

Review

Enhancing Anaerobic Digestion: The Effect of Carbon Conductive Materials

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Abstract: Anaerobic digestion is a well-known technology which has been extensively studied to improve its performance and yield biogas from substrates. The application of different types of pre-treatments has led to an increase in biogas production but also in global energy demand. However, in recent years the use of carbon conductive materials as supplement for this process has been studied resulting in an interesting way for improving the performance of anaerobic digestion without greatly affecting its energy demand. This review offers an introduction to this interesting approach and covers the different experiences performed on the use of carbon conductive materials proposing it as a feasible alternative for the production of energy from biomass, considering also the integration of anaerobic digestion and thermal valorisation.

Keywords: improving biogas yields; biochar supplementation; direct interspecies electron transfer; integrating pyrolysis-digestion

1. Introduction

Fossil fuels (coal, oil and natural gas) have been the conventional sources of energy that keep running the modern world. Yet, these fuels are finite and are a cause of great impact on the environment with relevant effects on climate change [1]. This has made the search for sustainable alternatives even more important—sources capable of producing renewable energy but also being economically feasible. Among these different processes, the conversion of biomass into biofuels plays a crucial role. The production of ethanol and biodiesel are just an example of the processes most widely applied for producing biofuels. On the other hand, anaerobic digestion is capable of generating biogas which can either be used directly or converted into energy by the use of a combined heat and power (CHP) unit, the process also produces stabilised organic material as a by-product which can be valorised as an organic amendment. This digested material is a nutrient-rich sludge which can serve as a fertiliser, if it is well handled by proper application operation and control of heavy metals in soils [2].

However, land spreading of digestates for agricultural purposes has been progressively restricted and often legally banned, with several difficulties when a proper way of final disposal is needed. In recent years, new ways of valorisation have been searched as it is the concatenation of the anaerobic digestion with thermal processes as pyrolysis units, using the digestate as input material of this latter process and generating as main products: pyrolysis gases, bio-oils and biochars [3,4]. Other ways of valorisation are the use of digestates as nutrient media for the culture of plant growth promoting microorganisms in an attempt to convert this digested material into a high value added product [5].

Anaerobic digestion is a well-known technology widely applied for the valorisation of high organic strength wastes. The process has been subjected to extensive research to increase the efficiency of the degradation and biogas yields. This technology has been traditionally applied in the treatment of

sewage sludge, chemical effluents, agricultural and livestock wastes, and has been extensively studied in an attempt to improve performance and overcome several difficulties frequently encountered when dealing with complex residues, including inhibitory conditions caused by toxic intermediaries, difficult assimilation of lignocellulosic material, and low assimilation rate of acid compounds.

In the present review, a description of the process and the different approaches for increasing biogas yields is presented, with special focus on the performance of the digestion when carbon conductive materials are used for favouring biomass proliferation and increasing degradation rates.

2. Fundamental Aspects of the Anaerobic Digestion Process

Anaerobic digestion is a complex process where a diverse group of microorganisms are involved in the sequential conversion of organic matter. In general, the process can be divided into four main steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis in which hydrolytic, fermentative bacteria, acetogens and methanogens play distinct roles [1]. A schematic representation of the different phases of the digestion process is shown in Figure 1. The first stage is usually regarded as the limiting phase, being a process characterised by surface and transport phenomena in close relationship with the biomass activity or concentration. The hydrolysis stage is carried out by extra-cellular enzymes secreted by fermentative microorganisms which are responsible of the conversion of complex substrates such as particulate carbohydrates, proteins and lipids, into soluble components. Among these compounds, volatile fatty acids and H_2 are formed which serve as precursors of biogas reactions [6].

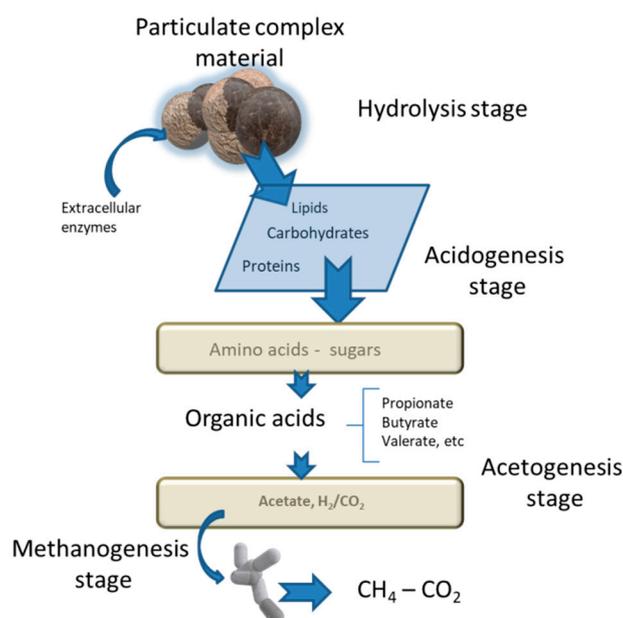


Figure 1. Schematic representation of the different stages of the anaerobic digestion process adapted from [1]. Organic matter is transformed into methane by a series of sequential microbiological reactions.

High levels of volatile fatty acids (VFAs) in the reactor may cause a decrease of process pH when the alkalinity level is not enough, resulting in a process failure. However, changes in VFA concentration in the digester do not necessarily induce a significant change in the reactor microbial population [7]. Short chain fatty acids, of a number of carbons greater than two, are traditionally monitored during the digestion process since they serve along with pH as an indication of the presence of imbalance of the process. Nonetheless, these changes should be properly interpreted as the result of process imbalance and not the cause of inhibition [8]. In addition, defining a proper level of VFA to be kept in a “healthy” anaerobic reactor is not an easy task, as different systems have their own “normal” levels of VFAs that can be considered as a cause of instability in a completely differing anaerobic reactor but not cause any problem in the other [9].

