Review

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Recent developments in biohythane production from household food wastes: a review

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Abstract

Biohythane is a hydrogen-methane blend with hydrogen concentration between 10 and 30% *v/v*. It can be produced from different organic substrates by two sequential anaerobic stages: a dark fermentation step followed by a second an anaerobic digestion step, for hydrogen and methane production, respectively. The advantages of this blend compared to either hydrogen or methane, as separate biofuels, are first presented in this work. The two-stage anaerobic process and the main operative parameters are then discussed. Attention is focused on the production of biohythane from household food wastes, one of the most abundant organic substrate available for anaerobic digestion: the main milestones and the future trends are exposed. In particular, the possibility to co-digest food wastes and sewage sludge to improve the process yield is discussed. Finally, the paper illustrates the developments of biohythane application in the automotive sector as well as its reduced environmental burden.

Keywords: biohythane; Anaerobic Digestion; Household Food Wastes; Review; Dark Fermentation; Applications; recirculation;

1. Gaseous biofuels form biomass: the advantage of biohythane

Hydrogen and methane are widely used in chemical and process industries (Ellaban et al., 2014) because of their high calorific value of 143 kJ/g and 55 kJ/g for hydrogen and methane, respectively (Roy et al., 2016), (Sharma et al., 2015). Hydrogen is recognised as a clean energetic fuel since it does not release CO_2 in the atmosphere during combustion (Roy et al., 2016). Unlike other fuels, methane and hydrogen combustion does not release any NO_x (nitrous oxide) and SO_x (sulphur dioxide), the major contributors to air pollution (Gaffney and Marley, 2009). Methane combustion, on the other hand, still generates the greenhouse gas CO_2.

The term hythane has been coined in the early 90s by the Hydrogen Component Inc. (HCI), a company which was conducting several studies concerning the feasibility of the use of a blend of Compressed Natural Gas (CNG) and hydrogen as a fuel for internal combustion engines. They showed that the lean burn of mixture of hydrogen (7% by energy or 20% by volume) and CNG can reduce the emission of pollutants (mainly NO_x) into the atmosphere, while maintaining the energy efficiency of CNG (Mishra et al., 2017). The use of this mixture does not require storage system neither particular changes both in the CNG engines and infrastructures. As a result HCI patented this mixture and the commercial name of this fuel was Hythane®.

Hythane displays remarkable advantages over CNG: it is a better vehicular fuel thanks to the presence of hydrogen, which improves the performance as far as the flammability range is concerned: hydrogen, in fact, it is characterized by a flame speed which is 8-fold that of methane (Moreno et al., 2012). Hydrogen stimulates methane combustion in the engine and, being an excellent reducing agent, contributes to a better catalysis also at lower exhaust temperatures, (Roy et al., 2016). From the environmental point of view, hythane has the great advantage to reduce the greenhouse emissions into atmosphere because of the hydrogen presence which reduces the carbon content of this gaseous blend. To accentuate the environmental friendly nature of hythane, the investigation of renewable sources for hydrogen and methane production has been encouraged in the last decade. In fact, hythane is currently produced in intensive way from no sustainable processes: for example hydrogen can be obtained as main gaseous output from syngas production and methane reforming (Liu et al., 2018). The term "biohythane" has started to be used to indicate hythane produced from organic substrates, such as food wastes and agriculture residues (Preeti Mishra et al. 2017), (Liu et al., 2018) by Anaerobic Digestion (AD) technology conducted in two separated phase. In this way, the production of biohythane, in comparison of the methane production by AD in a single stage, allows the reduction of the overall required fermentation time and, consequently, of the working volume of the reactors (Si et al., 2016). In addition, the attractiveness of producing hythane through a two-stage AD process, rather than the production of hydrogen alone, stems from the fact that the latter is not economically sustainable due to the low production rates and yields of dark fermentation (Valdez-Vazquez and Poggi-Varaldo, 2009) (Abreu et al., 2016). Theoretically, a hydrogen yield of 4 mol/mol glucose can be achieved through dark fermentation. However, the hydrogen yield is seldom above 2 mol/mol glucose due to the limited metabolic fluxes and the generation of higher fatty acids (such as propionate and butyrate) and alcohols (Zhang et al., 2011), which means that only about 7.5–15% of the energy contained in organic wastes is converted to H_2 (Si et al., 2016a), (Mamimin et al., 2017), (Luo et al., 2017).

Biohythane production advantages are well described by Life Cycle Assessment (LCA) models, which emphasise that single and two-stage AD processes allow for the reduction of the wastes led to landfills and the release of greenhouse gases (methane, carbon dioxide) to the atmosphere. AD, in fact, leads to a reduction of $CO_{2\ eq}$ emissions by 90%, compared to the scenario where organic substrates are released in urban landfill or simply disposed on soil (Franchetti, 2013). This is attributed to the fact that, when the biogas is burned to produce electricity, all the CH_4 is converted to CO_2. On the contrary, with conventional uncontrolled disposal systems, the methane from wastes degradation, is all emitted unaltered into the atmosphere (Coast et al., 2013), increasing significantly the equivalent CO_2 emissions, as methane is more than 20-fold effective as a greenhouse gas than CO_2.

Recent LCA researches on biohythane production demonstrates that a two-stage AD process, such as that used for the production of hythane, produces a methane amount similar to the single-stage configuration. However, the simultaneous production of hydrogen, which is a carbon free fuel, allows to reduce by an additional 10% the overall CO_2 equivalent emissions of the process (Coast et al., 2013), (Franchetti, 2013). Lastly, the solid-liquid output from biohythane production is represented by digestate, which is more stable because of the lower amount of acid, nitrogen and carbon content, since the major part of the organic matter used for the AD has been already converted into biogas. In this way, apart from global warming potential (GWP), additional important LCA parameters show a better performance than single-stage AD. For example, Acidification and Eutrophication, which measure respectively the soil and air acidification, which leads to acid rain and a vegetation growth reduction (Ecoinvent database 2013) , as well as a negative accumulation of nutrients (mainly N and P compounds) in the environment (Stranddorf et al., 2005).

2. Main aspects of the biohythane production process

Anaerobic Digestion for the production of biogas, can be conducted in a single reactor (single-stage AD), or in two separate tanks (two-stage AD). Biohythane production may be carried out readily by a two-stage Anaerobic Digestion process. Over 17,000 AD full scale plants are present in Europe: 10,000 of which only in Germany, for a total generation of about 8,293 MWel (European Biogas Report, 2015). However, it is estimated that less than 1% of these plants are represented by full scale two-stage AD process (Roy et al., 2016). The main reason is the higher capital costs required by a two-stage bioreactor system.

Two-stage AD occurs through the physical separation of hydrolysis and acidogenic (also called dark fermentation) which allow to obtained biogas rich in hydrogen, from the acetogenic and methanogenic phases which results in a biogas rich in methane. These two stages are characterized by the prevalence of different microorganisms which require specific operating conditions for their activity. Biohydrogen production via dark fermentation is carried out by various anaerobic bacteria, particularly *Clostridium spp., Thermoanaerobacterium spp., Enterobacter* and *Bacillus* (Reith et al., 2003), (USEPA, 2017) as a result of a chain of microbial activities and provided that environmental conditions, such as the pH and temperature, are favourable. Instead, biomethane production is supplied by more sensitive microorganisms, such as *Methanosarcina barkeri and Methanococcus,* which are characterised by a less thick cellular membrane and, consequentially, request more stable temperature, pH conditions, and a less vigorous agitation (Battista et al., 2016). Another difference is represented by the growth kinetics: acetoclastic methanogenic bacteria have a growth rate 4-5 times smaller than acetogenic microorganisms. This means that the methanogenic phase is the limiting control step of two-stage AD, whenever of course the hydrolysis step is not limiting.

