**Renewable Hydrogen Anaerobic Fermentation Technology: Problems and Potentials**

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**Abstract**

Hydrogen technology is essential to the decarbonisation of global economies because it addresses the variability and storage limitation of renewable energy. Several research literatures on hydrogen technology have focused on energy systems with minimum attention given to other fossil fuel driven sectors such as chemical and material production. For effective decarbonisation, the application of hydrogen in global economies must extend beyond the use of energy systems. Renewable hydrogen anaerobic fermentation is a suitable technology for converting the hydrogen substrate into gaseous fuel and precursors for material and green chemical production. The technology leverages on the well-established anaerobic digestion (AD) technology and can be selectively operated for a specific product. Although there are some problems associated with renewable hydrogen anaerobic fermentation, studies show different technological advancements in mitigating these challenges. This review focuses on the technological breakthroughs and limitations associated with renewable hydrogen anaerobic fermentation and provides insights on other products that could be derived from it, especially for a circular economy and the emerging market of green chemicals, sustainable agriculture, and bio-based product development.

**Highlights**

Challenges and opportunities for renewable hydrogen fermentation are introduced

Limitations and conditions for enhancing microbial hydrogen uptake are reviewed

Recent advances in increasing hydrogen gas dissolution are discussed

Potential applications for renewable hydrogen technology aside energy fuel is reviewed

**Keywords**

Anaerobic digestion

Biochemical

Bio-nutrient

Mass transfer limitation

Reactor configuration

Renewable hydrogen

1. **Introduction**

Renewable energy is a low carbon technology capable of facilitating the transformation of global energy systems to mitigate climate change. The potential of this technology to transform the future of global energy is dampened by its intermittent supply which has an unbalancing effect on the power grid and cannot provide constant energy supply and large storage [1]. These gaps can be bridged with the use of hydrogen as an energy carrier because hydrogen enables renewable energy transition, particularly for the difficult high-grade heat sectors such as transport and industry [2]. During periods of low energy consumption, excess or surplus renewable energy can be stored as hydrogen which can be used in periods of high energy consumption to supplement other energy sources hence balancing the power grid [2]. However, limiting the application of hydrogen to supplement other energy sources is not economically viable because the cost of storing and transporting hydrogen gas is expensive [3] . According to Schiebahn [4], diversifying the market for renewable fuel from electricity to other sectors like transportation has more economic potential. It is therefore imperative to direct resource and research beyond energy production and focus on other valuable products and precursors for green chemicals and bio-materials [4]. Currently, the energy and chemicals derived from fossil fuels are expected to continue to grow by more than 300 % by the end of the century [5]. Scarlat [6] reported that about 8.7 % of renewable raw materials were used for green chemical production in 2011 and this is expected to grow to 30 % by 2050. This projection precipitates the need to efficiently process hydrogen using the renewable hydrogen anaerobic fermentation, (RH2AF) technology based on existing industrial infrastructures for anaerobic digestion (AD).

 Renewable hydrogen (H2) anaerobic fermentation is simply the biological conversion of hydrogen as a gaseous substrate into methane, precursors for green chemicals and other valuable products. The conventional AD system can only attain about 55-60% of CH4 (g) and other chemical products [7]. However, to eliminate the excess carbon dioxide and increase the biogas energy density, an in-situ or ex-situ hydrogen substrate can be injected into the biological process [8, 9]. An established approach for increasing biogas production from AD is the utilization of hydrogen as a co-substrate [7-12]. Although the conversion of hydrogen to methane involves energy loss, it increases the energy density of the biogas. This process has been demonstrated in the literature using both ex-situ (matching biogas with H2-substrate) and in-situ (direct injection of H2-substrate) configuration [7, 13]. Irrespective of the approach, the technology maximised the conversion of available carbon to increase methane or acetate yield [7, 11, 12]. The conversion of excess carbon dioxide into methane will enhance the emerging biogas to the grid connection and reduce the cost implication of biogas upgrading system [13].

RH2AF has also benefited from a plethora of research focusing on several advancements in hydrogen gas dissolution, reactor geometry, establishing suitable operating conditions and as a co-substrate [7, 8, 10-12, 14-17]. Other advances such as co-digestion of hydrogen gas with the organic substrate in an in-situ fermentation operation and extended gas-liquid contact times for high rate reactors such as trickle bed and fixed bed reactors have been recorded in literature [17, 18]. Like the renewable energy discussed above, most of the RH2AF research has also focused on energy production. It will be more difficult to sustain a RH2AF which is solely for energy production without government incentives [19]. This review paper aims to investigate how RH2AF process can further the boundary for additional value recovery. The challenges and advancements in operating a RH2AF system are also discussed to identify the benefits of the system as it contributes to realizing a greener economy are also discussed.

