, or their combinations, have been explored for capturing organics at A-stage as much as possible [142, 143]. It is documented that >80% of organic carbons and >90% of phosphorus would be removed from a high-rate reactor through controlling solids retention times at 2.0-2.5 days and hydraulic retention times at 0.5-1 day [142]. Then, the effluent from the A-stage, containing low organic matters (i.e., low COD) but high ammonium, is further treated by mainstream deammonification (called B-stage) [144], while the captured organics are separated and passed onto hydrogen production units such as anaerobic fermenter for energy recovery. Compared with the conventional WAS, A-stage sludge is easier to be disrupted [142], which is beneficial to the subsequent hydrogen production.

To provide more soluble substrates for hydrogen production, pertinent pretreatment of sludge prior to hydrogen production is usually performed in the sludge treatment line. Although many pretreatment

methods were tested, most of them require considerable input of either energy or chemicals, as mentioned above. This is unsustainable in long-term operation and largely limits the applications in field situations. Here, FA-based sludge pretreatment may be a solution for sustainable operation. FA, which could be obtained from anaerobic fermenter effluent directly, is a waste-generated, renewable chemical that can be produced in situ in WWTPs as a byproduct of sludge treatment (Figure 3). Recent investigations demonstrated that FA not only facilitated the lysis of sludge cells but also promoted the biodegradability of organics released[145, 146]. Although FA is reported to cause a strong biocidal impact or inhibition on broad microbes, its inhibitions to hydrogen consumers were much severer than those to hydrolytic microorganisms and hydrogen producers, which benefited hydrogen accumulation [147].

After FA pretreatment, the pretreated sludge is suggested to be fed to anaerobic fermenter for the production of hydrogen and volatile fatty acids. Wang et al. reported that with an increase of FA from 34 to 254 mg/L, the maximum hydrogen yield from dark fermentation increased from 7.3 to 15.6 mL/g VSS without any addition of inoculum [147]. Zhang et al. demonstrated that when WAS was pretreated with 237.8 mg/L FA for 3 d, the organics released were largely enhanced, with the soluble COD of 3400 ± 120 mg/L and volatile fatty acids of 226.9 mg COD/g VSS being 4.5-fold and 2.7-fold that without FA pretreatment, respectively[148]. Then the fermented WAS is further fed to microbial electrolysis cell unit for secondary hydrogen production. In such processes, the accumulation stage of soluble organic matters and hydrogen production stage is separated, which is beneficial for microbial electrolysis cell to generate hydrogen. Although many components in WAS could use as substrates for hydrogen production in microbial electrolysis cells, volatile fatty acids are the preferred substrates that could achieve higher efficiency and hydrogen recovery [141]. In addition, the combination of dark fermentation with microbial electrolysis cell could also achieve cascade utilization of complex organics from WAS through the syntrophic interactions between fermentative bacteria and exoelectrogens. He et al. confirmed that the positive effects of such combination could enhance hydrogen production, as compared to microbial electrolysis cell alone[23].

And last, the effluent of dark fermentation-microbial electrolysis cell usually contains 200-600 mg/L ammonium and 100-300 mg/L phosphorus, which are mainly released from the disintegration of sludge cells [149, 150]. The phosphorus released can be recovered via either crystallization or precipitant as valuable

products. Phosphorus recovery from WAS, an alternative and renewable resource, is very significant, because the word's main P resource, i.e., phosphate rock, is non-renewable and may exhaust in next 100-250 years [149, 151]. Thus, this could not only fulfill world demand for phosphorus but also compensate for a part of WWTPs costs. In addition, phosphorus recovery from the fermentation liquid could further reduce its loading in mainstream wastewater treatment, which thereby benefits to acquire the cleaner effluent. The effluent of P recovery basin, which contains high levels of ammonium, is partially sent to the sludge pretreatment unit for free ammonia production. By adjusting proper pH and temperature, the desirable free ammonia concentration can be attained easily.