There is a complementary activity between the two microorganisms' species: methanogenic ones use the hydrogen produced in the acetogenic step, keeping its concentration low. Then the formation of methane and carbon dioxide takes place, under strictly anaerobic conditions. A recent study investigated on the microorganisms communities involved in the biohythane production, focusing the attention on the difference between the single stage and the two stage AD (Si et al., 2016b). Compared with mono stage system, the biohythane process had higher COD removal and energy recovery. In particular it was observed the improvements of dark fermentation's performances which influence positively the followed methanogenic phase. The analysis of microbial communities revealed the variation of biochemical pathways: a reduction of acidogenesis bacteria in biohythane system has been revealed, while the amount of acetogens (*Syntrophaceae, Syntrophomonadaceae and*

Desulfovibrionaceae) was higher. The archaea community remained stable, and mainly consisted of acetoclastic methanogens from family *Methanosaetaceae* (Si et al., 2016).

As reported, biohythane production includes the dark fermentation and the acetogenic- methanogenic phase, each one being conducted by specific microorganisms, which require different optimal conditions. Consequently, the biohythane production requires a sensitive balance between pH, temperature, partial pressure, HRT, OLR and nutrients. The most relevant operative conditions of these parameters have been summarized in Table 1

*** Insert Table 1***

Micronutrients, all the chemical substances which at low concentrations are necessary for the bacterial metabolism, are essential in the optimization of the two stages of AD. Table 2 summarizes the concentrations of some chemical substances which are able to sustain, to inhibit or to stop AD (Battista, 2015).

*** Insert Table 2***

After their production, Hydrogen and methane need of cleaning and upgrade phases in order to remove carbon dioxide, acid compounds, water, ammonia and siloxanes from biogas. The most widely used techniques are water scrubbing (41%), followed by chemical scrubbing (22%) and pressure swing adsorption (PSA) (21%). Membrane separation, which is receiving much attention in the recent years, has been employed by 10% of the upgrading units (IEA, 2014).

3. Feedstocks for biohythane production

3.1 Biohythane from household food waste

In the last decade, the increasing interest in the good performances of biohythane has attracted the attention on this technology with a consequent increase of laboratory and pilot-scale experiences. In particular, two types of wastes have been chosen by scientific community for the experimentation: food wastes and sewage sludge. The reason is due to their large worldwide availability: it has been estimated that the 72% of the single stage AD plants treating waste, actually treat food wastes, for a total of 8 million tons digested within the EU countries, and another 28% of the AD plants receive sewage sludge as feed (Micolucci et al., 2016). The huge amount of food wastes is easily explicable considering that it is produced all along the food life cycle: 42% of it is derived from households (HFW, which represents the fraction treated along this review work), 38% from food processing, and 20% from the whole chain. In the food industry, waste generation comes from processing raw vegetable and animal materials into foodstuffs (Baiano, 2014). AD represents a good solution to reduce the environmental impact of HFWs: in fact, most of the HFW ends up in landfills, making the disposal practice unsustainable, terminating the option for resource recovery and leading to the release of greenhouse gases (Sarkar and Mohan, 2017). The first AD plant treating HFWs was opened in the USA in 1939. In Europe a large number of AD facilities came in operation only over the past few decades. In particular, Germany, Switzerland, Denmark and Italy are the European pioneers of AD technology with over 150 million tons per year of HFWs treated. The estimations report that biogas production capacities will grow up to 20 x 10^9 m^3 by 2030 (Kharthikeyan et al., 2017). According to their provenance and the period of the year in which they are produced, HFWs present a different chemical composition and physical characteristics. Generally, HFWs are richer in proteins during the winter season, while more carbohydrates are present in the summer, when the meat consumption decreases in favour of more fruits and vegetables (https://www.arpal.gov.it). Although carbohydrates and proteins are ideal substrates for hydrogen and methane synthesis, cellulose and hemicellulose are more recalcitrant without a prior pre-treatment stage (Seghezzo et al., 1998), (Si et al., 2016). Recent studies focused on the controversial effect of lipid content in HFWs. Wang et al. (2014) found that when lipid concentration is higher than 25% *w/w* the methane formation is inhibited for several reasons: formation of long chain fatty acids and consequent increasing of HRT, as consequence of the more recalcitrant nature of these compounds. In addition they observed the formation of oil flocs which are responsible to microorganisms' adsorption, reducing the organic matter degradation and the AD process yield. But other researchers (Yong et al., 2015; Jin et al., 2016) recorded a higher biogas production at high lipid content condition. Wu et al (2016) understimated the importance of the lipids effect on AD. In fact, even if a reduction of the proteins and carbohydrates' degradation can be possible in presence of high lipids concentrations, they concluded that process yield is fundamental due to the C/N ratio of the HFWs mixture. Nitrogen is the fundamental element for the correct metabolism of all microorganisms. Fruit wastes are characterized by higher C/N ratio (> 20) than meat products (< 5) and their mixing ratio with other food products influences the final C/N ratio of HFW. The typical C/N of HFW varies between 14 and 37 (Karthikeyan et al., 2017). These ratios are too low compared to the ideal for the AD for the methane production which requires a C/N ratio of about 50. On the contrary, low C/N ratios are fit for hydrogen and VFAs synthesis.

The pH is another key parameter to be controlled, especially if treating heterogeneous substrates such as HFWs; in fact, in order to obtain the best performances of the hydrogenase enzyme activity, the pH must range between 5 and 6 (optimum value at 5.5). There are several strategies available to control the pH, for example the addition of alkaline substances or the use of high protein-containing HFWs (Lay et al., 2003). It was verified that the alkalies addition can affect the operation conditions, such as OLR and HRT. Algapali et al. (2016), in particular, suggested to increase the alkaline substance's dose when HRT is low, condition which favours the acidification and consequentially the methanogens washout from the reactor. It is also fundamental to consider that the ions dissociation from alkalies, such as Na^+ and Ca^{2+}, can become toxic for microorganisms as reported in Table 2.

An advanced strategy for the pH control is the recirculation of the digested effluent, rich in buffer agents, from the methanogenic phase to control the dark fermentation pH. The recirculation allows to exploit the residual buffer capacity (ammonium, bicarbonate) of digestate to supply nutrients and dilute the feedstock used (Reith et al., 2003), (Kataoka et al., 2005). Cavinato et al. (2011) and Micolucci et al. (2014), explained the reason for the good performances achieved through the recirculation strategy: VFAs accumulation during dark fermentation generally decreases the pH below the optimal value which is set at 5.5. They exist in an un-dissociated form and partly in a dissociated form, depending on the pH. Un-dissociated acids have a greater inhibitory effect because they penetrate into cells due to their lipophilic properties, where they denature cell proteins. The reaction medium recirculation from the second stage of AD, rich in ammonia and other buffer agents, has a doubly favourable effect allowing for the pH control in the first stage. It has been observed that recirculation is particularly impacting when HFWs were used as substrates (Kobayashi et al., 2012), while no improvements were observed with the sewage sludge recirculation (Cheng et al., 2011). It is fundamental to remark that an excess of the recirculation may be toxic for AD. Gottardo et al. (2017) demonstrated how working with an excessive recirculation may result in accumulation of ammonia in the system with consequent inhibition of both methanogenic and the hydrogenogenic processes. This theory has been also confirmed by Wu et al. (2018) who showed that, even if recirculation increased alkalinity within the reactor, keeping a good pH for methanogens, an excessive ammonia accumulation, which could cause the biochemical pathways alteration of the hydrolytic bacteria and methanogens. Conversely, too low recirculation ratios may be insufficient to control the pH of the reaction medium where the hydrogenogenic process occurs. To find the optimal ratio, Micolucci et al. (2014) treated HFWs at pilot scale plant composed by a 200 L reactor for the dark fermentation and A 380 L for the second stage of AD. They saw that it is not convenient to keep constant the recirculation along the whole AD time, mainly when the system has not still reached steady-state conditions. The use of a variable recirculation flow makes possible to control the whole process, preventing ammonia inhibition in the second stage reactor and avoiding VFAs accumulation in the dark fermentation reactor. A good way to regulate the recirculation flow is provided by the monitoring of VFAs / alkalinity ratio which should not overcome the 0.3 value, when inhibition starts to occur (Battista et al., 2015).