1. **H2 -Substrate Uptake**

Renewable H2 uptake via anaerobic fermentation occurs at a stage in the biological process and in an AD system, this occurs in three main pathways to produce either a chemical or a gaseous product (Fig 1). There are three main pathways for H2 uptake in the AD system to produce either a chemical or gaseous product (Fig 1). The uptake of H2 occurs naturally during the AD process mainly as counter measures to regulate the hydrogen partial pressure of the system[20]. Hydrogen is naturally produced during the acidogenic phase of the AD process and if not consumed, the H2-partial pressure of the system will increase and inhibit the cleavage of simple organic molecules [20]. The first pathway as shown in Fig 1 is the conversion of the H2-substrate into acetate [21]. Apart from the production of acetic acid, the H2-substrate can also be used to produce other metabolic products such as methane and hydrogen sulphide. The conversion of H2 and CO2 into CH4 has been well documented in the literature [7-12].

The success of the fermentation process relies on the solubility of the H2-substrate and availability of microbial biomass. The H2-substrate is problematic, partly because of its poor solubility in water [8]. Poor solubility limits the mass transfer of H2- substrate from gas to the liquid phase of the medium thereby limiting the conversion efficiency of the process and microbial growth [22]. Several studies on biological gas treatment have identified the mass transfer of H2 substrate and microbial biomass as important parameters capable of influencing the biological uptake of H2- substrate [22-25]. A few of the quickest ways to determine whether the H2- substrate uptake of an AD operation is mass balance or kinetically limited is to carry out the following tests in isolation: (i) increase the microbial biomass, (ii) reduce the operating temperature (iii) check the gas treatment efficiency at different inlet loading and (iv) increase the gas velocity [26, 27]. For instance, when a change in the H2- substrate input results in an increase in biomass concentration, then the operation of the system is expected to be kinetically limited. This is because, the poor dissolution of the H2- substrate in a solely gaseous feed AD system will result into low metabolic rate and stress on the microbial biomass. However, if the increase in H2- substrate does not result in higher microbial biomass under favourable operating conditions, then the process is mass transfer limiting. There are several approaches recorded in literature on how to mitigate these limiting effects and enhance the uptake of the H2- substrate [7-12, 16]. The following section focuses on the dissolution of H2-substrate and several reported limitation and approaches to mitigating these challenges.

* 1. **H2-Substrate Dissolution**

The anaerobic uptake of H2-substrate is only possible when the gaseous substrate is readily available as a dissolved gas in solution and this is realised after overcoming the mass transfer constraint [28]. The transfer of H2-substrate into the liquid phase has been well described using the two-thin film theory [29]. The H2-substrate is transferred from a rising bubble into the liquid phase and finally diffused through the cell membrane of the microbial cell [28]. According to the two-thin film model in Fig 2, H2-substrate can only solubilize in water after successfully overcoming the resistance created by the liquid film (concentration of liquid phase, CL < concentration at intermediate phase, Ci). It should be noted that gases which are more soluble in water will experience more resistance at the gas film and vice versa for less soluble gases (Fig 2). The H2-substrate will experience more resistance in the liquid phase due to its relatively low solubility in water. After the successful adsorption of H2 into the liquid phase, it becomes readily available for microbial uptake. The dissolved gas diffuses through the cell membrane of the microbes. However, for biofilm and other immobilised cell systems, the diffusion limitation between the biofilm and medium environment has to be weakened to avoid additional resistance to the adsorption of H2 into the individual microbial cell [30]. This is because the substratum in which the cells are anchored adjust connective transport and increase the diffusion time which is longer than dispersed cells [31]. To overcome mass transfer limitation for microbial uptake of H2- substrate, several studies have been carried out and reported in the literature [7-12, 16]