VFAs have been reported to cause inhibition of the cellulolytic activity at concentrations greater than 2 g/L, and therefore of the rate of cellulose hydrolysis, whereas glucose degradation seems to be more tolerant to the presence of high levels of these acids [10]. Propionic acid has been reported to have a significant inhibitory effect at concentrations much lower than those reported for acetic or butyric acid (900 mg/L of propionic acid for observing negative effects, versus values of 2400 mg/L for acetic and 1800 mg/L for butyric acid [11]). However, the presence of propionic acid itself as a consequence of the metabolism of the substrate does not necessarily exert a negative influence on the performance of the process.

Pullammanappallil and co-workers [12] reported on stable operation of a digester treating phenol. Propionic acid was formed from the metabolism of this compound, reaching levels of 2750 mg/L. The digester continued to convert all the glucose, which was also supplied to methane, showing that propionic acid accumulation to such high values did not inhibit the conversion of glucose to methane. Therefore, propionic acid accumulation may be an effect and not a cause of inhibition of the anaerobic digestion process. In a similar way, Fierro et al. [13] reported on stable performance of a high loaded reactor treating swine manure and glycerine as co-substrate. In this case, the propionic concentration reached values greater than 5000 mg/L and its accumulation was explained by the fastest rate of glycerol degradation, which was higher than that of propionate consumption; thus, accumulation of the acid was to be expected [14].

The transformation of organic acids with a number of carbons greater than two is the next stage in this complex transformation process. Acetogenic organisms produce acetate from organic acids, and waste electrons as hydrogen. This hydrogen is converted by hydrogenotrophic methanogens, while the acetic acid is converted to methane by acetoclastic methanogens [15]. The acetotrophic methanogens can compete with the acetate oxidizing bacteria which in time convert acetate to hydrogen and carbon dioxide, or they can also use the reverse reaction to produce acetate. At high hydrogen content (e.g., ≥ 500 Pa) acetogenesis is favoured whereas at low hydrogen content (≤ 40 Pa), the oxidation of acetate takes place [16].

The final stage involves the production of methane. Methanogens are the microorganisms that produce methane as the end-product of their anaerobic respiration; they are strictly anaerobic archaea belonging to the *Euryarchaeota* with a restricted capacity for utilizing substrates: CO₂, methyl-group containing compounds and acetate [17]. The use of CO₂ as main substrate necessarily involves H₂ electron donor, thus type of methanogens are called hydrogenotrophs. *Methanosaeta* species can convert acetate to methane but are unable to use hydrogen or formate as an electron donor for producing methane from carbon dioxide [18]. It is surprising that only two genera *Methanosaeta* and *Methanosarcina* are capable of assimilating the major intermediary of the digestion process (acetate) and are also responsible for at least two-thirds of the biologically generated methane [17].

The balance of the different stages in the anaerobic digestion process has been usually explained based on the levels of hydrogen in the reactor, with low levels of this gas being considered necessary to make the conversion into methane thermodynamically favourable [19]. The exchange of hydrogen between syntrophs and methanogens is a delicate step and any disruption may cause the accumulation of this gas and a subsequent build-up in VFA. However, other mechanisms for the transfer of electrons between different species have been proposed recently, based on experimental work with pure cultures and mixed microflora where hydrogen or formate were not available as electron carriers to explain the methane yields of the system [20–22].

3. Improving the Performance of the Anaerobic Digestion Process

The intrinsic complexity of the digestion process is associated with the diverse groups of microorganisms involved and the strict balance that should be kept between the different stages. For this reason, improving degradation rates of complex substrates has been traditionally associated with the application of pre-treatments whereas the balance of nutrients has been traditionally attempted

with the addition of co-substrates for avoiding accumulation of toxic intermediaries and increase biogas yields.

The enhancement of the digestion process has been performed using different approaches. The most studied in literature cover the application of pre-treatments to facilitate the hydrolysis of the organic material. Figure 2 represents some of the different pre-treatments available and some of their possible combinations for increasing the efficiency of the solubilisation and disruption of the organic compounds. Thermal and microwave pre-treatments, the use of alkaline and acid media, mechanical (ultrasound, high pressure and lysis) chemical oxidation and electrooxidation, as well as the combination of the above processes, are just some examples of the wide variety of developed processes intended for improving the degradation rate of the substrates [23–25]. An extensive review of the different available pre-treatments and results can be found in Khanal et al. [26], Tyagi and Lo [27] and Pilli et al. [28].