6.2. Benefits of the hybrid process

With a reasonable integration of various available technologies in WWTPs, it could achieve substantial technical, environmental and economic benefits. The hybrid technologies could use a minimal energy input to address some key questions occurred in current WWTPs, in particular, some key challenges faced by hydrogen production from WAS. Firstly, the A-stage could capture much organic matters to improve WAS characteristics. Generally, the destruction of WAS in anaerobic digester is around 35% whereas the corresponding value of A-stage sludge could achieve at 70% [152]. Secondly, FA pretreatment could efficiently break the rigid structure of WAS microbial cell to release more extracellular and intracellular organics for hydrogen production, which is usually thought to the rate-limiting step. In addition, FA pretreatment could efficiently inhibit hydrogen consumption, i.e., homoacetogenesis, methanogenesis and sulfate-reducing processes. Microbial electrolysis cell could further utilize most of the organics (e.g., volatile fatty acids, proteins and carbohydrates) left in the liquor of anaerobic fermenter to produce hydrogen. All
these aspects may enable hydrogen yield from WAS at desirable levels.

This hybrid technologies could also achieve considerable environmental benefits. WAS adsorbs and concentrates substantial exogenous pollutants such as heavy metal and micro-pollutants [153]. The improper disposal of sludge would make these toxic and harmful contaminants reenter natural environments, posing the serious threat to the ecological environment. Substantial reduction and reuse of WAS through this hybrid technologies is conducive to the final disposal of sludge and avoids the secondary pollution of the environment. In addition, the extensively use of hydrogen rather than methane as energy reduces carbon

dioxide production, thereby slowing down the greenhouse effect. Moreover, the recovery of P-based fertilizers from WAS reduces the exploitation of phosphate deposits, avoiding environmental damage [15].

In addition, such operational paradigm also has enormous economic benefits. For example, mainstream deammonification could save the extra addition of carbon source which are usually applied in conventional WWTPs to enhance nutrient removal [140]. Free ammonia is renewable and waste-produced, which could be produced *in situ* from sludge fermenter effluent. In comparison, other pretreatments require high chemicals or energy input [147]. Combination of dark fermentation with microbial electrolysis cell could improve hydrogen yields largely from WAS, i.e., recover more energy from sludge and create more values for WWTPs [147]. Moreover, the P-based fertilizers from the effluent of dark fermentation-microbial electrolysis cell through either crystallization or precipitant could enhance the income of WWTPs and offset part of the cost of wastewater treatment [15]. And last, such operation could also enhance sludge reduction, thus decreasing the cost of sludge transport and disposal [154].

Here, the economic benefit of this hybrid strategy with taking a 500 000 population equivalent (i.e., 100 000 cubic meters per day) WWTP was estimated as an example (please see Table S1 for details, Supporting information). In China, a conventional WWTP is often operated without the process of sludge anaerobic digestion, which account for >80% of total WWTPs. In such a WWTP, wastewater treatment would consume \$15000~\$20000 per day (i.e., \$5475000~\$7300000 per year), depending on the organic carbon, nitrogen, and phosphorus available in the wastewater. In addition the disposal of excess sludge would consume about \$10000 per day (i.e., \$3650000 per year). By incorporating these hybrid technologies into the conventional WWTPs, a same size WWTP could save the expense of using extra organic carbon (i.e., about \$547500), \$2254000), and also produce more $\sim 2.64 \times 10^5$ kg of hydrogen per year. It is estimated that this combined strategy would save ~\$3.83 million in a WWTP with a capacity of 10 m^3/d per year, as compared with the traditional operation paradigm (without anaerobic digestion process). Considering thousands of the conventional WWTPs existing in the world, this hybrid strategy should have huge economic benefits. However, it should be noted that the technical and economic merits presented here are only indicative, which need to be further clarified at real-world situations in the future.

recover about 2.26×10 tons of P-based fertilizers, reduce the portion cost of sludge disposal (i.e., about $2254000), and also produce more ~2.64×10^5 kg of hydrogen per year. It is estimated that this combined strategy would save ~$3.83 million in a WWTP with a capacity of 10 m^3/d per year, as compared with the traditional operation paradigm (without anaerobic digestion process). Considering thousands of the conventional WWTPs existing in the world, this hybrid strategy should have huge economic benefits. However, it should be noted that the technical and economic merits presented here are only indicative, which need to be further clarified at real-world situations in the future.