This challenge has been tackled in research via reactor reconfiguration and adjustment of operating conditions (Table 1). This is because, conventional reactor design such as continuous stirred tank reactor (CSTR) is marked unsuitable for achieving the mass transfer rate required for an efficient H2–substrate uptake, partly because of low H2 retention time necessary for microbial uptake [32, 33]. This set back is common with the CSTR model but can be overcome through high-speed liquid agitation which requires additional energy input to achieve an efficient H2-substrate dissolution rate. According to Bredwell [23], the energy required to achieve an efficient dissolution of the gas substrate via medium agitation and microbial biomass distribution is unsustainable on a commercial scale. Apart from the associated cost of continuous medium agitation, the risk of damaging the microbial cells is higher and could further reduce the uptake of H2-substrate. To address the cost and problems associated with high-speed mechanical mixing, a low energy system operated with immobilised microbial cells, otherwise called high rate reactors have been developed and evaluated [8]. High rate reactors lack mechanical mixers hence the energy input is relatively lower than the CSTR model and is often used for treating low solid effluents [34]. High rate reactors are often impregnated with cell immobiliser to facilitate biofilm formation and retention. This high load of microbial biomass will drive faster uptake of gaseous substrates and depending on the geometry of the reactor, this can increase the retention time of the H2-substrate and the contact time with microbial cells [34]. While high rate rectors reduce washout due to biomass retention, some are also prone to clogging and channeling problems which can eventually lead to pressure loss. To overcome clogging and channeling, maintaining a thin layer of biofilm is effective [35] but as the biofilm layer toughens, diffusion limitation may set in (Fig 2).

 In addition, the reactor geometry of high rate reactors reduces homogeneity an essential feature for faster distribution of substrates for microbial uptake [36]. An example of such a reactor is the fixed bed model which does not encourage the mixing of gas and this is essential to facilitate substrate uptake. An upgraded version is the trickling bed model with mixing mechanisms, but the contact time between microbes and H2- substrate is too short to improve uptake [16].

 A proposed advancement to a biofilm reactor system is the hollow fibre membranes [12]. Past findings have demonstrated the impact of membrane types (hydrophobic and hydrophilic), membrane configuration (close and open end) and membrane porosity on overcoming mass transfer limitation of H2 dissolution [36, 37]. Hollow fibres have been shown to increase the solubility of H2- substrates for improved diffusion into the microbial cell but like fixed bed [12] reactors, hollow fibre reactors are susceptible to clogging. In a recent study by Strübing [35], the thermophilic operated trickle bed reactor outperformed thermophilic hollow fibre (79% CH4 at 9.6 m3CH4/(m3·d)) and fixed bed reactor (75% CH4 at 6.4 m3CH4/(m3·d)) with maximum methane production rate of 98% CH4 at 15.4 m3CH4/m3d [38, 39]. Savvas [16] showed that biofilm impregnated plug flow reactor is an improvement on hollow fibre, fixed and trickling bed reactor and a methane evolution rate of 40 v/v/d was achieved. The reactor improved both the H2–substrate residence time and the microbial contact time. This configuration should eliminate clogging but will require a significant energy input to facilitate gas-liquid mixing and recirculation.

1. **H2–Substrate Uptake Conditions**

The dissolution of the H2-substrate does not translate into an effective microbial uptake as there are several operating conditions that must be considered and operational regimes that must be established to ensure maximum H2 conversion rate. In the next section, emphasis will be made on the effect of microbial inoculum, gaseous substrate, product, redox potential, temperature, and pH on microbial uptake of H2-substrate.

* 1. **The effect of inoculum selection**

The biological uptake of H2 to produce a chemical or gaseous product is mediated by specific groups of microorganisms present in the fermentation culture [40, 41]. These microbes can be sourced from a mixed or isolated pure inoculum. The seeding stage for H2–substrate uptake using a pure inoculum is easier to operate because of its predictable metabolic pathway and performance rate of the microbes [40, 41]. On the other hand, to adopt a mixed culture, it is essential to have an enrichment stage that selects the desired microbes. The mixed inoculum approach to seeding for H2–substrate uptake facilitates the growth of different types of microbes and this makes the process robust and less vulnerable to microbial contamination [16, 35]. Mixed inoculum is abundant with facultative anaerobes which can help maintain anaerobic conditions during oxygen contamination and a strong redox potential within the system. Several commercial AD systems for treating different wastes such as sewage, livestock manure, food waste, and agricultural residues are rich in H2 utilising microorganisms and can serve as a source of mixed inoculum [16, 17, 42]. AD set up for treating food waste is expected to be the most abundant with hydrogen utilising microbes because it is abundant in syntrophic acetate oxidising bacteria and hydrogenotrophic methanogens which are essential in mitigating ammonia stress a condition prominent with food waste fermentation [43, 44]. Aside from the flexibility of using mixed culture for product specific enrichment, the diverse microbial community can help mitigate against contamination and removal of unwanted by-products. In the case of acetate build up in a hydrogenotrophic dominant reactor, the presence of acetoclastic methanogen can help convert excess acetate to methane (Fig 1). Initiating an H2–substrate uptake process with a robust inoculum source is essential but it does not guarantee the overall stability of the system in the long run. The stability of the H2 anaerobic fermentation can be easily influenced by changes in operating parameters and other factors that will be discussed in the subsequent sections.