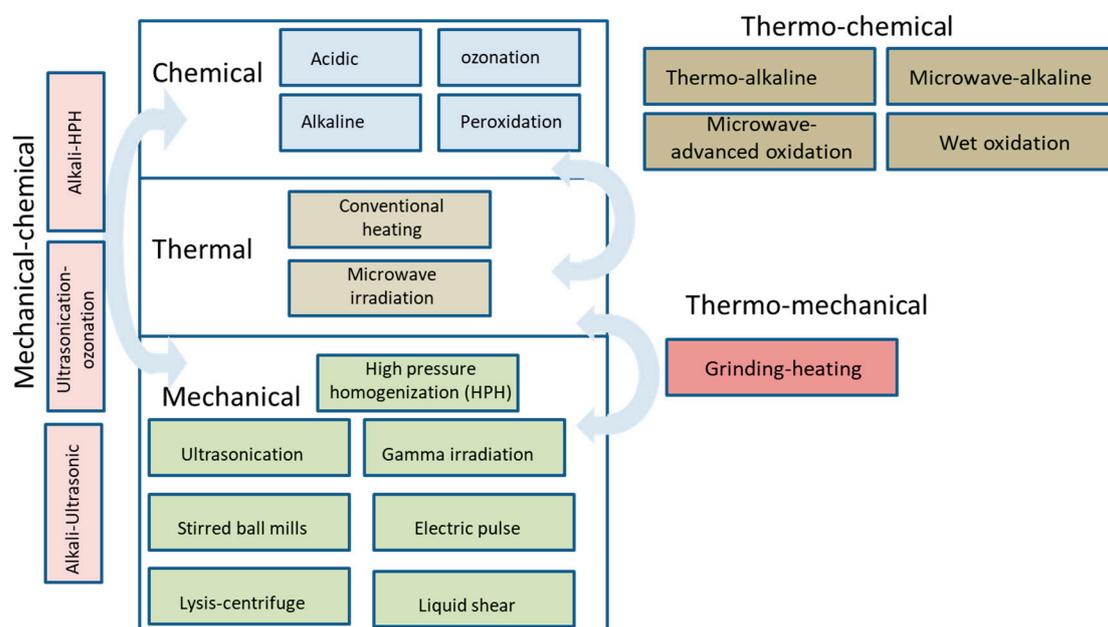


Figure 2. Classification of the different pre-treatment methods available for increasing substrate degradability and therefore the performance of the digestion process. Adapted from classification of pre-treatment processes of [27,28].

Thermal pre-treatments have been extensively implemented at an industrial scale because they greatly increase the solubilisation of the organic matter [29] and offer the additional advantage of obtaining a stabilised sludge free of pathogens [30]. The internal recycling of heat allows for the recovery of energy inside the same process, which counterbalance its high energy demand for increasing the temperature to that of the thermal hydrolysis stage (150–180 °C). Other thermal processes, as it is microwave pre-treatments, have been proposed as a feasible technical alternative to attain a high degree of solubilisation and enhance the rate of degradation during anaerobic digestion with low energy demand [31]. Microwave irradiation can rapidly produce focused direct heat, which lowers energy losses during transmission. In addition, the changing dipole orientation of polar molecules that takes place causes “athermal” (non-thermal) effects which, when combined with thermal effects, greatly aids in denaturing complex biological molecules [32]. In spite of these advantages, the energy balance is not as favourable as in the traditional thermal pre-treatment due to the impossibility of recovering energy by recycling of heat, and also the fact that conventional thermal process is capable of accumulating heat for adjusting the high peak demand with the production stage; the latter is, however, not available for any other technology in which the energy demand is associated with the direct use of electricity [33].

The addition of bases or acids, to improve thermal degradation, has demonstrated to be a feasible option, particularly in the case of thermo-alkaline pre-treatments, which can improve biogas production up to a 54% when evaluating the digestion of waste activated sludge [34] and has proven to be the preferred choice when the pre-treatment is intended for attaining the delignification of biomass [35].

In general, mechanical methods present a lower enhancement in biogas production since the effect in the solubilisation of the organic material has a lower impact when compare with thermal pre-treatments. Advanced oxidation methods, on the contrary, are capable of attaining a great extent of disintegration of organic components, but its costs and energy demand (in the case of electrooxidation) makes this process a feasible alternative only for the cases where the presence of recalcitrant molecules is the main feature of the waste stream as it would be the case of complex industrial wastewaters [36,37]. Different types of electrodes have been tested in an attempt to reduce the costs associated with this process and attain successful treatment of waste activated sludge [38].

Table 1 presents a list of different pre-treatments applied for improving anaerobic degradation of the organic matter. The studies of these different technologies have been widely applied to increase the digestibility of waste activated sludge (WAS). This organic material is generated in wastewater treatment plants as a result of the assimilation of the soluble compounds found in wastewaters by aerobic microorganisms. The excessive biomass growth in aerobic reactors favours a fast turnover of the organic compounds, but requires disposing the excess biomass as sludge. This sludge consists of a concentrated suspension of solids and biodegradable compounds [39]. In large scale wastewater treatment plants the traditional treatment of sludge is performed by anaerobic digestion. However, WAS has demonstrated to need high retention times in digesters and low degradability associated with the complex structure of their organic constituents. This brings, as result, the need for improving hydrolysis and acidification of this material in order to increase its degradation rate [40].

Table 1. Gas yields reported in literature regarding the effect of different pre-treatments on anaerobic digestion of waste activated sludge (WAS). Biogas or methane production data are reported either per unit of volatile solid (VS) or chemical oxygen demand (COD).

Pre-Treatment	Biogas Yield	Improvement (%)	Reference
Ultrasound	261 mL CH ₄ /g VS	30	[24]
Thermal (170 °C)	333 mL CH ₄ /g COD	50	[41]
Ozonation	259 mL CH ₄ /g COD	17	[41]
Microwave	183 mL biogas/Lr ¹ d (HRT ² = 10 d)	37	[42]
Fenton	547 mL CH ₄ /g VS	37	[43]
Electro-oxidation (Fe electrode–H ₂ O ₂)	147 mL biogas/g VS	256	[44]
Electrochemical (Ti/RuO ₂)	647 mL biogas/g VS	63.4	[45]
Surfactant addition and mechanical disintegration	180 mL CH ₄ /g VS	260	[46]

¹ Lr: reactor volume in liters, ² HRT: Hydraulic retention time.

The reduction of the energy demand of the different pre-treatment processes is a subject of on-going research. The effect on biogas enhancement and sludge minimisation must compensate for the high energy demand of pre-treatment methods. Recent research has been devoted to the evaluation of enzymatic hydrolysis and surfactant addition as a means for increasing digestibility [47]. However, the cost associated with this type of process represents a significant limitation. The use of fungal mash rich in hydrolytic enzymes was proposed by Yin and co-workers [48] as a way of reducing treatment costs. This seems a feasible approach, since these authors reported an improvement in methane production and a 54.3% reduction of biosolids when co-digesting sludge with food wastes.