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6.3. Challenges of the hybrid process and future directions

Although many endeavors have been done, microbial electrolysis cell has not yet achieved practical application, which is mainly tested at lab-scale. Moreover, some other individual technologies of this hybrid system (e.g., phosphate removal based-high rate activated sludge process) are also not applied in real-world. Hence, scaling up this hybrid system would be therefore inevitable. This requires not only enhancing the size and treatment capacity of this hybrid system to a full-scale level, but also enabling desirable energy recovery. Thus, the biggest challenge for a practical situation is how to scale up the system size and guarantee the performances of each individual simultaneously.

Microbial electrolysis cells, which play important roles in this hybrid system, face some specific limitations in full-scale application. First, in a full-scale reactor, long distance between electrodes and pH gradient would lead to high internal resistances. Second, high capital costs of expense electrodes and membrane materials also limit its application. Third, there are still lacking comprehensive understanding of microbiology involved in microbial electrolysis cells including fermentative bacteria, exoelectrogens and biocathode microorganisms. By further designing microbial electrolysis cell reactors, developing new materials, deeply understanding and taking control over microbiology are expected to address these challenges.

Apart from improving the performance of individual units, rational integration of individual technologies is also important for achieving full-scale application of such hybrid systems. Mathematical modeling could be useful to understand, design, and operate such complex hybrid system. In addition, on-line monitoring and real-time process control is indispensable in practice. These monitoring parameters such as ammonium and nitrite concentrations in the nitritation reactor, FA concentration in pretreatment unit, and electrode potential in microbial electrolysis cells could use as valuable indicators to manipulate the whole system, ensuring the stability and robustness of such hybrid system.

Finally, it's crucial to acquire government support to develop such emerging technologies. The ignored environmental costs and emerging technologies, which have not yet benefited from application scale, lead to the expensive cost of recover energy and resource from WWTPs, as compared with the use of fossil-fuel energy. More investment in WAS treatment and disposal is required, particularly in the developing world. For

example, in China, the investment in WAS treatment and disposal (5.6 billion USD/year) was much less than that in wastewater treatment (68.8 billion USD/year) [155]. Government must try to put the emerging technologies, which could save energy even produce energy, into priority position through improvement of existing regulatory frameworks to contain or enhance the costs of greenhouse gas (e.g., carbon dioxide) emission and waste treatment and disposal. Government and relevant enterprises should also offer enough funds and suitable infrastructure to develop these emerging technologies available in real-world situations.

7. Conclusions

In this paper, the principles and potentials, microorganisms, possible technologies, and process parameters of hydrogen production were reviewed. Besides, both the opportunities and challenges of hydrogen production were analyzed. Microbial electrolysis cells were regarded as the prospective technology for hydrogen production due to high theoretical hydrogen yield and the ability of using varieties of organics as substrate. Nonetheless, the low organics utilization from WAS and the quick depletion of generated H_2 limited hydrogen accumulation. Based on systematic summarization and critical analysis, a possible paradigm for hydrogen production and wastewater treatment by rationally integrating dark fermentation with several other treatment technologies, including MEC, high-rate activated sludge process, mainstream deammonification, phosphate recovery, are presented. This hybrid process could achieve substantial technical, environmental and economic benefits. In addition, the limitations and future directions for application of such hybrid processes were discussed. More efforts are required to achieve the application of this hybrid process in real-world.