Lately, a novelty relating to the literature is the adoption of statistical methods in the study of the process variability. It allows the possibility of preventing instability situations in the system (Micolucci et al., 2018) and in this case determining the right range of recirculation ratio to be implemented (Gottardo et al. 2017). Based on this parameter, the recirculation ratio can be modified in the optimal range 0.45-0.65. In this way Micolucci et al. (2014) and Gottardo et al. (2017) achieved a very high biohythane production of almost 3 L H_2 per L reactor per day with a volumetric concentration of 7% hydrogen, 58% methane and 35% of carbon dioxide. Thus, recirculation can be considered a fundamental parameter in the control and optimization of biohythane production from HFWs and sewage sludge because of the ammonia capability to buffer the first reactor.

Temperature and the reactor configurations are other two important factors which can require different conditions between dark fermentation and methanogenic phase. Ventura et al. (2014) optimized the two stage AD adopting mesophilic and thermophilic conditions respectively for acidogenic and methanogenic phases. Bong et al. (2018) explained that it permits the optimization of hydrogen and methane production, remarking the need to avoid the sudden variations of temperature which can inhibit the methanogen metabolism. The increasing in biohythane yield has not been recorded when the two different temperature ranges have been inverted, that means thermophilic and mesophilic conditions for dark fermentation and methangenic stage, respectively, as happened in the study by Xia et al. (2018) who recorded almost the same yield for the two stage AD and the AD conducted as single stage on HFWs.

Regarding the biohythane production from HFWs testing different reactor configurations, the possibility to adopt the Continuously Stirred Tank Reactor (CSTR) and the Anaerobic Fixed Bed Reactor (AFBR) for dark fermentation and the methanogenic phase, respectively, has been investigated. The CSTR configuration is the most used not only because its design simplicity but also for the possibility to have a simple washout of some AD bacteria, such as methanogens during dark fermentation, by low HRT or high OLR (Angeriz-Campoy et al., 2015). On the contrary, the second stage of AD is characterized by more vulnerable and slow methanogens (Battista et al., 2016). A valid alternative for these more sensible microorganisms are AFBRs able to immobilize them on porous supports assuring a large surface area, high loading capacity, resistance to hydraulic and organic shocks and no need of mixing systems (Van Lier et al., 2015). Taking into account these consideration Yeshanew et al. (2016) tested a two stage AD adopting a CSTR for the dark fermentation and an AFBR for the methanogenic phase. The reactors were operative for 200 days and were fed with HFWs whose composition was prepared considering an average of the European HFWs: 79% of vegetables and fruits, 5% of cooked pasta and rice, 6% of bread and bakery, 8% of meat and fish and 2% of dairy products. Hydrogen production had a good performance in CSTR recording a maximum of 115 L H_2 per Kg VS fed. During the test it was observed the important role of pH, able to shift the production from hydrogen to solvents when it drops below 4.5. The pH decrease, in fact, can be caused by a VFAs accumulation due to too short HRT or high OLR which accelerate their synthesis. It represents a serious problems during the second stage of AD which results to be inhibited by high VFAs concentration when it is conducted in a CSTR, where a minimal HRT of 15-30 days is recommended (Schmidt et al., 2014). Instead, Yeshanew et al. (2016) demonstrated the AFBR's resistance to wash out even in presence of a HRT of 1.5 days with a methane production higher than 330 L CH_4 per Kg VS .fed.

3.2 Household food waste codigestion with sewage sludge

Codigestion of substrates having different origin and composition is a common practise to optimize the two stages of AD. It is a solution to supply a lack of macro or micronutrients in a specific substrates, which are useful for the microorganisms' metabolism. In addition, codigestion is a good strategy to provide to the reactors a continuous feed also in presence of seasonality substrates. HFWs are often codigested with sewage sludge in order to improve the biohythane production. The term 'sewage' refers to the wastewater produced by a community, which may originate from three different sources: (a) domestic wastewater, generated from bathrooms and toilets, and activities such as cooking, washing, etc.; (b) industrial wastewater, from industries using the same sewage system for their effluents (treated or not), and (c) rain-water (Seghezzo, 1998). The wastewater's origin is fundamental to predict the nutrients content. Goberna et al. (2018) tested different typology of sewage sludge observing very variable chemical elements concentrations and chemical properties: total organic carbon (21.5–49.5%), nitrogen (2.4–8.1%) and phosphorous (6–20 g kg^{-1}), as well as water contents (70–87%), electrical conductivity (0.7–4 dSm^{-1}) and pH values (6.4–7.9). Moreover, it was found that heavy metals concentrations, responsible of the AD inhibition, are higher in industrial sewage sludge than municipal one. Instead, the microbial community composition seemed to not be influenced by the sewage sludge origin, but it depended on the pretreatments nature, by the HRT duration and the ammonia concentration (Goberna et al., 2018). Sewage sludge has attracted increasing interest in biohythane fermentation because of its huge amount, stable source, low cost and high organic content (> 60% of dry matter) (Yang et al., 2015). It has been estimated that in the EU the per capita production of sewage sludge is about 90 g per person per day, which means 10 million tons for year (Davis, 1996). Sewage sludge can be treated by aerobic and anaerobic technologies: the aerobic way requires a too high cost to supply air inside the reactor, and it is not economically sustainable (USEPA, 2017). On the contrary, sewage sludge AD is a cost-effective technology to valorise sludge for bioenergy but hydrogen fermentation of sewage sludge alone is usually not efficient. The hydrogen yield from sludge normally ranges between 10 and 90 mL/gVS$_{added}$ (Table 3), which is much lower compared with other feedstocks such as macroalga (29.5–158 mL/gVS$_{added}$), crude glycerol (29.2–219.1 mL/gVS$_{added}$), and HFW (100-

250 mL/gVS$_{added}$) (Yang et al., 2015) (Kim et al., 2011). The essential constraint for hydrogen fermentation of sewage sludge is its low carbohydrate content (<10% of dry weight), which cannot provide sufficient substrate for hydrogen producers. Furthermore, the C/N ratio of sewage sludge is commonly in the range of 4-9, which is much lower than the optimal value for hydrogen fermentation (12-17) (Yang et al., 2015). Yoon et al. (2018) reported that with low *C/N* ratio, ammonia concentration can rise in the process of anaerobic digestion, causing, as already described, the microorganisms inhibition. Instead HFWs have a high C/N (usually superior than 20) and consequentially can be used for codigestion with sewage sludge.

Co-fermentation for hydrogen and methane production has some unique advantages including better substrates condition, the dilution of inhibitors and more balanced nutrients condition (Xie et al., 2017).

*** Insert Table 3***

Table 3 underlines how hydrogen and methane yields depend essentially on the feedstock nature. The low performances in biohythane production from sewage sludge are attributable to the low C/N ratio with consequent high ammonia formation (Mamimin et al., 2017), (Khongklian et al., 2015). Although ammonia is an essential nutrient for bacterial growth, it may inhibit AD, mainly the methanogens during the second stage of AD. Several mechanisms for ammonia inhibition have been proposed, such as a change in intracellular pH, an increased maintenance energy requirement, and the inhibition of specific enzyme reactions. Ammonium ions (NH_4^+) and free ammonia (FA) are the two principal forms of inorganic ammonia nitrogen in aqueous solution. FA has been suggested to be the main cause of inhibition because it is freely membrane-permeable. One of the best strategies for increasing the sewage sludge AD yield is represented by codigestion with HFWs (Cheng et al., 2016) because of their high carbohydrates content. Maragkaky et al. (2018) observed that a 5% addition of HFWs in sewage sludge comported an improving in biogas production of about 150 *v/v*, passing from a daily biogas production of 230 mL/L to more than 570 mL/L with a methane content from 60% to 70% *v/v*. The organic matter removal, expressed ad VS, passed from an efficiency of 45% to about 55%, which means the obtaining of a more stabilize digestate. Thus, the authors demonstrated that, even if HFWs additions comport an increasing of OLR, microorganisms had good adaptability to a higher load. Several studies reported that codigestion did not cause variation in VFAs concentration when HFWs additions happen in little ratios. In this condition, VFAs are not present in the reaction medium, which means they are completely converted in biogas at the end of the AD process, demonstrating the codigestion's good performances (Maragkaky et al., 2018), (Yoon et al., 2018). At the same time, it was observed that if the HFWs (mainly with high lipid substrates, as previously commented) in codigestion with sewage sludge content is elevated, VFAs may become inhibiting for the methanogenic phase (Nguyen et al., 2015). Anyway, it is not possible to determine the limit beyond which HFWs addition compromise the stability of AD because of the heterogeneity of the substrates and of the microorganisms involved in the process (Riviere et al., 2009). Biohythane production, as commented, is a complex process which involves a series of synergetic biochemical reactions, whose stability of depends on a synergistic effort of two groups of microbial communities. A recent study (Xu et al., 2017) investigated on the interaction between the microorganisms involved in the two stage of AD, affirming that acetogenic microbe convert intermediate products from hydrolysis into acetate and H_2 through β-oxidation process in dark fermentation. Finally, acetate and H_2 are used by methanogenic Archaea to generate methane. In particular, the methanogenic Bacteria and methanogenic Archaea are more sensible to operative variations (Jang et al., 2016). Riviere et al. (2009) found that changes in HFWs and sewage sludge ratio complicates the interaction of these microorganisms. In fact, even if they observed a strong positive correlation between archaeal ratios and methane yield, with the increasing of HFW content, an excess of HFWs addition resulted in a considerable decreases in archaeal (from 20–25% to 15–20%), suggesting an evident inhibiting effect. Xu et al. (2017) affirmed that individual parameters, such as TVFAs/TA ratio or pH, are often unable to timely indicate the beginning of the process' inhibition and suggested to monitor the archaeal numbers for evaluate the AD effective status.