Another way to improve performance of anaerobic digestion is by increasing the treatment efficiency through the addition of a co-substrate. Balancing nutrients is usually an approximation used for avoiding the accumulation of ammonia inside the digester which may cause a decrease in biogas production when inhibitory levels are reached. This is the reason for supplementing digesters treating manures with carbohydrate rich substrates. Co-digestion of swine manure, cattle and poultry manure have been studied for several years reporting better performance of the system when an organic material is added for increasing the organic loading of the reactors. The co-digestion of manures has

led to numerous research works related with the evaluation of biogas yields using different types of co-substrates [49,50].

Readily degradable materials, such as residual glycerine, cheese whey and food wastes, are added to digesters to increase biogas production rates [51–53]. However, the presence of these organic compounds can affect the fate of digestion; accumulation of VFAs may cause partial inhibitions which may finally affect the biogas yield [54]. Also, the presence of an abundant material which is easily converted by microorganisms may cause its preferential degradation causing a detriment in the stabilisation degree of the main residue. This is a relevant aspect which should not be overlooked. Anaerobic digestion is a process which is also focused on the treatment of wastes and on reducing their putrescible potential. However, when boosting biogas production, the degradation of rich carbohydrate materials is preferred by microorganisms over the degradation of more complex components such as proteins and lipids. Therefore, under the dynamic of fluids in continuously stirred tank reactors, the resulting digestate obtained at the hydraulic retention times typically applied in large scale digesters may need a post-stabilisation stage to further decrease its volatile solid content and its biological activity [13,55].

The increase in VFA production when a readily biodegradable substrate is added to the digester can be seen as a new alternative for the valorisation of wastes. Although, the present application of anaerobic digestion is focus on energy production and waste stabilisation, the recovery of valuable compounds can be proposed as a new way for increasing revenues from the traditional digestion process. This is the case of the recovery of VFA by means of filtration membranes. The use of micro and nanofiltration membranes allows for the production of a stream free of particles and microorganism with a high content of acids [56]. In addition, the coupling of membranes for the treatment of digester liquid effluents allows not only for the recovery of green chemicals, but also facilitates the disposal of a liquid stream which is becoming increasingly expensive [57]. The conditions of the digestion can be easily optimised in the desired direction by changing hydraulic retention time or organic loading rate, therefore the dreaded VFA build-up in reactor operation can become a desired step if the aim of the digestion becomes obtaining green chemicals with multiples applications. VFA recovered from waste streams can be used for obtaining biodegradable polymers [polyhydroxyalkanoates (PHA), polyhydroxybutyrate (PHB), polylactate (PLA)], biodiesel (by means of fermenting processes), or either be used for increasing the treatment efficiency by coupling the digestion process with nutrient recovery systems, struvite recovery or for producing hydrogen [58–60].

Improving the performance of anaerobic digestion has been attempted by coupling different biological processes. The production of hydrogen can be performed by dark fermentation, which is usually coupled to anaerobic digestion as a first stage where hydrogen and short chain acids are produced [61,62]. The first fermentative stage usually operates at low hydraulic retention time which results in small reactors being needed and reduced installation costs. In addition, the capacity for using the whole substrate is limited in dark fermentation due to the high production of organic acids as by-products; thus, the application of this process as an intermediate stage becomes a reasonable option with the digestion process dedicated to the conversion of the hydrolysed material. The energy efficiency of the global process (coupling dark fermentation and anaerobic digestion) has been demonstrated to be increased by 13% when compared with the stand alone digestion configuration [63]. A similar approach has been also reported for integrating microbial electrolysis cells and anaerobic digestion, in this case for either increasing the efficiency of the organic matter degradation and thus methane production or increasing the production of energy by obtaining hydrogen [64,65]. In microbial electrolysis cells the oxidation of the organic matter is mediated by electrodes in an anodic chamber where a microbial biofilm catalysis this reaction. Hydrogen is produced in the cathode thanks to the application of small external voltage obtaining efficiencies of production higher than 90% [66]. The combined approximation allows for an oxidation of the organic material with the advantage of being operated under similar temperature conditions. Hydrogen can be directly used for energy production, or when handling this gas is not feasible due to incompatibility of the piping installation (because of the small

volume of the hydrogen molecule), the direct injection of this gas in anaerobic digesters has been proposed for increasing methane production to up 30% by favouring the action hydrogenotrophic methanogenes [67,68].

Other types of substrates such as cellulosic and lignocellulosic biomasses have also been studied as suitable co-substrates in anaerobic digestion to balance the carbon to nitrogen (C:N) ratio. The addition of cellulosic biomass as crop residues, grass and recently algae material has demonstrated to increase the methane production rate thanks to the increase attained in the organic load of the reactor, and no synergistic effect is clearly demonstrated from the co-digestion with manures. The addition of this material does not cause an increase in the specific methane production of the mixture compared with the biogas yields obtained for the individual substrates [69].