* 1. **The effect of gaseous substrate**

The choice of gaseous substrates for H2 -substrate uptake depends on the approach used in the fermentation process. For in-situ systems involving matching solid and gaseous substrates, the fermenter is already fed with the solid substrate and the CO2 produced is only matched with H2 –substrate [11]. For example, Tao [17] demonstrated that in-situ RH2AF can be achieved by matching CO2 with H2-substrate and recorded volumetric methane production from 0.86 to 1.51 v/v/d. The in-situ system is suitable for simultaneous biogas production and upgrade yet presents a higher risk to the buffering stability of the system. To avoid this occurrence, it is important to match the CO2 production in a way that avoids excessive CO2 scrubbing and alkaline buildup [17]. The key here is to establish a suitable H2-substrate loading rate for both biomethanisation and CO2-acid buffering. Unlike the in-situ system, ex-situ operations often require a mix of H2, CO and CO2 substrate in the appropriate ratio either for the chemical or gaseous product [7, 12]. In the ex-situ regime, the gas mix can be used to manipulate the pH of the medium, especially when the alkalinity of the medium is high [16].

 The H2-substrate loading, or flow rate is an important parameter to monitor and aside its influence on the buffering capacity of the system, it can select the dominant metabolic pathway and eventual products. A low loading rate often favours the hydrogenotrophic methanogens for methane production because of their moderate affinity for  H2-substrates [45]. Results from Cord-Ruwisch [46] showed that at high H2 levels of 520-950 ppm, the homoacetogens were more competitive for the H2-substrate compared to the hydrogenotrophic methanogens. Acetate accumulation has been observed during the high level of H2-substrate injection using enriched thermophilic hydrogenotrophic methanogens in a hollow fibre membrane reactor and when using hollow fiber membrane [12, 47]. The only downside to the continuous production of acetate in an homoacetogenic dominated fermenter is its potential to drive down the pH of the fermenter due to the accumulation of organic acid [48]. This can be mitigated by acetate recovery and an understanding of the kinetics of acetate production will provide a better approach to stabilise the system [47, 49]. For a hydrogenotrophic methanogenic dominant fermenter, the presence of acetoclastic methanogens within the microbial matrix will facilitate the conversion of acetate produced into CH4.

* 1. **The effect of gaseous Products**

Products such as CH4, H2S, NH3, CO2, alcohol, organic acid, and H2O can be produced during RH2AF. Irrespective of the dominant metabolic pathway, for a mixed culture operation, by-products are often more than one even though the dominant pathway accounts for the highest conversion rate. The downside with by products is the interferences they have on the production process, but this still needs to be further investigated. Some of these impacts are expected to be specific to the selected fermentation process. For instance, the impact of water vapour production does not depend on the metabolic pathways as water is produced for every molecule of organic acid or methane (Fig 1). The implication of water as a product is the diluting effect on the nutrient-rich medium and the possibility of biomass washout for dispersed microbial cell reactors [50]. On the other hand, the effect of CH4 production is peculiar to hydrogenotrophic dominant fermentation processes because it can interfere with the mass transfer of H2-substrate into the medium. This can be attributed to the proximity between the solubility of the hydrogen and methane in water (Fig 3). A study has shown that CH4 can reduce the H2 partial pressure and decrease hydrogen mass transfer flux [51] although the effect of product gases are expected to be more prevalent in a reactor agitated through gas substrate/product or operating medium. This could be explained by the poor solubility of H2 gas and the competition with other gaseous products, which are more soluble with less resistance to the liquid film (Fig 3). Similarly, acetic acid production is expected to drive the pH of the medium acidic in a homoacetogenic fermentation process. However, if a wastage regime is put in place or an alkaline co-substrate is used in the case of an in-situ system, then the pH can be regulated to avoid short retention of CO2, a usefulsubstrate for methane and organic acid production [11].