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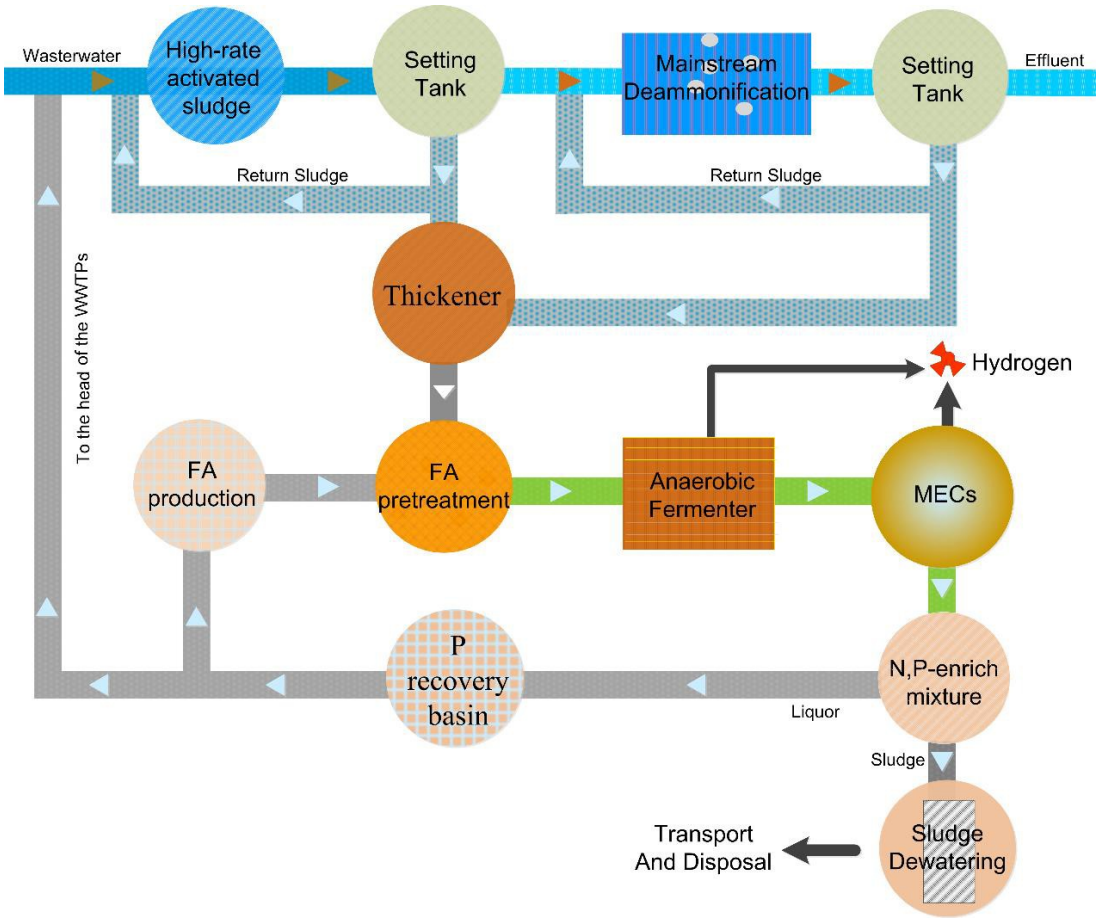
graphical abstract