The use of lignocellulosic biomass is also another option, but in this case, several challenges need to be overcome, prior to consider it as a real alternative. The use of lignocellulosic biomass is hindered by the high energy demand associated with the pre-treatments needed to make accessible the cellulosic and hemicellulosic compounds to the anaerobic microflora [70]. In addition, based on the operating conditions of the reactor, some components as hemicellulose may be rapidly converted into methane, while living behind cellulose and lignin in the fibrous digested material [71]. One way of increasing the efficiency of the conversion process is by increasing the solid content of the organic material. Taking into account that the treatment of lignocellulosic biomass under anaerobic conditions will leave unconverted a fraction of the solid material, performing the process under a high solid configuration seems to be the most obvious way for attaining the digestion of this material. These high solid technologies (with solid content material greater than 15%) are gaining great interest because of the advantage of the smaller size of the plant, but also lower heating energy demand, easiness of post-treatment of the digested material and lower liquid production [72,73]. The co-digestion of switchgrass with swine, poultry and dairy manure has shown promising results for the first one with methane yields similar to those obtained for submerged fermentation systems (337 mL CH₄/g VS) [72]. Although results for poultry manure are not as encouraging, the combination of co-digestion and high solid digestion technology may open a new feasible way for improving performance. This is the case of percolating systems for the co-digestion of poultry manure and sludge, where the single digestion of poultry manure may lead to poor results but the addition of the co-substrate exerted a positive influence by the increase in volatile solid destruction and biogas production [74].

Recent approaches for improving the performance of anaerobic reactors also include operation under high pressure. The reasoning behind this type of technology is associated with the need of up-grading biogas to be used as transport fuel. The behaviour of all systems under high pressure is governed by LeChâtelier's principle, which predicts that the application of pressure shifts equilibrium towards the state that occupies a smaller volume, and accelerates processes for which the transition state has a smaller volume than the ground state [75]. Therefore, it is highly expected that the microbial conversion of CO₂ into CH₄ would be favoured under high pressure conditions.

Recent research activities on high pressure anaerobic systems have demonstrated the feasibility of methanogenesis in a high-temperature petroleum reservoir. The incubations experiments were conducted by Mayumi and co-workers [76] without any substrate amendment and at 55 °C and 5 MPa. Results demonstrated the dominance of methane production by syntrophic acetate oxidation coupled to hydrogenotrophic methanogens in this environment. Considering the limits of life in terms of temperature (122 °C for *Methanopyrus kandlerii*) and pressure (130–150 MPa for *Pyrococcus yayanosii*, thus occurring ca. 4 km below ground), the high pressure biotopes contribute significantly to the production and recycling of organic carbon on earth, representing about 50% of the primary biomass production [77–79]. Microorganisms isolated from these biotopes are often able to grow more efficiently under high hydrostatic pressure than under atmospheric pressure. These are referred to as piezophiles having optimal growth rates at pressures greater than 1 atm or 0.1 MPa. In addition, their inhibitory pressures are higher than those of surface organisms, often exceeding 100 MPa [79]. These types of microbes belong to a wide variety of eubacterial and archaeal populations [80].

The feasibility of operating methanogenic reactors under high pressure condition has been demonstrated by Mayumi and co-workers [81]. These authors reported that the increase in CO₂ pressure accelerates the rate of methanogenesis to more than twice. Isotope tracer and molecular analyses showed that high CO₂ conditions invoke acetoclastic methanogenesis in place of syntrophic acetate oxidation coupled with hydrogenotrophic methanogenesis that typically occurs at low CO₂ conditions. These findings would also explain why acetate is often the most abundant organic acid found in oil reservoirs.

On the other hand, Chen and co-workers [82] reported on the operation of anaerobic digestion at increasingly biogas pressure conditions inside the reactor (3–90 bar). This phenomenon led to an increase in the solubilisation of CO₂ in the liquid phase which may have had a detrimental effect on the pH of the system but as additional advantage offered an enrichment of methane in biogas to values close to 90–95%, lowering, based on the same reason, the content of other contaminants as H₂S and NH₃ [83]. These preliminary investigations will presumably lead to the development of new technologies intended to increase the degradation rate of organic wastes and facilities that can overcome the present constraints associated with organic loading rates of digesters capable of attaining biogas up-grading at much lower costs.

4. The Use of Carbon Conductive Materials for Improving Anaerobic Digestion

The first demonstration of electrodes serving as a direct electron donor for anaerobic respiration was performed by Gregory et al. [84]. Their findings had great implications for the harvesting of electricity from anaerobic sediments and the bioremediation of oxidised contaminants. In previous reports, the capability of *Geobacteraceae* to growth attached to electrodes and use them as electron donors was reported by Bond et al. [85] and Tender et al. [86]. These authors used graphite electrodes as anodes and identified two anodes reactions: the first one was the oxidation of sediment sulfide (a by-product of microbial oxidation of sedimentary organic carbon) and the second one, the oxidation of sedimentary organic carbon catalysed by microorganisms colonising the anode.

Pure cultures within this family, such as *Geobacter sulfurreducens*, *G. metallireducens* and *Desulfuromonas acetoxidans*, have demonstrated the capability of supporting growth by oxidizing acetate or other organic compounds to carbon dioxide and transferring the electrons to the surface of a graphite electrode [85,87]. It is accepted that organisms other than *Geobacteraceae* may be also able to use electrodes as an electron acceptor without the need of requiring an exogenous electron shuttle. Summers and co-workers [20] introduced the concept of direct interspecies electron transfer (DIET) when evaluating the coculture of *Geobacter metallireducens*, an ethanol-oxidizing Fe(III) reducer, and *Geobacter sulfurreducens*. In their research, these authors tested the aforementioned organisms, because the latter one is incapable of metabolising ethanol, and was added to the culture to act as a hydrogen-consuming partner with the additional benefit that it can use fumarate as the electron acceptor, which cannot be used by *G. metallireducens*. The coupling of these two organisms led to the formation of an interesting aggregate being electrically conductive and suggested therefore the mechanism of DIET.