There are other benefits of the gaseous products to the H2-substrate uptake operation. Gaseous products such as CO2 provide buffering against alkalinity, H2S increases the redox potential of the system while NH3 is an important source of nitrogen for microbial growth [51-53]. Regulating the alkalinity of the H2-substrate uptake system is important because of the continuous stripping of CO2 from solution and this explains why CO2 should be further applied to regulate pH changes [16]. Luo and Angelidaki [12] recorded reactor failure when the pH increased to 8.3 as a result of high alkalinity. They also showed that co-digestion with an acid substrate like whey permeate could be used to counteract the effect of increase in alkalinity. There are other situations where the pH will reduce due to the production of organic acid and ammoniacal nitrogen or co-digestion with the protein-rich substrate (such as slurry) should mitigate pH drop [54]. Although the threshold for ammoniacal nitrogen inhibition of homoacetogen is yet to be established since ammonia toxicity has mostly been observed with methanogens [55]. It is therefore important to acclimatize the mixed culture for a metabolic pathway that will yield the desired product.

* 1. **The effect of redox potential**

Redox potential is the measure of the oxidizing or reducing capacity of a chemical solution. It is an essential parameter for H2-substrate uptake (Kumar et al., 2010; Søndergaard, 2009). In a well-oxidized system, the redox potential will be positive while in a reduced environment like the AD system the redox potential will be negative [56, 57]. The redox potential is a suitable parameter for enriching mixed microbial biomass to select for a favourable chemical or gaseous product. According to Vongvichiankul [58], the redox potential of -284 ± 32.7 mV is most suitable for acidogenic microbial community responsible for the formation of acetate through H2-substrate uptake. However, the production of CH4, a gaseous product from H2-substrate uptake will require a much lower redox potential with a minimum and maximum value of (-387 and -452 mV) [56]. An unsuitable redox potential level specifically influences the biochemical activity of the microorganisms and for H2-substrate uptake, the electron transport system will be disrupted. The role of lower redox potential is well described in the Wood-Ljungdahl pathway, which represents the main biochemical stages for H2-substrate uptake. The Wood-Ljungdahl pathway is a mediator for chemiosmosis and free energy harvesting. Free energy is harvested as hydrogen ions/protons diffuse into the microbial cell to produce ATP from ADP [59]. The formation of ATP depends on the concentration of hydrogen ion and sodium motive force because this will drive the transportation of electron from CO2 (CO2 reduction) [60]. The electron transfer reaction is the main function of Nicotinamide adenine dinucleotide (NAD), a coenzyme. On accepting the electron, the NAD is reduced to form NADH which drives the formation of methyl H4MPT from the methenyl group, an essential step necessary to produce CH4. A lower redox is needed to attain an NADH: NAD+ ratio, required to drive the biochemical reaction [45]. H2S is a reducing agent and is produced during sulfidogenesis, one the many pathways for hydrogen utilisation (Fig 1). Aside the sulfidogenic activity, facultative anaerobes from a mixed culture system can also remove excess oxygen to further increase the redox potential.

* 1. **The effect of pH**

Fermentative metabolites like VFAs, ammoniacal nitrogen and bicarbonates contribute to the fluctuations in the pH value of the AD system [61, 62]. pH is always a critical factor in the AD system because it could shift the metabolic pathway and change the product formed. For H2-substrate uptake, acidic pH is most favourable for a chemical product such as acetate while neutral or slightly alkaline pH is most suitable for the gaseous product (CH4). For chemical products, Ju [40] found that acidic pH of 5.9-6.6 was favourable for acetate production and a dominant homoacetogenic microbial community. Though acidic pH is favourable for the chemical product there are some consequences to this operating condition, especially for the H2-substrate system. As acidity increases, the probability for the conversion of carbonate into carbon dioxide also increases. When this happens, a weak buffering capacity and low carbon situation is created [42, 55]. Apart from organic acid production, there are findings that show that the degree of acidity in the fermenter can also select for a specific type of an organic acid (Kim et al., 2011; Temudo et al., 2007). For instance, it has been reported that under the alkaline and acidic condition, butyrate production is more favourable because the NAD/NADH ratio in the AD system decreases [63, 64]. Although some of the findings were not consistent with Jiang [65] which showed that acetate was a dominant product at pH 5 followed by butyrate and propionate. There is no consistent report on the influence on pH on the composition of VFA production as there are other interfering parameters. However, for the gaseous product, alkaline pH of 7.2-8.0 have been reported to be most favourable for CH4 production via hydrogenotrophic methanogenesis [8, 16].