Lastly, Chiu and Lo (2018) conducted a LCA to biohythane production from codigestion of HFWs and sewage sludge confirming the beneficial environmental and energetic effects of the two stage of AD. Anyway no improvements has been showed between the case of two stage of AD, where HFW and sewage sludge were treated as separated substrates, and the case where they were codigested in the same reactor. Their study, in fact, exhibited similar CO_{2eq} reduction of about 40 tons for the entire life cycle of the reactors.

4. Recent and innovative strategies for biohythane production

With the increasing interest in biohythane, innovative techniques to improve the overall yield of the two-stage AD have been investigated. As previously commented, the hydrogen molar yield is lower than the theoretical one. An alternative which is receiving great interest by the scientific community is represented by the combination of dark fermentation with a photo fermentation process. Contrary to dark fermentation, where hydrogen production occurs under anoxic or anaerobic conditions, during photo-fermentation, nonoxygenic photosynthetic bacteria use sunlight and biomass to produce hydrogen. This way, the products from dark fermentation can be further converted to hydrogen through photo-fermentation according to the following reaction (Akroum-Amrouche et al., 2013) (Chen et al., 2010):

$CH_3COOH + 2\ H_2O \rightarrow 2\ CO_2 + 4\ H_2$

The main disadvantages of photo-fermentation are the high costs and small efficiencies of photochemical reactors and the large amounts of required nutrients by the anoxic microorganisms (Abreu et al., 2016).

In alternative, biohythane production can be increased by the reinforcement of the pretreatments phase. Borg et al., (2018) reported the recent interest for the thermal pretreatments which consists in the substrates heating at high temperature (>120°C) for a time period varying according the nature of organic matter, usually about 30 minutes. It was demonstrated that this strategy is particularly efficacy for lignocellulosic materials, since cellulose solubilisation results to be greatly improved. Sarkara and Mohan (2017) achieved significant results with an aeration stage before the feeding. HFWs were treated through 400 L oxygen injection for 60 minutes. Molecular oxygen present in air suppresses the methanogens, which compete in hydrogen conversion to methane (not desired in dark fermentation stage), reducing the overall hydrogen production. In addition, pre-aeration helps the hydrolysis of complex food waste to simpler molecules. The pre-aeration operation resulted in 97% improvement in hydrogen conversion efficiency and 10% in VFAs production. The major drawback of this technique is the possible oxygen infiltration in the next methanogenic stage of AD, conducted by very oxygen-susceptible microorganisms. Lastly, one of the last frontiers in pretreatments for biohythane production, is represented by lipid extraction from HFWs by a methanol-chloroform solution. Lipid, as seen, favours the long fatty acids production which can inhibit hydrogen and methane production. The extracted lipid have been introduced to a secondary anaerobic bioreactor fed by sewage sludge, where the acids production does not represent an possible inhibiting factor, as sewage sludge organic content is lower than HFWs (Algapani et al., 2017).

Other ways used to improve the biohythane process from HFWs have been tested For example, working in thermophilic conditions accelerates microorganisms' kinetics, allowing the HRT reduction, the working volume of the reactor and, consequently, the capital and installations costs. However, low HRTs result in significant methanogenic biomass washout, affecting methane production, substrate degradation and digestate quality. Several lab-scale thermophilic anaerobic digesters have shown low methane yield values approximately 0.15 m^3/ $kgVS_{fed}$ with 8-9 days HRT (Nges and Liu, 2010), (Braguglia et al., 2015). Karadag et al., (2010) proposed to vary simultaneously two parameters: HRT and mixing. They proposed mixing interruptions 2 h before feeding, in order to provide a quiet environment where methanogens, very sensitive to mechanical stresses, can grow. This operational mode allowed to compensate for methanogen washout, even at low HRTs, resulting in a biohythane production was of 350 L/Kg VS, with a methane content over the 65 % *v/v*. Finally, another strategy to improve biohythane production is the addition of Fe, Mg, P, Cu and Zn salts the ions of which are able to improve the microorganisms' metabolism, especially when the reactor digests a specific substrate and not a mixture of different HFWs. Recent studies have shown the strong influence of some metals on the production of biohythane from HFW. Facchin et al. (2013), in particular, demonstrated that Mo concentrations in the range of 3–12 mg/kg dry matter and Se concentrations of 10 mg/kg dry matter increased methane production to as high as 30–40%. Supplementation with a metal mixture (Co, Mo, Ni, Se and W) increased the methane production to the range 45–65%. In addition, Climenhaga and Banks (2008) underlined that the presence of sub stoichiometric amounts of ferric hydroxide reduced the sensitivity of acetoclastic methanogenesis to inhibition by fatty acids, while an adequate mix of nickel and cobalt is able to play a role in supporting AD. As previously discussed, the ions act as cofactors of the different enzymes involved in the fermentation pathway. Therefore, the supplementation of such micronutrients is essential for the improvement of the hydrogen and methane yields up to double those for the case without micronutrients supplementation (Karadag et al. 2010), (Preeti Mishra et al., 2017).

5. Applications of biohythane

As previously described, hydrogen in combination with methane from clean organic biomasses, has several environmental benefits contributing to reduced CO_2 equivalent and NO_x emissions to the atmosphere, being a carbon free fuel. In addition, hydrogen is able to improve the performance of internal combustion engines, usually fed by methane from fossil sources, to reduce the methane number, which is expressed as the percentage of methane in the biohythane and is related to the knock resistance. Furthermore, the lower ignition energy of hydrogen in air with respect to methane (0.02 mJ vs 0.29 mJ, at stoichiometric conditions) helps to burn better, but makes the mixture susceptible to pre-ignition by contact with hot spots or residual gases. The turbulent flame speed propagation's increasing in internal combustion engine is achieved when hydrogen is added to methane, in accordance to the stoichiometric laminar speed, which is 1.9 m/s for hydrogen and only 0.3 m/s for methane. Moreover, biohythane offers the possibility to expand the lean burn limit, because of a more stable combustion (De Simio et al., 2016). These advantages have made biohythane particularly attractive for the automotive sector, which is the most important sector where this blend has received more attention. Many car manufacturers, such as Toyota, have already developed hythane vehicles with interesting advantages in energy consumption (Genovese and Ortenzi, 2016). Hythane offers the possibility to use already existing engines in the automotive markets, without requiring heavy changes in designs, but only small operating adaptations on engines and adjustments on combustion control. In addition, hythane distribution can be supplied by the natural gas network or, alternatively, hythane may be produced directly at the refilling station. Recent studies on hythane performances over diesel fuel combustion demonstrated the absence of sulphur and toxic compounds, such as benzene and higher molecular weight hydrocarbons, or highly reactive olefins. Even particulate matter in exhausted gas emissions is very low compared to diesel-fuelled vehicles. The low carbon content in hythane allows to decrease the CO_2 emissions from an average of 3.2 kg CO_2/kg fuel of diesel combustion to 2.8 kgCO_2/Kg fuel (Genovese and Ortenzi, 2016).