This mechanism has also been suggested to take place in anaerobic digesters. Morita and co-workers [21] proposed that microorganisms within some natural methanogenic aggregates may directly exchange electrons, instead of producing hydrogen or formate as an intermediary electron carrier. These authors evaluated the performance of an up-flow anaerobic sludge blanket reactor treating brewery waste to methane. This reactor contained aggregates with a low capacity for converting hydrogen into methane, and even though these aggregates were capable of converting formate into methane, the rate of conversion was too low to explain the high capacity of the reactor for assimilating ethanol. Aggregates in this type of experiment demonstrated that methanogenic species were electrically conductive and were predominantly enriched in anaerobic microorganisms most closely related to the acetate-utilizing *Methanosaeta concilii*. A similar suggestion was also reported by Rotaru et al. [88], who found that *Methanosaeta* species can make direct electrical connections with

Geobacter species, accepting electrons for the reduction of carbon dioxide to methane. It seems that this collaboration within these two species is responsible for the recurrent finding of enhanced performance of anaerobic digesters when electrodes are introduced in anaerobic systems.

This statement was later corroborated by Zhao et al. [22], who reported that the introduction of graphite-brush anodes as electrodes in an anaerobic digester treating sewage sludge resulted in the predominance of *Geobacter* species being enriched in the digestion system. These species belonged to *Geobacter metallireducens*. The prevailing presence of *Methanosaeta* species in the microbial communities along with the presence of *Geobacter* species, capable of performing the direct exchange of electrons, was the reason for the “unknown” increase in methane production reported. Conductivity of the sludge in this electric-anaerobic digester was about 30% higher than that of the sludge in a control digester without electrodes.

On the other hand, the addition of Iron has a similar effect on anaerobic digestion, by increasing the production of methane and the reasoning behind this effect may keep a close relation with the improvement observed when carbon conductive material is supplemented. The addition of Iron (II) as a salt has been demonstrated to increase acetic conversion into methane [89], whereas the addition of zero valent iron (Fe^0) and iron scraps (Fe(III)) has been reported to increase the performance of the digestion of waste activated sludge. The addition of Fe^0 favoured the presence of homoacetogens and hydrogenotrophic methanogens, while oxidized iron enhanced the diversity of acetobacteria and enriched iron-reducing bacteria [90,91]. *Geobacter* species are known to reduce iron oxides and their presence have been commonly reported in sediments and bioelectrochemical systems where their predominance is associated with its characteristic feature of using solid state compounds as electron acceptors [92,93]. Therefore, the enhancement in anaerobic digestion when carbon conductive materials are added to a conventional digester seems to be associated with the bio-augmentation of anaerobic species capable of providing a different route for electron transport as it is the collaboration between methanogens and *Geobacter* species [94].

Different carbon conductive materials have been evaluated in the digestion of complex wastes. Results reported by several authors when allowing the presence of an external material to serve as electrons transport have led to open an innovative path for improving anaerobic digestion. The performance of digestion is reportedly improved when taking advantage of this feature. In particular, the use of carbon based materials, as it is carbon cloth, carbon felt and granular activated carbon has proven to enhance the treatment of high organic loading streams and favour the recovery of sour reactors [95].

The use of activated carbon along with different types of adsorbents has demonstrated to enhance the performance of digestion. Desai and Madamwar [96] studied the addition of silica gel, bentonite, among others, to evaluate the effect of methane production when digesting manures and cheese whey. Their results (about two-fold enhancement) were also in agreement with those previously reported by Patel et al. [97], studying the addition of different types of adsorbents in the digestion cattle dung. This initial line of research has led to extensive studies on the use of activated carbon and consequently, because of the imperative need of reducing treatment cost, the use of biochar.

There exist numerous reports on the performance of reactors operating with the use of different adsorbents. Adsorbents can be defined as chemically inert materials with properties that allow the accumulation of atoms, ions or molecules on their surface. This is a surface based interaction between a solid and a fluid with the rate of sorption being dependent on the adsorbent (the material used as the adsorbing phase) and the adsorbate (the material being adsorbed) [98]. Therefore, the implication of these materials in the microbial degradation of wastes has been traditionally associated with their capacity of removing toxic compounds from the culture lowering the inhibitory burden microorganisms need to overcome.

Zeolites have been traditionally studied in digestions systems to enhance the performance of reactors with high nitrogen levels thanks to its natural capacity to exchange cations (Ca^{2+} and Mg^{2+}) and remove ammonium [99–101]. The use of bentonite was tested by Angelidaki and co-workers [102]

for reducing the inhibitory effect of long chain fatty acids. The performance of reactors is reported to be significantly improved but the cost of using adsorbents seems to set an important burden to the process.

Similarly, the benefits in the use of activated carbon have also been explained by its adsorption capacity of inhibitory compounds [103]. However, recent reports demonstrated that its ability for improving digestion performance should be explained based on a combination of different factors. This statement was demonstrated by Cuetos and co-workers [104] who studied the digestion of poultry blood. This waste stream is an organic material of difficult degradation due to its high carbon and protein content which leads to a rapid accumulation of VFAs and ammonium under anaerobic conditions, causing a complete inhibition of the process. The addition of granular activated carbon to the digestion of blood as sole substrate resulted in a better assimilation of C₃–C₅ acid forms, indicating that an enhancement in syntrophic metabolism may have taken place. The addition of granular activated carbon (GAC) may favour the adsorption of ammonium, offering protection to the microbial biomass by limiting mass transfer of toxic compounds, and also acting as a conductive material. These features may explain the successful digestion of residual blood as the sole substrate obtained by Cuetos et al. [105].

The addition of GAC has also been studied in digesters treating food wastes, where the presence of this carbon material favoured the growth of archaea and syntrophic bacteria, suggesting that interactions between these microorganisms were enhanced with the predominance of hydrogenotrophic methanogens [106]. The combination of factors, as it is the transport of electrons and the important role of this material associated with the removal of inhibitory compounds by adsorption phenomena has led to the extensive search for alternative materials with similar qualities.