Figure

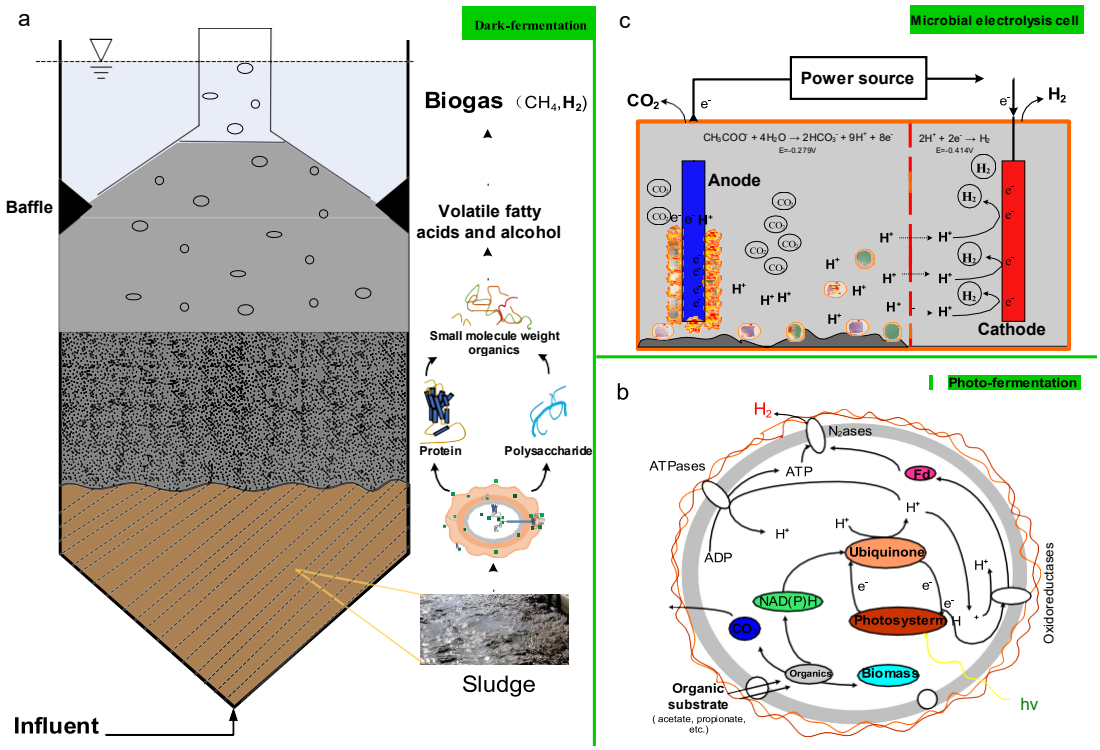


Figure. 1. Schematic diagram of hydrogen production from dark-fermentation (a), photo-fermentation (b) and microbial electrolysis cells (c).