The first experiences of hythane concerned the bus sector in Montreal in 1995. The project, called Montreal Hythane Bus Project, used hythane having 10% *v/v* of hydrogen and achieved a decrease in NOx emission of 45% compared to the methane fuelled buses (www.arb.ca.gov). The SunLine Transit Agency project in California overpassed this performance, exhibiting a NOx decrease higher than 50% using hythane with 20% *v/v* hydrogen concentration. Similar results have been recorded in Sweden and in China by the Beijing Hythane Bus Project. In Italy the first experiments on hythane use for automotive application have been conducted in the framework of the EU project BONG-HY (Blend of Natural Gas and Hydrogen in internal combustion engines) (www.dmf.unicatt.it). The vehicle was a light-duty commercial car Euro III which showed good performances in NO_x and CO_2 equivalent reductions. In 2008 the Italian research centre ENEA tested an 8 m long bus fuelled with hythane having different hydrogen concentrations, from 5 to 25% *v/v*. The bus was tested on the road demonstrating that hydrocarbons and CO emissions decrease with the increase of hydrogen content. More recently in the framework of the European Life plus program, Mhybus project provided the technical and admistrative steps to bring the first hythane-fuelled bus in Italy to circulate on public roads. Finally, in September 2007, Fiat presented the “Fiat Panda Aria”, a car equipped with a 900 cc twin-cylinder engine. It is able to use a mixture of methane and hydrogen (30%), with emissions of 69 g / km of CO_2. The innovative aspect of this technology is represented by the possibility to return to gasoline thanks to the flex-fuel engine (www.omniauto.it).

Although these improvements, the major obstacle to a largely biohythane adoption as automotive fuels is represented by the gas distribution system. Methane distribution system is mature and extensive in numerous countries and 12 millions of methane fed vehicles are already circulating in the world (Murphy and Thamsiriroj, 2011). But hydrogen presence in biohythane requires some modifications of the pipelines. For example, it is necessary the adoption of steel that is less prone to hydrogen embrittlement under pressure Modification of the distribution system from natural gas to hythane may require significant infrastructural investment cost and massive infrastructure projects over several years (Xia et al., 2016).

Thus, biohythane as automotive fuel has to adapt the distribution system to make possible the automotive transition from fossil to renewable fuels.

Even if biohythane has an immediate application as automotive fuel, it can be also considered an interesting intermediate for liquid fuels and value added products (Ge et al., 2014). Methane is conventionally used for methanol synthesis which represents the main feedstock for formaldehyde and methyl butyl ethers production through the activation of methane C-H bond by methanotrophs (Patel et al., 2016). Anyway, methane upgrading in methanol is limited to high cost of pure methane. In addition, Caceres et al. (2014) demonstrated that methanothrophs are able to work also in presence of high ammonia and hydrogen sulphide concentration, typically present in raw biogas, even if an inhibition of the conversion was observed. The recent challenge consisted in the improvement in methanol synthesis through biohythane at different hydrogen concentrations. Patel et al. (2017) immobilized methanotrophs, such as *M. tundrae*, through different adsorption and covalent immobilization methods on solid support (chitosan, amberlite XAD-4, dualite A-7). Almost a 2 fold increasing in methanol production, from biohythane compared to the pure methane or biogas, emerged from their study. The beneficial effect of biohythane can be explained by the hydrogen presence which has as an important role as electron source for the enzymatic reaction involved in the methanol biological pathway. Hydrogen helped also to reduce the inhibiting effects of ammonia, carbon dioxide and hydrogen sulphide. In particular the optimal CH_4:H_2 ratio for biohythane conversion into methanol was in the range 7:1 – 4:1. Another way for methanol production consists in biohythane conversion by Fischer-Tropsch (FT) reactions, previous carbon dioxide and water removal. Hydrogen and carbon monoxide are sent to a FT reactor where reactions over a catalyst produces a range of straight-chain alkanes and alcohols, such as methanol. The FT liquids are distilled to separate olefins and alkanes, the latter of which are refined to naphtha and diesel range hydrocarbons (Hu et al., 2012). A more innovative biohythane use is represented by the Polyhydroxyalkanoates production. The reactions were based on the metabolism of *Rhodospirillum rubrum*, which can utilize CO, produced by the steam reforming of biohythane, under anaerobic conditions as a sole carbon and energy (Revelles et al., 2016) (Basset et al., 2016). Of the carbon monoxide anaerobically oxidized by the bacteria, 80% of it was assumed to be consumed in the biologically mediated water gas shift reaction. The remaining 20% of the metabolized carbon monoxide was assumed to be incorporated into bacterial cell biomass, of which 40% is PHA (Bereketidou and Goula, 2012). PHA was assumed to be poly-3-hydroxybutyrate (P3HB), with the repeating unit of C4H6O2, whose synthesis is assured by the reaction given by the following reaction $9CO + 3H_2O \rightarrow C_4H_6O_2 + 5CO_2$

A crucial limitation to the mentioned process is linked to the limited CO solubility in the aqueous culture media, which can be solved operating at high pressure (10 bar).

6. Conclusions

Biohythane is a gaseous blend, composed of 10-30 % *v/v* hydrogen and 70-90% *v/v* methane generated by two-stage AD. Good performances have been achieved with HFW-sewage sludge codigestion. Biohythane is currently used to replace methane in the automotive sector, because hydrogen presence improves the combustion yield and reduces the CO_2 equivalent and NO_x emissions in the atmosphere. Anyway, to favour the adoption of cars and buses biohythane fuelled, bigger investments in the optimization of the biohythane distribution are required. National incentives could help in this transition, mainly in Countries which are already good markets for CNG cars, as Italy.

References

1. Abreu, A., Tavares, F., Alves, M.M., Pereira, M.A., 2016. Boosting dark fermentation with co-cultures of extreme thermophiles for biohythane production from garden waste. Bioresource Technology 219, 132–138.
2. Algapani, D.E., Qiao, W., Su, M., Pumpo, F.D., Wandera, S.M., Adani, F., Dong, R., 2016. Bio hydrolysis and bio-hydrogen production from food waste by thermophilic and hyperthermophilic anaerobic process. Bioresour. Technol. 216, 768–777.
3. Algapani, D.E., Wang, J., Qiao, W., Su, M., Goglio, A., Wandera, S.M., Jiang, M., Pan, X., Adani, F., Dong R., 2017. Improving methane production and anaerobic digestion stability of food waste by extracting lipids and mixing it with sewage sludge. Bioresource Technology 244, 996-1005.
4. Angeriz-Campoy, R., Álvarez-Gallego, C.J., Romero-García, L.I., 2015. Thermophilic anaerobic co-digestion of organic fraction of municipal solid waste (OFMSW) with food waste (FW): enhancement of bio-hydrogen production. Bioresour. Technol. 194, 291–296.
5. Baiano A., 2014. Recovery of Biomolecules from Food Wastes - A Review. Molecules 19(9), 14821-14842
6. Basset, N., Katsou, E., Frison, N., Malamis, S., Dosta, J., Fatone, F, 2016. Integrating the selection of PHA storing biomass and nitrogen removal via nitrite in the main wastewater treatment line. Bioresource Technology 200, 820-829.
7. Battista, F., 2015. Resolutions for the anaerobic digestion of agro-food wastes: the case od olive oil production's refuses. Lap Lambert Academic Publishing.
8. Battista, F., Fino, D., Erriquens, F., Mancini, G., Ruggeri, B., 2015. Scaled-up experimental biogas production from two agro-food waste mixtures having high inhibitory compound concentrations. Renewable Energy 81, 71-77.