Anaerobic reactors treating complex wastes may be often submitted to stress conditions as it is high concentration of inhibitory compounds and acid intermediaries. These are common features frequently found in industrial scale reactors, hence developing strategies for the rapid recovery of these systems seems crucial in order to prevent the complete failure of the process. With this aim, the behaviour of anaerobic microbial systems was evaluated using carbon felt electrodes as supporting material when the digestion was forced to inhibitory acid conditions by organic overloading. The mere presence of the electrodes delayed the accumulation of volatile fatty acids, being indicative of the effect this type of materials has on the enhancement on the metabolism of VFA [107]. The performance of combined digestion and microbial electrolysis systems was previously studied by De Vrieze et al. [108]. These authors evaluated the digestion of molasses and indicated a stabilising effect on the process when bioelectrochemical systems are coupled to the reactor. However, the reported benefit was explained on the capacity of electrodes for retaining anaerobic microflora rather than an effect associated with the application of a cell potential.

Following this same line of research, the introduction of bioelectrochemical systems to anaerobic reactors was proposed as an alternative capable of integrating two anaerobic microbial processes which could benefit from the presence of electrodes and mediate in the conversion of organic materials. This combined reactor was tested in the stabilisation of waste activated sludge reporting a significant improvement in the rate of methane production (up to 3 times compared to the digestion stand-alone configuration) [109]. These results were also in agreement with those published by Feng and co-workers [110], studying this same organic substrate in a similar configuration of microbial electrolysis cell reactor and anaerobic digestion. In this later work high solid content was a differentiating feature. Results obtained under these conditions proved the superior performance of the combined approach by reporting a 22.4% increase in methane production when iron–graphite electrodes were used. More impressive results were described for the use of a single chamber reactor also integrating both processes and carbon felt electrodes. Methane content in excess of 98% was achieved from the microbial electrolysis cell and anaerobic digestion coupled process. An increase in methane yield of 2.3 times was also obtained being explained by the in situ conversion of CO₂ into methane on the anode by hydrogenotrophic electromethanogens (e.g., *Methanospirillum*), which were

the dominant population. These microbes could utilize hydrogen gas generated at the inner surface of stainless steel reactor, which also served as cathode of the microbial electrolysis cell [111].

However, the success of the different reactor configurations and alternatives for improving digestion performance are intimately linked to the installation and operating costs. Searching for techniques capable of producing low-cost adsorbents or low-cost microbial electrolysis systems have become a wide field of research. Lignocelulosic biomass, agricultural by-products and wastes have traditionally served as cheap raw materials for the production of energy from biomass and for the recovery of low-cost adsorbents [112–114].

Biochar is a carbon-rich by-product resulting from the burning of biomasses at high temperatures (300–1000 °C) either under low levels of oxygen (gasification) or under its complete absence (pyrolysis) [115]. This material is known to present adsorption properties and special features as soil amendments having positive effects on plant growth, associated with the capacity of retaining soil nutrients, increasing soil-water retention capacity and enhancing cation exchange capacity [116,117]. Different feedstocks serve as raw materials for obtaining biochar, as it is; organic wastes derived from agriculture and forestry, or urban wastes including sewage sludge [118]. However, its properties are greatly dependent on the feedstock used and the process conditions (temperature, atmosphere and heating rate) [119,120].

For several years the effect of biochar in soils has been studied, reporting different benefits which may be associated with its chemical and physical characteristics. Only in recent years have researchers focused their attention on extrapolating its use to anaerobic digestion systems, considering its capacity of acting as a low-cost adsorbent of inhibitory compounds and reporting relevant results regarding the improvement obtained in the performance of the digestion which could not be explained just by the removal of toxic compounds [105]. Table 2 presents a list of different experiences regarding the effect of supplementing anaerobic digestion systems with biochar. Improvements in the rate of degradation and increase in methane yields have been reported by several authors [121–123]. Addition of biochar to anaerobic digestion systems is suggested to reduce inhibitory stages and favours stability of the process thanks to its capacity for adsorbing toxic compounds, increasing buffering capacity and aiding in the immobilisation of anaerobic microflora [98].

Table 2. Results obtained from biochar supplemented reactor when evaluating the effect on biogas yields.

Source of Biochar	Substrate	Main Results	Reference
Fruitwood	Glucose (4–8 g/L)	Biochar shortened the methanogenic lag phase and increased maximum methane production rate in 86.6% at 4 g/L of glucose concentration and just 5.2% at the highest glucose concentration (8 g/L)	[121]
Fruitwood	Food wastes (4–10 g Dry Weight/L)	Biochar shortened the methanogenic lag phase and increased maximum methane production rate by 123%	[122]
Almond shell	Swine manure	Increased in methane yield of 39%. Addition of biochar enhanced the degradation of proteins	[123]
Fruitwood	Glucose N: 0.26–7 g/L	Biochar accelerated the initiation of mechanisation during anaerobic digestion under double inhibition risk from both ammonium and acids. Increased maximum methane production rate by 38%	[124]
Corn Stover	Sewage sludge	Biochar-amended digesters produced near pipeline-quality biomethane (>90% CH ₄ and <5 ppb H ₂ S). The biochar addition also increased alkalinity and mitigated ammonia inhibition. Increased maximum methane production rate by 27.6%	[125]
Clean forestry wood residue (Holm Oak)	Organic fraction of municipal solid waste (solid state fermentation)	Methane yield increased around 5% with biochar addition	[126]
Vineyard pruning	Orange peels	Increased in methane yield of 56%. Biochar addition favoured the electro-active microorganisms consortia creating a syntrophic metabolism between eubacterial and archaeal populations	[127]
Wheat bran pellets, coppiced woodlands, and orchard pruning	Food waste fermentate from acidogenic reactor	Conversion of VFAs proceeded at a rate up to 5 times higher than that observed in the unamended controls. Biochar affected the composition of the microbial consortium. Positive effect observed was directly related to the electron-donating capacity (EDC) of the material, but was independent of its bulk electrical conductivity and specific surface area	[128]