Figure

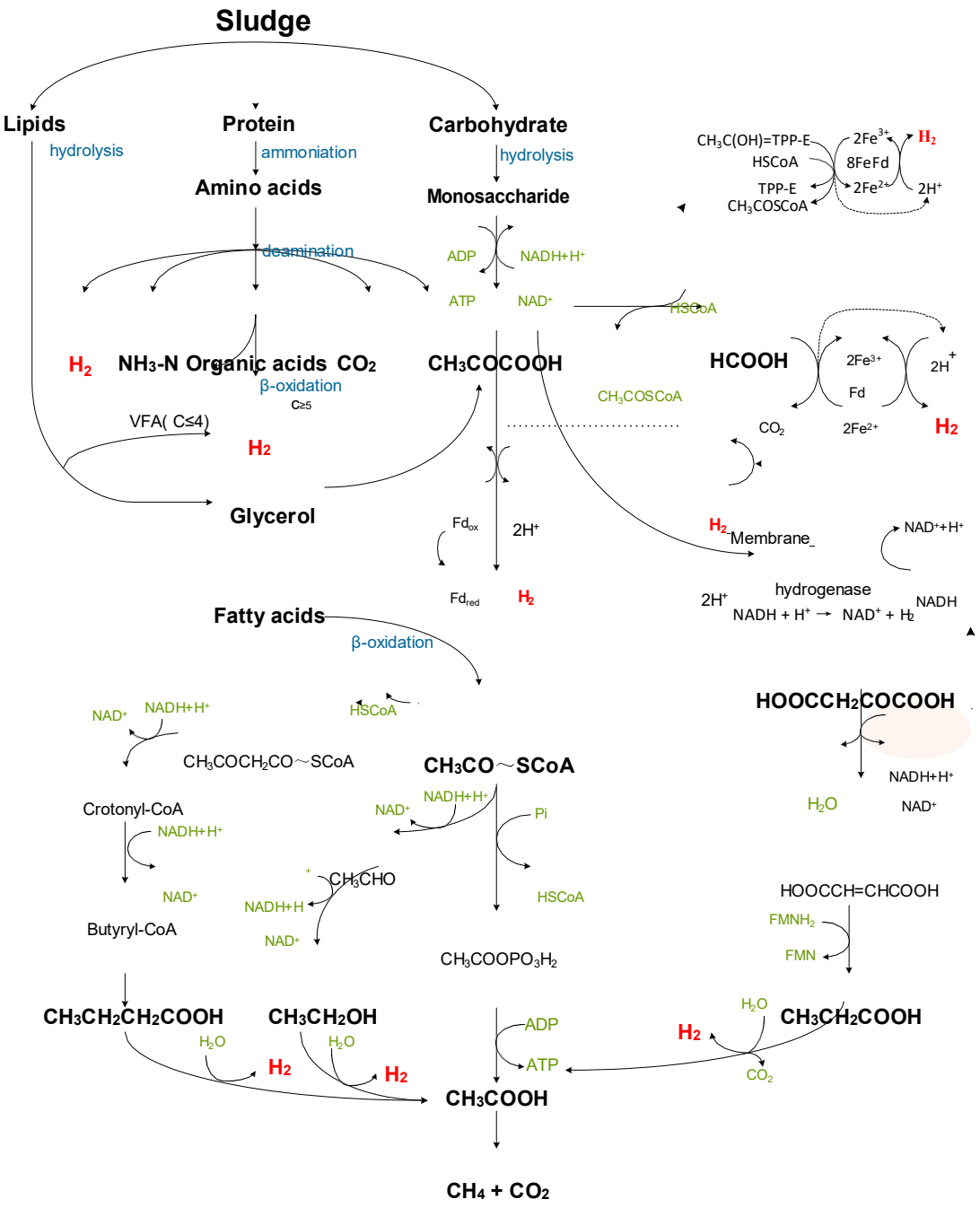


Figure. 2. The pathways of hydrogen production from sludge dark fermentation

Figure

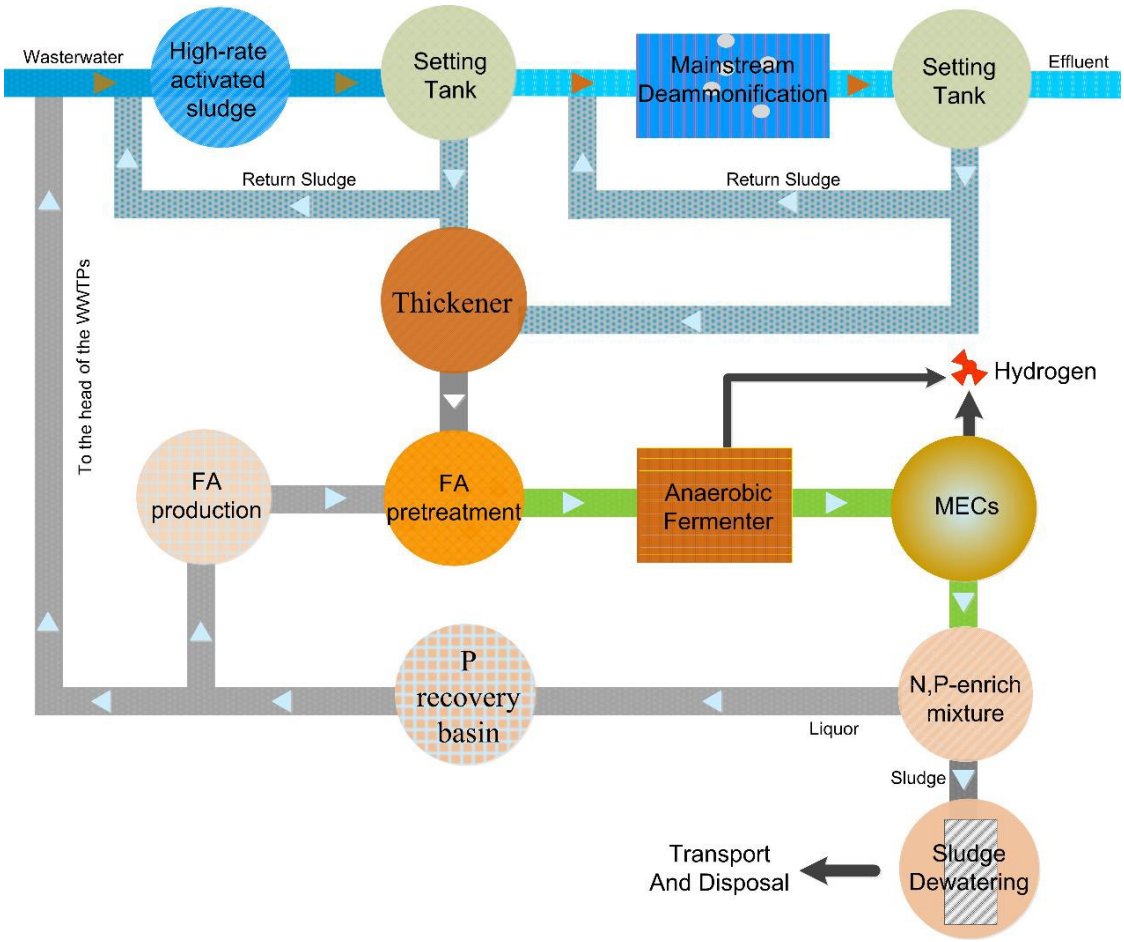


Figure. 3. The conceptual operation of WWTPs with hybrid technologies for hydrogen and other energy recovery.