9. Battista, F., Fino, D., Mancini, G., Ruggeri, B. 2016. Mixing in digesters used to treat high viscosity substrates: the case of olive oil production wastes. Journal of environmental chemical engineering 4, 915–923
10. Bereketidou, O.A., Goula, M.A., 2012. Biogas reforming for syngas production over nickel supported on cera-alumina catalyst. Catal Today 195(1), 93–100.
11. Bong, C.P.C., Lim, L.Y., Lee, C.T., Klemeš, J.J., Ho, C.S., Ho, W.S., 2018. The characterisation and treatment of food waste for improvement of biogas production during anaerobic digestion – A review. Journal of Cleaner Production 172, 1545-1558.
12. Braguglia, C.M., Gianico, A., Gallipoli, A., Mininni, G., 2015. The impact of sludge tretreatments on mesophilic and thermophilic anaerobic digestion efficiency: role of the organic load. Chem. Eng. J. 270, 362-371.
13. Caceres, M., Gentina, J.C., Aroca, G., 2014. Oxidation of methane by Methylomicrobium album and Methylocystis sp. in the presence of H2S and NH3. Biotechnol. Lett. 36, 69–74.
14. Cavinato, C., Bolzonella, D., Fatone, F., Cecchi, F., Pavan, P., 2011. Optimization of two-phase thermophilic anaerobic digestion of biowaste for hydrogen and methane production through reject water recirculation. Bioresour. Technol. 102, 8605–8611.
15. Chen, C.Y., Yeh, K.L., Lo, Y.C., Wang, H.M., Chang, J.S., 2010. Engineering strategies for the enhanced photo-H2 production using effluents of dark fermentation processes as substrate. Int J Hydrogen Energy 35, 13356-13364.
16. Cheng, J., Ding, L., Lin, R., Yue, L., Liu, J., Zhou, J., Cen, K., 2016. Fermentative biohydrogen and biomethane co-production from mixture of food waste and sewage sludge: effects of physiochemical properties and mix ratios on fermentation performance. Appl. Energy 184, 1–8.
17. Cheng, S.S., Chao, Y.C., Yang, K.H., Bai, M.D., 2011. Process recovery of bio- hydrogenation in a pilot plant from methanogens invasion. Int. J. Hydrogen Energy. 36, 8779-8784.

18. Chiu, S.L.H, Lo, I.M.C., 2018. Identifying key process parameters for uncertainty propagation in environmental life cycle assessment for sewage sludge and food waste treatment. Journal of Cleaner Production 174, 966-976.
19. Chu, C.F., Li, Y.Y., Xu, K.Q., Ebie, Y., Inamori, Y., Kong, H.N., 2008. A pH and temperature-phased two-stage process for hydrogen and methane production from food waste. Int. J. Hydrogen Energ. 33, 4739–4746.
20. Climenhaga, M. A., Banks, C. J., 2008. Anaerobic digestion of catering wastes: effect of micronutrients and retention time. Water Science and Technology (2008) 57 (5), 687-692
21. Davis, R.D., 1996. The impact of EU and UK environmental pressures on the future of sludge treatment and disposal Water Environ J 10, 65-69
22. De Simio, L., Gambino, M., Iannaccone, S., 2016. Using natural gas/hydrogen mixture as a fuel in a 6-cylider stoichiometric spark ignition engine. Enriched Methane: the first step toward the hydrogen economy. Springer International Publishing Switzerland. ISBN978-3-319-22191-5
23. Ecoinvent.Ecoinvent database v3. Swiss Centre for Life Cycle Inventories 2013.
24. Ellabban, O., Abu-Rub, H., Blaabjerg, F., 2014. Renewable energy resources: current status, future prospects and their enabling technology. Renew. Sust. Energ. Rev. 39, 748–764.
25. European Biogas Report 2015. http://european-biogas.eu/2015/12/16/biogasreport2015/
26. Facchin, V., Cavinato, C., Fatone F., Pavan, P., Cecchi, F., Bolzonella, D., 2013. Effect of trace element supplementation on the mesophilic anaerobic digestion of foodwaste in batch trials: The influence of inoculum origin. Biochemical Engineering Journal 70, 71– 77.
27. Franchetti, M., 2013. Economic and environmental analysis of four different configurations of anaerobic digestion for food waste to energy conversion using LCA for: A food service provider case study. Journal of Environmental Management 123, 42-48.

28. Gaffney, J.S., Marley, N.A., 2009. The impacts of combustion emissions on air quality and climate–from coal to biofuels and beyond. Atmos. Environ. 43, 23–36.
29. Ge, X., Yang, L., Sheets, J.P., Yu, Z., Li, Y., 2014. Biological conversion of methane to liquid fuels: Status and opportunities. Biotechnol. Adv. 32, 1460–1475.
30. Genovese, A., Ortenzi, F., 2016. Enriched methane for city public transport buses. Enriched Methane: the first step toward the hydrogen economy. Springer International Publishing Switzerland. ISBN978-3-319-22191-5
31. Goberna, M., Simón, P., Hernández, M.T., García, C., 2018. Prokaryotic communities and potential pathogens in sewage sludge: Response to wastewaster origin, loading rate and treatment technology. Science of the Total Environment 615, 360–368.
32. Gottardo, M., Micolucci, F., Bolzonella, D., Uellendahl, H., Pavan, P., 2017. Pilot scale fermentation coupled with anaerobic digestion of food waste - Effect of dynamic digestate recirculation. Renewable Energy 114, 455-463
33. http://www.dmf.unicatt.it/bong-hy/doc_finali/bong-hy_final_rep.pdf. Parallel application of Blends Of Natural Gas and Hydrogen in internal combustion engines and fuel cells. Final report Last access October 2017.
34. http://www.omniauto.it/magazine/4626/fiat-panda-aria
35. https://www.arb.ca.gov/msprog/bus/zbus/meetings/hythane_062806.pdf Last access October 2017.
36. https://www.arpal.gov.it/files/rifiuti/ANPA_Merceologia.pdf
37. Hu, J., Yu, F., Lu, Y., 2012. Application of Fischer–Tropsch synthesis in biomass to liquid conversion. Catalysts 2, 303–326.
38. IEA, 2014. Key world energy statistics. Available from http://www.iea.org/publications/freepublications/publication/key-world-energy-statistics-

39. Jang, H.M., Ha, J.H., Kim, M.-S., Kim, J.-O., Kim, Y.M., Park, J.M., 2016. Effect of increased load of high-strength food wastewater in thermophilic and mesophilic anaerobic co-digestion of waste activated sludge on bacterial community structure. Water Res. 99, 140–148.
40. Jin, Y., Li, Y., Li, J., 2016. Influence of thermal pretreatment on physical and chemical properties of kitchen waste and the efficiency of anaerobic digestion. J. Environ. Manag. 180, 291-300
41. Karadag, D., Puhakka, J.A., 2010. Enhancement of anaerobic hydrogen production by iron and nickel. Int. J. Hydrogen Energ. 35, 8554–8560.
42. Karthikeyan, O., Trably, E., Mehariya, S., Bernet, N., Wong, J.W.C., 2017. Carrere,H. Pretreatment of Food Waste for Methane and Hydrogen Recovery: A review. Bioresource Technology doi: http://dx.doi.org/10.1016/j.biortech.2017.09.105
43. Kataoka, N., Ayame, S., Miya, A., Ueno, Y., Oshita, N., Tsukahara, K., Sawayama, S. & Yokota, N. 2005. Studies on hydrogen-methane fermentation process for treating garbage and waste paper. ADSW 2005 Conference Proceedings, 2, Process Engineering
44. Khongkliang, P., Kongjan, P., Sompong, O., 2015. Hydrogen and methane production from starch processing wastewater by thermophilic two-stage anaerobic digestion. Energy Procedia 79, 827–832.
45. Kim, D.H., Kim, S.H., Kim, H.W., Kim, M.S., Shin, H.S., 2011. Sewage sludge addition to food waste synergistically enhances hydrogen fermentation performance. Bioresour. Technol. 102, 8501-8506
46. Kobayashi, T., Xu, K.Q., Li, Y.Y., Inamori, Y., 2012. Effect of sludge recirculation on characteristics of hydrogen production in a two-stage hydrogen-methane fermentation process treating food wastes. Int. J. Hydrogen Energy. 37, 5602-5611.