* 1. **The effect of operating temperature**

The role of temperature on the AD system is three dimensional as it affects the microbial growth rate, hydrolysis of complex material and enzymatic action [66]. There are three main types of operating temperature used in the AD process and they provide selective pressure for thermophiles above 50 °C, mesophiles around 30-45 °C and psychrophiles below 20 °C [67]. For H2-substrate uptake, the temperature can be used to select for a desired metabolic pathway, particularly when using a mixed culture. For example, there are findings showing that lower operating temperature increases the competition between H2 utilizing methanogens [68-70]. At a mesophilic temperature of 37 °C, sulfate-reducing bacteria (SRB) can outcompete hydrogenotrophic methanogens while at the thermophilic condition of 55 °C, the hydrogenotrophic methanogens are most dominant microbial community [71]. Ho [72] showed that increasing the thermophilic temperature to 65 °C favoured CH4 production through acetate oxidation and hydrogenotrophic methanogenesis while increasing the efficiency from 60% to 100%. The relevance of temperature in AD operation is not limited to selecting a suitable microbial community. The thermophilic temperature has been reported to be suitable for increasing the microbial growth rate which further reduces the hydraulic retention time of the system [7, 38, 39, 72]. However, the two downsides to operating at thermophilic temperature is the relative increase in the parasitic load and low diversity of microorganisms [73, 74]. Table 1 showed that thermophilic operation of H2-substrate uptake recorded higher gaseous product values when compared to the mesophilic condition. This shows that the operating temperature of the reactor is critical for selecting a dominant metabolic pathway and increasing the metabolic activity of the H2 utilising microbes.

1. **H2–Substrate Benefits**

H2-anaerobic fermentation has several benefits and provided its application is extended beyond energy recovery, it can be used to produce a precursor for green chemicals, bioplastic, and biological soil nutrient. In addition, H2-substrate can be used to mitigate ammonium toxicity in conventional AD process and carbon sequestering. The benefit of using H2-Substrate is discussed in the following sections.

* 1. **As a mitigator of climate change**

CO2 is one of the primary greenhouse gas in the earth’s atmosphere with a high global warming potential and a fossil fuel based global economy is one of the main contributors to this carbon footprint and the main driver of climate change [75, 76]. Studies show that atmospheric CO2 concentration has been on the increase with the current value of 411.04 ppm [75, 76]. This could be attributed to the continuous dependence on coal and crude oil for energy supply. However, there has been an increased awareness of environmental issues and climate change leading to extensive research aimed at finding alternative uses for carbon dioxide [77-79]. Numerous technologies for CO2 utilization are currently under research and development including membrane separation, adsorption and mineralization [76] but H2-substrate uptake via CO and CO2 as carbon sources for gaseous and chemical products provides a sustainable approach [80]. The use of homoacetogenic and methanogenic microorganisms for biofuel production from a mixture of CO2, CO and H2 has received a lot of attention in recent years [81-83]. Acetogenic bacteria such as *Clostridium carboxidivorans* and *Clostridium ragsdalei* can metabolize H2 and CO2 to produce chemical products such as acetate, ethanol and other products [81-83]. The overall reaction of CO and CO2 conversion into a chemical products is represented in equations 1-4 via the Wood-Ljungdahl pathway and presented in Fig 4.

$6CO+3H\_{2}O=C\_{2}H\_{5}OH+4CO\_{2}$ Δ*G*o = − 217.4 kJ mol−1 (1)

$6H\_{2}+CO\_{2}=C\_{2}H\_{5}OH+3H\_{2}O$ Δ*G*o = − 97 kJ mol−1 1 (2)

$12CO+5H\_{2}O=C\_{4}H\_{9}OH+8CO\_{2}$ Δ*G*o = − 486.4 kJ mol−11 (3)

$12H\_{2}+4CO\_{2}=C\_{4}H\_{9}OH+7H\_{2}O$ Δ*G*o = − 245.6 kJ mol−11 (4)

* 1. **As a mitigator of ammonia** **toxicity**

Ammonia toxicity is most prevalent with methanogens and is triggered by fermentation of protein-rich feedstocks causing inhibition to methane production [84, 85]. The increase in ammoniacal nitrogen is accompanied by rising propionic acid concentration, with progressive instability observed from 2.5 gN/l [86]. A recent report showed that when the total ammonia nitrogen approached 5 gN/L the pH of the reactor dropped to 6.5 with a significant rise in acetic acid concentration [87]. The pattern of VFA accumulation indicates a failure of acetoclastic methanogenesis and acetate oxidation. The traditional measures for ammonia toxicity include temperature control, microbial acclimation, co-digestion, and dilution [88, 89]. Co-digestion is the only traditional method still widely used by AD operators. High energy food waste is often co-digested with cardboard or paper waste [90]. However, co-digestion relies on accessibility to the appropriate co-substrates, which is a problem for AD operators [91]. In recent times, a more sophisticated development such as stripping ammonia using water electrolysis or electrodialysis, hollow fibre membrane contactor, zeolite, activated carbon and enriching the inocula with syntrophic acetate-oxidizing bacteria have been investigated [92-98].