Table

Table 1. Endeavors that have been done to improve hydrogen yields from WAS

Type of H_2 production	Regulating strategy	Brief description of operation	Result	Reference
Dark fermentation	Fermenter operation	Alkaline fermentation	Facilitated the disruption of WAS and suppressed the activities of hydrogen consuming microorganisms	[11, 93]
Dark fermentation	Fermenter operation	High fermentative temperature	Facilitated the hydrolysis of substrates and simplified microbial diversity	[95]
Dark fermentation	Fermenter operation	Shorter hydraulic retention time	Wash out H_2-consuming bacteria (e.g., propionic acid bacteria)	[94]
Dark fermentation	WAS characteristics	Increased PHA content	Enhanced the cell disintegration, the hydrolysis of organics, and the soluble protein conversion of non-PHA biomass	[22, 96, 98]
Dark fermentation	WAS characteristics	Co-fermenting WAS with other carbon-rich organics	Pertinent increases of carbon/nitrogen ratios was helpful to the conversion of protein	[97, 99, 100]
Dark fermentation	Pretreatment methods	Thermal pretreatment	Accelerated the disruption of floc structure and microbial cells.	[87, 91]
Dark fermentation	Pretreatment methods	Ultrasonic pretreatments	Accelerated sludge solubilization, and enhanced the release of biodegradable organics,	[89, 91]
Dark fermentation	Pretreatment methods	Alkaline pretreatment	Enhanced sludge disintegration and inhibited the activity of methaogens	[6]
Dark fermentation	Pretreatment methods	Free nitrite acid pretreatment	Promoted the biodegradability of organics released and inhibited activities of hydrogen consumers	[7, 90]
Dark fermentation	H_2-producer	Metabolic engineering	Modify hydrogen-producers in fermenters and enhanced hydrogen production by 3.5 fold	[102, 103]
Dark fermentation	H_2-producer	Constructed a microbial consortium	inoculating with three established bacteria and improved hydrogen production by 1.5-4 times	[101]
Photo fermentation	Fermenter operation	Temperature optimization	The suitable temperature ranges of photo-fermentative hydrogen generation were 31-36 ℃	[118]

Photo fermentation	Fermenter operation	High transparent materials	Glass and polymethyl methacrylate are considered the suitable materials for constructing photo-bioreactor	[74]
Photo fermentation	Fermenter operation	pH optimization	The suitable pH ranges of photo-fermentative hydrogen generation were 6.8-7.5	[118]
Photo fermentation	Light	Light wavelengths and intensities	The suitable light wavelength and intensity were in the range of 400-1000 nm and 6-10 klux, respectively.	[121]
Photo fermentation	H_2-producer	Strain improvement and replacement of nitrogenase with hydrogenase	Strains with a 41%-49% decrease in LH2 (LH: light-harvesting) content by UV irradiation produced 50% more hydrogen than untreated strains.	[119, 120]
Microbial electrolysis cell	Electrode improvement	Anodic material selection	Many new, cheap and effective anode materials (e.g., carbon nanotubes and graphene) were found	[125, 126]
Microbial electrolysis cell	Electrode improvement	Cathode material selection	many new, cheap and effective cathode materials (e.g., carbon nanotube and bio-cathode) were developed	[127, 128]
Microbial electrolysis cell	Electrode improvement	Anode modification	Many treatments such as heat treatment and acid treatment on anode materials surface have been used to improve the properties of anode surface (e.g., electron transfer).	[132, 133]
Microbial electrolysis cell	Reactor configurations	Incorporated membranes	Reduced the possibility of hydrogen diffusing to the anode to be used by electrogenic or methanogenic bacteria, and made the collected hydrogen more pure	[134]
Microbial electrolysis cell	Reactor configurations	Membrane-free designs	Lead to both high hydrogen recoveries and production rates	[134]
Microbial electrolysis cell	Microbial manipulation	Adding methanogen inhibitors	Added 2-bromoethanesulfonate in MEC enhancing H_2 production efficiency from 56.1± 5.7 to 80.1± 6.5%.	[135]