47. Lay, J.J., Fan, K.S., Chang, J., Ku, C.H., 2003. Influence of chemical nature of organic wastes on their conversion to hydrogen by heat-shock digested sludge. Int. J. Hydrogen. Energy. 28, 1361-1367.
48. Lee, D.Y., Ebie, Y., Xu, K.Q., Li, Y.Y., Inamori, Y., 2010. Continuous H2 and CH4 production from high-solid food waste in the two-stage thermophilic fermentation process with the recirculation of digester sludge. Bioresour. Technol. 101, 42–47.
49. Liu, Z., Si, B., Li, J., He, J., Zhang, C., Lu, Y., Zhang Y., Xing X.H., 2018. Bioprocess engineering for biohythane production from low-grade waste biomass: technical challenges towards scale up. Current Opinion in Biotechnology 50, 25–31.
50. Luo, S., Jain, A., Aguilera, A., He, Z., 2017. Effective control of biohythane composition through operational strategies in an innovative microbial electrolysis cell. Applied Energy 206, 879–886.
51. Mamimin, C., Prasertsan, P., Kongjan, P., O-Thong, S., 2017. Effects of volatile fatty acids in biohydrogen effluent on Biohythane production from palm oil mill effluent under thermophilic condition. Electronic Journal of Biotechnology 29, 78–85.
52. Micolucci, F., Gottardo, M., Bolzonella, D., Pavan, P., 2014. Automatic process control for stable bio-hythane production in two-phase thermophilic anaerobic digestion of food waste. Int. J. Hydrogen Energ. 39, 17563–17572.
53. Micolucci, F., Gottardo, M., Cavinato, C., Pavan, P., Bolzonella, D., 2016. Mesophilic and thermophilic anaerobic digestion of the liquid fraction of pressed biowaste for high energy yields recovery. Waste Management 48 (2016) 227–235
54. Micolucci, F., Gottardo, M., Pavan, P., Cavinato, C., Bolzonella, D., (2018). Pilot scale comparison of single and double-stage thermophilic anaerobic digestion of food waste. Journal of Cleaner Production 171, 1376-1385.

55. Mishra, P., Balachandar, G., Das, D., 2017. Improvement in biohythane production using organic solid waste and distillery effluent. Waste Management 66, 70-78.
56. Moreno, F., Munoz, M., Arroyo, J., Magen, O., Monné, C., Suelves, I., 2012. Efficiency and emissions in a vehicular spark ignition engine fuelled with hydrogen and methane blends. Int J Hydrogen Energy 37, 11495-11503.
57. Murphy, J.D., Thamsiriroj, T., 2011. What will fuel transport systems of the future? Materials today 14, 518-524.
58. Nges, I.A., Liu, J., 2010. Effects of solid retention time on anaerobic digestion of dewatered-sewage sludge in mesophilic and thermophilic conditions. Renew. Energy 35, 2200-2206
59. Nguyen, D., Gadhamshetty, V., Nitayavardhana, S., Khanal, S.K., 2015. Automatic process control in anaerobic digestion technology: a critical review. Bioresour. Technol. 193, 513–522.
60. Patel, S.K.S, Singh, R.K., Kumar, A., Jeong, J.H., Jeong, S.H., Kalia, V.C., Kim I.W., Lee, J.K., 2017. Biological methanol production by immobilized Methylocella tundrae using simulated biohythane as feed. Bioresource Technology 241, 922-927.
61. Patel, S.K.S., Mardina, P., Kim, D., Kim, S.-Y., Kalia, V.C., Kim, I.-W., Lee, J.-K., 2016. Improvement in methanol production by regulating the composition of synthetic gas mixture and raw biogas. Bioresour. Technol. 218, 202–208.
62. Q. Liu, Z.J. Ren, C. Huang, B. Liu, N. Ren, D. Xing, 2016. Multiple syntrophic interactions drive biohythane production from waste sludge in microbial electrolysis cells. Biotechnology for Biofuels 9,162
63. Reith, J.H., Wijffels, R.H., Barten, H., 2003. Bio-methane & bio-hydrogen, status and perspectives of biological methane and hydrogen production. Dutch Biological Hydrogen Foundation.

64. Revelles, O., Tarazona, N., García, J.L., Prieto, M.A., 2016. Carbon roadmap from syngas to polyhydroxyalkanoates in Rhodospirillum rubrum. Environmental Microbiology, 708-720.
65. Rivière, D., Desvignes, V., Pelletier, E., Chaussonnerie, S., Guermazi, S., Weissenbach, J., Li, T., Camacho, P., Sghir, A., 2009. Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge. ISME J. 3 (6), 700–714.
66. Roy, S., Das, D. 2016. Biohythane production from organic wastes: present state of art. Environ Sci. Pollu Res 23, 9361-9410.
67. Sarkar, O., Mohan, S.V., 2017. Pre-aeration of food waste to augment acidogenic process at higher organic load: Valorizing biohydrogen, volatile fatty acids and biohythane. Bioresource Technology 242, 68–76.
68. Schmidt, T., Ziganshin, A.M., Nikolausz, M., Scholwin, F., Nelles, M., Kleinsteuber, S., Pròter, J., 2014. Effects of the reduction of the hydraulic retention time to 1.5 days at constant organic loading in CSTR, ASBR, and fixed-bed reactors: performance and methanogenic community composition. Biomass Bioenergy 69, 241–248.
69. Seghezzo, L., Zeeman, G., van Liel, J.B., Hamelers, H.V.M., Lettinga, G. 1998. A review: the anaerobic treatment of sewage in uasb and egsb reactors. Bioresource Technology 65, 175-190.
70. Sharma, S., Ghoshal, S.K., 2015. Hydrogen the future transportation fuel: from production to applications. Renew. Sust. Energ. Rev. 43, 1151–1158.
71. Si, B., Liu, Z., Zhang, Y., Li, J., Shen, R., Zhu, Z., Xing X., 2016b. Towards biohythane production from biomass: Influence of operational stage on anaerobic fermentation and microbial community. International Journal of Hydrogen Energy 41, 4429-4438.
72. Si, B.C., Li, J.M., Zhu, Z.B., Zhang, Y.H., Lu, J.W., Shen, R.X., Zhang, C., Xing, X.H., Liu, Z., 2016a. Continuous production of biohythane from hydrothermal liquefied cornstalk biomass via two-stage high-rate anaerobic reactors. Biotechnol Biofuels 9:1.

73. Stranddorf, H.K., Hoffmann, L., Schmidt, A., 2005. Impact categories, normalisation and weighting in LCA.
74. Valdez-Vazquez, I., Poggi-Varaldo, H.M., 2009. Hydrogen production by fermentative consortia. Renewable and Sustainable Energy Reviews 13, 1000-1113.
75. Van Lier, J.B., Van der Zee, F.P., Frijters, C.T.M.J., Ersahin, M.E., 2015. Celebrating 40 years anaerobic sludge bed reactors for industrial wastewater treatment-Review paper. Rev. Environ. Sci. Biotechnol., 1–20
76. Ventura, J.R.S., Lee, J., Jahng, D., 2014. A comparative study on the alternating mesophilic and thermophilic two-stage anaerobic digestion of food waste. J.Environ. Sci. 26, 1274-1283
77. Wang, L., Shen, F., Yuan, H., Zou, D., Liu, Y., Zhu, B., Li, X., 2014. Anaerobic co-digestion of kitchen waste and fruit/vegetable waste: lab-scale and pilot-scale studies. Waste Manag. 34, 2627-2633
78. Wu, C., Huang, Q., M. Yu , Ren, Y., Wanga, Q., Sakai, K., 2018. Effects of digestate recirculation on a two-stage anaerobic digestion system, particularly focusing on metabolite correlation analysis. Bioresource Technology 251, 40–48.
79. Wu, L.J., Kobayashi, T., Kuramochi, H., Li, Y.Y., Xu K.Q., 2016. Improved biogas production from food waste by co-digestion with de-oiled grease trap waste. Bioresour. Technol. 201, 237-244
80. Xiao, B., Qin, Y., Zhang, W., Wu, J., Qiang, H., Liu, J., Li, Y.Y., 2018. Temperature-phased anaerobic digestion of food waste: A comparison with single-stage digestions based on performance and energy balance. Bioresource Technology 249, 826-834.
81. Xie, S., Wickham, R., Nghiem, L.D., 2017. Synergistic effect from anaerobic co-digestion of sewage sludge and organic wastes. International Biodeterioration & Biodegradation 116, 191-197