With exception to enriching with inocula with syntrophic acetate-oxidizing bacteria, the recent advances in techniques for counteracting ammonia inhibition is not reliable because of cost, complexity and risk of second-hand pollution remain high. Although the enrichment process with syntrophic acetate oxidation is promising, it can only thrive in a system with a surplus population of hydrogenotrophic microbes (Fig 6). This process would also require the injection of an external H2 substrate into the setup over time whenever an inoculum supplement is not used or not available. The enrichment process will help quicken and drive metabolic activity from microbial groups that are less susceptible to ammoniacal nitrogen. The syntrophic acetate-oxidizing bacteria (SAOB) and hydrogenotrophic methanogens are more tolerant to ammoniacal nitrogen and their metabolic pathways are different from acetoclastic methanogenesis [43, 44]. The activity of the SAOB is often triggered by an elevated level of ammoniacal nitrogen to convert acetate into H2 and CO2 which is then further reduced into CH4 by hydrogenotrophic methanogens [99]. However, the association between SAOB and hydrogenotrophic methanogens is not immediate and sometimes need to be facilitated through nutrient supplementation [100, 101]. Studies have shown that the addition of certain trace metals such as selenium, iron and tungsten can facilitate the transition of methane production pathway to mainily SAOB and hydrogentrophic methanegensis [100, 101]. The addition of trace metals is often associated with the production of metal sulphide which reduces its bioavailability and can only be compensated with high doses. A high dose of metals can render the digestate unfit for land spreading because of the PAS 110 regulation. An alternative method is to feed H2-substrate into the AD system to build up the hydrogenotrophic methanogens. According to Wang [102], the hydrogenotrophic methanogens are essential for methanogenesis during ammonia stress. It is therefore necessary to build up their microbial population. In doing so, the microbial biomass required to sustain the second phase of the SAOB pathway is adequately available to enable a successful shift to the SAOB mediated community in the event of elevated ammonia concentration. An active population of hydrogen utilizing methanogens is required to facilitate the activity of acetate-oxidizing bacteria [103].

* 1. **Precursor substrate for bioplastic production**

Bioplastics have the potential to replace traditional hydrocarbon derived plastic material because it is biodegradable and creates a solution to the environmental problems associated with traditional plastic products. Bioplastic production has continued to attract increasing scientific interest, especially when 4.9-12.7 million tonnes of hydrocarbon derived plastic products were estimated to have entered the ocean as at 2010 and expected to increase by an order of magnitude by 2025 [104, 105]. Bioplastic is derived from renewable source biomass sources like vegetable oils, cellulose, corn starch, organic acids and alcohols (Fig. 7). However, because bioplastics can be made from a variety of biomass materials, the nature of the material influences the physicochemical properties of the bioplastic product. Depending on the substrates used, there are different types of bioplastic materials and the common types are polylactic acid (PLA), poly-3-hydroxybutyrate (PHB), polyamide 11 (PA11) and Polyhydroxyalkanoates (PHA) [106-109]. Currently, PLA and PHA are produced on an industrial scale although cost remains an issue as the price is still relatively higher than traditional plastics [110]. The cost of material has been identified as one of the contributors to the low economic value of biopolymers and the adoption of waste derived VFA as a precursor for biopolymer production has been proposed and still at the research and development stage [111-113]. These types of biopolymer are called PHAs (PHB, PHBV). PHA derived bioplastic is UV stable and can withstand high temperature perhaps this explains why this biodegradable polyester is attracting more research interest with the potential to replace traditional plastics products [114]. When compared to other bioplastic materials, the PHA is the cheapest to produce because it can use low-cost feedstock, eliminate sterile condition and rely on the efficient selection of enriched PHA-accumulating organisms [115].

VFA is a widely used substrate for PHA production and it has been well researched [116, 117]. The substrate is an intermediate product of the AD process, specifically the acidogenic phase of the biological reaction. Depending on the operating conditions and other parameters, the VFA produced ranges from acetate, butyrate, and propionate [118]. These are the common types of VFA and the most abundant during the AD process. The VFA production process is not only limited to the use of organic substrates, but studies also show that H2-substrates can be used for VFA production [21, 119]. This is achieved through homoacetogenesis [120].