The enhancement obtained in anaerobic digestion with the supplementation of biochar leads to considering as reasonable the coupling of pyrolysis and digestion technologies for the global treatment of waste organic material. This combined approach has been studied by Monlau and co-workers [129] and Hübner and Mumme [130] either by considering the production of biochar from anaerobic digestate or by the degradation of the aqueous phase of pyrolysis liquor in the anaerobic digester. The approach seems promising and this is particularly true if the enhancement in the global efficiency of the combined approach is considered.

Figure 3 is a schematic representation of the different possibilities available for an integrated management of waste organic material considering anaerobic digestion and pyrolysis. Salman and co-workers [131] evaluated a simulation of the combined approach integrated into a bio refinery concept to establish the benefits obtained from the energy and exergy point of view. These authors reported a global efficiency of 59% for the combined configuration against a 52% for the anaerobic digestion stand-alone process. Hence, the use of biochar as supplement in the digestion process has led to consider as a feasible option the full integration of biological and thermal valorisation of organic wastes bringing out as additional benefit the significantly reduction in the amount of material needing final disposal.

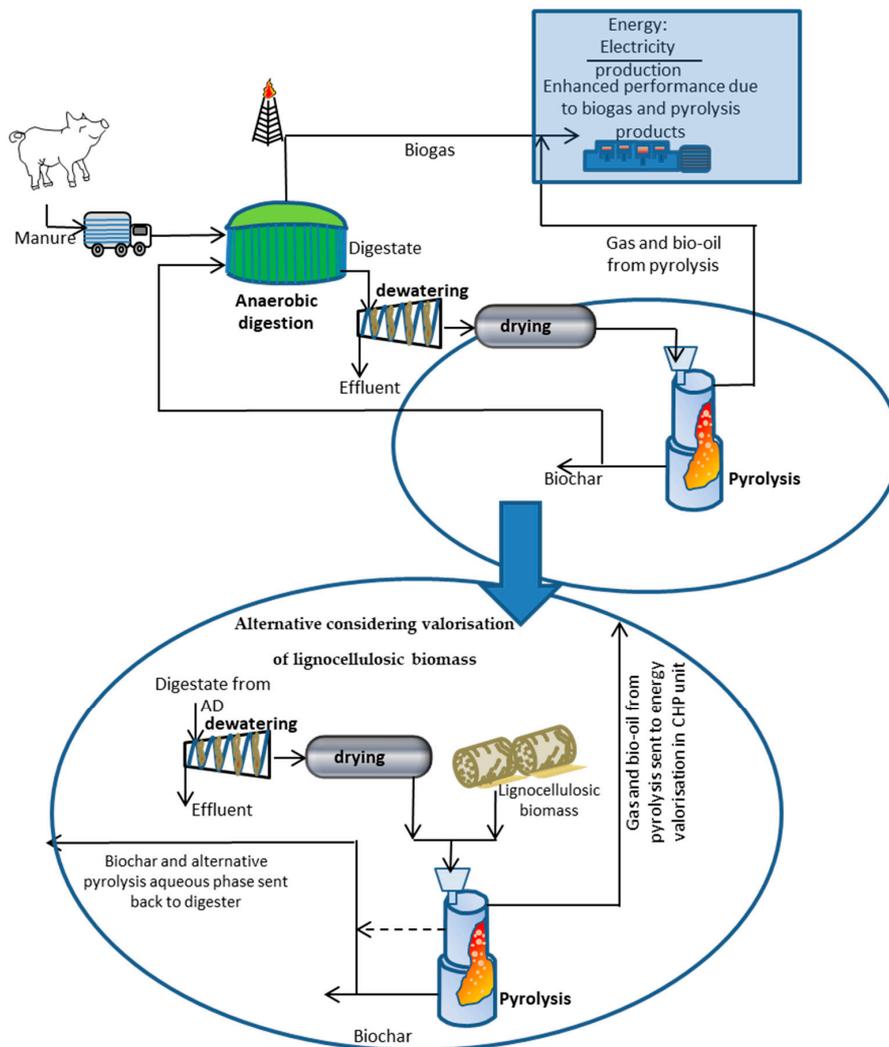


Figure 3. Schematic representation of the valorisation of waste and lignocellulosic biomass for energy production. The configuration considers the integration of anaerobic digestion and pyrolysis for the treatment of swine manure. Biochar produced by pyrolysis is used as supplement in the digestion process. Digestion of the aqueous phase is proposed also as alternative.

5. Conclusions

Anaerobic digestion is a well-known technology presenting a wide application in the treatment of high loaded organic effluents and in the valorisation of biomass into biogas. The enhancement of biogas yields of organic substrates mostly keep relation with the increase in the degradation rate. This can be performed by the application of different types of pre-treatment to the organic compounds, but this implies a high energy demand. This review also presents the effects reported on by different research works when evaluating the performance of the digestion process supplemented by carbon conductive materials, with the main focus on the use of biochar. The use of carbon conductive materials aids in the degradation rate of substrate by allowing an alternative route for electron transport. The use of biochar and recently hydrochar seems to be a feasible way for increasing the performance of anaerobic digesters and opens a new approach for the global valorisation of organic wastes by the integration of anaerobic digestion and thermal processes. This combined configuration allows for increasing the efficiency of the whole treatment process and greatly reduces the amount of material needing final disposal.

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