82. Xu, R., Yang, Z.H., Zheng, Y., Zhang, H.B., Liu, J.B., Xiong, W.P., Zhang, Y.R., Ahmad, K., 2017. Depth-resolved microbial community analyses in the anaerobic co-digester of dewatered sewage sludge with food waste. Bioresource Technology 244, 824-835.
83. Yang, G., Zhang, G.M., Wang, H.C., 2015. Current state of sludge production, management, treatment and disposal in China. Water Research 78, 60-73.
84. Yeshanew, M.M., Frunzo, L., Pirozzi, F., Lens, P.N.L, Esposito, G., 2016. Production of biohythane from food waste via an integrated system of continuously stirred tank and anaerobic fixed bed reactors. Bioresource Technology 220, 312-322.
85. Yong, Z., Dong, Y. , Zhang, X., Tan T., 2015. Anaerobic co-digestion of food waste and straw for biogas production. Renew. Energy 78, 527-530
86. Yoon Y., Lee S., Kim K.H, Jeon T., Shin S., 2018. Study of anaerobic co-digestion on wastewater treatment sludge and food waste leachate using BMP test. J Mater Cycles Waste Manag 20, 283–292.
87. Zhang, C., Lu, F.X., Xing, X.H., 2011. Bioengineering of the Enterobacter aerogenes strain for biohydrogen production. Bioresour Technol 102, 8344-8349.

CAPTIONS

Tables

Table 1. Reproduction kinetic for acidogenic, acetogenic and methanogenic microorganisms.

Table 2. Micronutrients concentration for inhibition of the microorganisms involved in two stage AD (Battista, 2015)

Table 3 Hydrogen and methane yields from different organic wastes

Table 1

	Dark Fermentation	**Methanogenic phase**	**References**
Temperature ranges	psychrophilic (0-20°C), mesophilic (20-42°C) and thermophilic (42-75°C)		Valdez-Vazquez et al., 2005; Hung et al., 2011)
Optimal pH	5.5-6.5	7.0 -8.0	Calli, 2008; Cavinato et al., 2011
Beginning of inhibition 'pH	3.8-4.2	< 6.5	Khanal et al., 2004; Micolucci et al., 2014
Operative pressure	atmosphere condition; preferable 10-20 mbar		Calzata et al., 1984
Hydraulic retention Time (HRT)	low HRT (from some hours to 3 days, depending on operative conditions) in order to favour the wash out of methanogens	High HRT (usually > 15 days)	Fan et al., 2006; Roy et al., 2016
Organic Load Rate (OLR)	High OLR favours the VFAs accumulation, inhibiting methanogens	Lower OLR	Lee et al., 2010
C/N ideal ratio	15-35	> 50	Battista, 2015; Karthikeyan et al. 2017
N/P ideal ratio	about 7		Vismara et al., 2011; Battista, 2015

Table 2

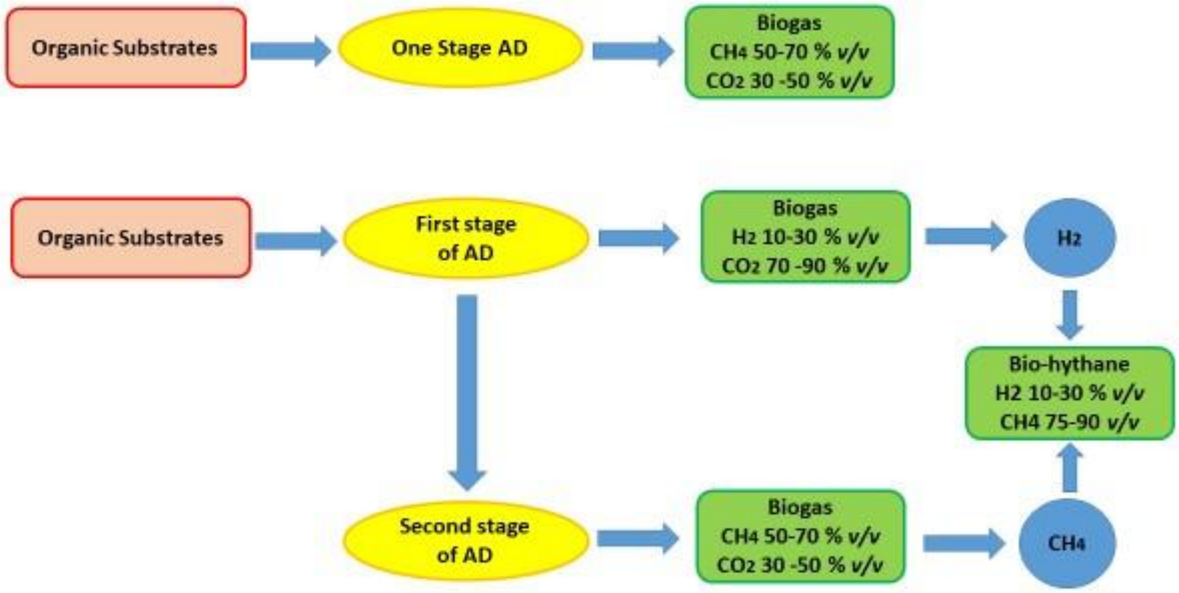
Element	Present as micronutrient (mg/L)	Beginning of inhibition (mg/L)		Toxic (mg/L)
		As Ions	As carbonate	
Na	45-200	5000-30000	nd	60000
Mg	10-40	1000-2400	nd	nd
Fe	1-200	nd	1750	nd
Ni	0,005-30	10-300		30-1000
Co	0,06-20	nd	nd	nd
Mo, W, Se	0,1-0,35			
Zn	0-3	3-400	160	250-600
Cr	0,005-50	28-300	530	500
Cu	nd	5-300	170	170-300
Cd	nd	70-600	180	20-600
Pb	0,02-200	8-340	nd	340
Ca	nd	2500-7000	nd	nd

Table 3

Test description	Hydrogen yield (L/Kg VS)	Methane yield (L/kg VS)	Reference
HFW treated at thermophlic condition during dark fermentation with HRT of 1.5d. Mesophilic condition and short HRT (5 d) for the methanogenic phase	205	464	Chu et al., 2008
HFW treated at thermophilic conditions for the both phases. OLR was changed during test	270	287	Lee et al., 2010
HFW treated at thermophilic condition with a HRT of 3 d for dark fermentation and 12.5 d for the methanogenic phase	52	410	Cavinato et al., 2011
HFW treated at thermophilic condition with a HRT of 3 d for dark fermentation and 12.5 d for the methanogenic phase with recirculation	220	710	Micolucci et al., 2014
HFW and sewage sludge codigested at 5 different ratios at mesophilic condition	174	264	Cheng et al., 2016
Sewage sludge treated at thermophilic condition (60°C) with HRT of 6 and 18 days for dark fermentation and methanogenic phase, respectively	81.5	310	Khnongkliang et al., 2015
Sewage sludge tretated at mesphilic condition	75	187	Liu et al., 2016

Highlights

- Biohythane is a gaseous blend constituted by methane and 10-30% *v/v* hydrogen
- Biohythane has better combustion performances and lower emissions than other fuels
- Dark fermentation and second stage of AD allow hydrogen and methane production
- Biohythane from food wastes has been deeply discussed during this review work
- The major potential of biohythane application in automotive sector have been reported


Organic Substrates
One Stage AD
Biogas
CH4 50-70 % v/v
CO2 30 -50 % v/v
Organic Substrates
First stage of AD
Biogas
H2 10-30 % v/v
CO2 70 -90 % v/v
H2
Bio-hythane
H2 10-30 % v/v
CH4 75-90 v/v
Second stage of AD
Biogas
CH4 50-70 % v/v
CO2 30 -50 % v/v
CH4