$4H\_{2}+2CO\_{2}=CH\_{3}COOH+2H\_{2}O$ Δ*G*o = − 105 kJ mol−1  (5)

$4H\_{2}+2HCO^{-}\_{3}+H^{+}=CH\_{3}COO^{-}+2H\_{2}O$ (6)

[121]

The application of H2-substrates to produce acetate precursor for PHA production can positively impact the hydrogen economy [122, 123]. H2-substrate production from wind and solar technology are intermittent and expensive to store but can be stored as PHAs (Fig. 8). The application of hydrogen as a precursor substrate for PHA production is advantageous because it eliminates some of the challenges encountered in the use of biomass material. Unlike the H2- substrate, other feedstock require a pre-treatment stage to meet the specification for PHA production [124-126].

**Precursor substrate for Lipid production**

Microbial lipid is facilitated by the activities of a group of microorganisms called as oleaginous. These microorganisms are able to accumulate lipids of 20-70% of their total cell biomass [127]. The lipids are stored intracellularly as a reserve supply of energy and nutrients. Several studies have shown that the lipid accumulating ability is triggered by short of supply of phosphorus or nitrogen depending on the microorganisms [128-130]. The microbial lipid produced by oleaginous microorganisms provides an alternative way of producing green chemical products such as biofuel and omega 3 fatty acids without putting pressure on the finite human resources [131]. Although the practicability of the production of microbial lipids on a commercial scale has been questionable for some time because of the cost of feedstock required to sustain the process [132]. Among the various possibilities, the application of VFAs stand out and has gained the attention of several types of research [132, 133]. VFAs are used as carbon sources although the composition influences the yield and composition of lipid produced and there are several studies to show that acetate is more favourable [134, 135]. The production of VFA using organic waste materials often result in mixed VFAs which are predominantly, acetate, butyrate, and propionate [136]. On the other hand, homoacetogenesis of H2-substrate produces predominately acetate which has been identified as a more suitable carbon source for lipid production. There are limited studies however on the effect of acetate on lipid production by different groups of oleaginous microbes.

* 1. **Precursor substrate for digestate nutrient stability**

The plant available nutrient content of the digestate makes it an attractive soil conditioner and provided it meets the quality protocol standard, it can be spread on agricultural land. The nutrient composition of a digestate is attributed to the feedstock and AD process operation. During the fermentation process, the nutrients are mineralized through microbial activities to form inorganic compounds that are readily available for plant uptake [137]. Through chemical and biological manipulation of the AD process, to promote acidogenesis and as opposed to methanogenesis in conventional AD schemes, organic waste material can be broken down into fermentative intermediates such as reactive nitrogen (N) and phosphorus (P) and the risk of nutrient loss can be minimized [138]. For an homoacetogenic regime, the build-up of acetate is expected to reduce the pH of the medium to 5.5-6.0 which provides a suitable condition for digestate storage. Maintaining an acidified digestate is essential because it helps reduce nutrient loss during handling and drying of digestate material [139, 140]. Ammonia volatilization appears to be unavoidable, especially during thermal drying and this can trigger the shift in dynamic equilibrium toward ammonia release [141]. An additional benefit from acidifying digestate is the increase in solubility of phosphorus. Phosphorus availability increases with a decrease in the pH of the medium and for an homoacetogenic AD system, acidification can be easily achieved [142, 143]. Plants can only absorb P as free orthophosphate ions and the rate of phosphate uptake decreases as the pH of the medium increases [142]. Acidification of digestate can also be achieved using other chemical agents such as a sulphuric, chloric and nitric acid [143]. The sulphuric acid is capable of degrading the organic matter while the nitric acid promotes the N2O emissions [144, 145].

1. **Conclusion**

The technology for H2 production and AD technology is mature, developing and already commercialised. Several advancements in mass transfer, reactor configuration and suitable operational conditions have been recorded for RH2AF. However, the commercialisation of this technology is still dependent on the availability of cost effective methods that will maximise the use of hydrogen for energy production and other valuable products. It is difficult to sustain a RH2AF for solely energy production without government incentives. Although there is still room for advancement in technology, a wider application strategy could help drive more innovation and development. Application of the technology as a precursor for other viable products such as bioplastic, bio-nutrients, bio-lipids, and carbon sequestration are highly needed to help increase widespread application especially for a circular economy and the emerging market of green chemicals, sustainable agriculture and bio-based products development. The diversification of RH2AF technology could help attract investment and development.

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