The challenges of anaerobic digestion and the role of biochar in optimizing anaerobic digestion

Michael O. Fagbohungbe*a Ben M.J. Herbert*b; Lois Hurst*b, Cynthia N. Ibeto*c, Hong Li*a, Shams Q. Usmani*d and Kirk T. Semple*a

a*Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YQ, United Kingdom
b*Stopford Energy and Environment, Merseyton Road, Ellesmere Port, Chester, CH65 3AD, United Kingdom
c*National Centre for Energy Research and Development, University of Nigeria Nsukka, Enugu State, Nigeria
d*Ariva Technology, The Heath Business and Technical Park, Runcorn, Cheshire WA7 4EB

*Corresponding author: m.fagbohungbe@lancaster.ac.uk
Abstract

Biochar, like most other adsorbents, is a carbonaceous material, which is formed from the combustion of plant materials, in low-zero oxygen conditions and results in a material, which has the capacity to sorb chemicals onto its surfaces. Currently, research is being carried out to investigate the relevance of biochar in improving the soil ecosystem, digestate quality and most recently the anaerobic digestion process. Anaerobic digestion (AD) of organic substrates provides both a sustainable source of energy and a digestate with the potential to enhance plant growth and soil health. In order to ensure that these benefits are realised, the anaerobic digestion system must be optimised for process stability and high nutrient retention capacity in the digestate produced. Substrate-induced inhibition is a major issue, which can disrupt the stable functioning of the AD system reducing microbial breakdown of the organic waste and formation of methane, which in turn reduces energy output. Likewise, the spreading of digestate on land can often result in nutrient loss, surface runoff and leaching. This review will examine substrate inhibition and their impact on anaerobic digestion, nutrient leaching and their environmental implications, the properties and functionality of biochar material in counteracting these challenges.

Keywords: biochar; inhibition; nutrient leaching; digestate; anaerobic digestion
1. Introduction

The number of anaerobic digestion (AD) systems has increased rapidly because of various factors including financial incentives for renewable energy facilities, governmental policies on climate change, landfill and an increasing energy need (Zglobisz et al., 2010; Klavon et al., 2013). Currently, in Europe and Asia, there are over 30 million large and small-scale anaerobic digesters for both commercial and domestic applications (Chen et al., 2010; De Baere, 2010; Donoso-Bravo et al., 2011; Ferrer et al., 2011). AD is the stepwise breakdown of an organic substrate by a consortium of mutually dependent groups of microorganisms (Fig 1). If the correct conditions are maintained, the AD process will be stable with high energy recovery (Dechrugsa et al., 2013). However, the technology still faces two major challenges: (i) operational instability and (ii) the quality of the digestate produced (Holm-Nielsen et al., 2009; Appels et al., 2011).

Organic substrate selection plays an important role in the stability of an AD system as some feedstocks can have inhibitory effects on AD processes. Substrate-induced inhibition (SII) in AD can occur when the constituent fraction(s) or metabolic intermediate product(s) from organic substrates inhibit microbial activity. These forms of inhibition have been reported for organic substrates containing high amounts of protein, lipids, limonene, furans, metals, pesticides, antibiotics and other organic compounds (El-Gohary et al., 1986; Palmqvist & Hahn-Hagerdal, 2000; Lallai et al., 2002; Wilkins et al., 2007; Alvarez et al., 2010; Sousa et al., 2013; Yangin-Gomec & Ozturk, 2013). SII is either through the direct addition of inhibitory compounds, such as limonene, or indirectly through the production of inhibitory intermediates, such as ammonium and hydrogen sulphide from protein (Table 1). Microbial adaptation to potential inhibitors and co-digestion with two or more substrates are commonly used
to reduce inhibition (El-Mashad & Zhang, 2010; Zhang & Jahng, 2012). During microbial adaptation, the inhibitor can be transformed into metabolites with a similar or lower level of toxicity while the application of co-digestion reduces the concentration of the inhibitor by increasing the ratio of the co-substrate (Athanasoulia et al., 2014). In most cases, AD operators prefer co-digestion of two or more substrates in order to reduce possible inhibition that might result from the treatment of individual feed-stocks (Cheng & Zhong, 2014). However, an alternative approach to reducing inhibition in AD is to remove or reduce the mobility/bioavailability of the inhibitors without affecting with the AD process.

Another major concern with AD is how to retain the nutritive value of the digestate before and after application to land (Mihoubi, 2004; Mangwandi et al., 2013). In most cases, digestate has a high moisture content and in an attempt to reduce this, phase separating equipment is utilised. According to Vaneeckhaute et al. (2013), 43% of the total nitrogen (N) and 25% of the total phosphorus (P) will be lost if the liquid fraction of pig slurry digestate is separated. Further nutrient and metal losses can occur during and after the spreading of the digestate on farmland via transfer to the surrounding watercourses or to the atmosphere. The volatilization of ammonium, leading to ammonia emission, and the leaching of heavy metal as diffuse pollution, are examples of losses that have a negative impact on the environment and crops (Svoboda et al., 2013; Page et al., 2014). Nutrient recovery from digestate has been considered as an option to reduce the nutrient loss from the digestate. However, this approach might reduce the economic value of the digestate (Verstraete et al., 2009; Batstone et al., 2015).
A better approach may be to focus on increasing the nutrient retention capacity of the digestate material. There is a growing interest in the use of biochar in AD to both increase the recovery rate of the process during SII and decrease the nutrient loss before and after land application (Mumme et al., 2014; Dicke et al., 2015; Cai et al., 2016; Lü et al., 2016; Sunyoto et al., 2016). This will potentially increase the operation of mono-substrate AD, which is often used by single substrate onsite AD operators, increase nutrient availability during digestate application to land and reduce the environmental implications of diffuse pollution and nutrient leaching. This review examines substrate-induced inhibition and its impact on anaerobic digestion, nutrient leaching and its environmental implications, and the properties and functionality of biochar material in counteracting these challenges.

2. The Challenges with anaerobic digestion of organic substrate

AD is the breakdown of complex organic material under anoxic conditions by a consortia of microorganisms via a multistep process (Fig 1) (Chen et al., 2008). The microorganisms that drive AD are divided into two groups: (i) acid producers (acidogens and acetogens) and (ii) methane producers (methanogens). These two groups of microorganisms differ physiologically and have different growth rates and sensitivities to operational conditions (Ruiz & Flotats, 2014). The inability to maintain a population balance between these two groups of microorganisms often results in AD process failure. If conditions such as temperature, hydrogen partial pressure, pH and organic loading rate are favourable for both microbial populations, the AD process should be stable (Rudolfs & Amberg, 1952).

In addition to the controls exerted by the operating conditions, the stability of the AD system can also be disrupted if metabolic intermediates of a substrate are
inhibitory to microbial activity (Palmqvist & Hahn-Hagerdal, 2000; Wilkins et al., 2007; Sousa et al., 2013; Yangin-Gomec & Ozturk, 2013). This form of instability is substrate-induced and is called substrate-induced inhibition (SII). According to Ruiz and Flotats (2014), a chemical or metabolite can be termed inhibitory when it causes a shift in microbial population or inhibits microbial activity. There is a wide variety of biodegradable organic materials that have been classified as inhibitory to microbial growth, particularly at higher concentrations (Fig 2 and Table 1). SII can be classified into two categories, direct and indirect sources of inhibition. Direct inhibitors are those that are supplied directly from substrates in the feedstock whilst indirect inhibitors are metabolic intermediates produced during the AD process (Fig 2). The following sections (2.1 and 2.2) describe the types of direct and indirect inhibitors commonly associated with AD and the mechanisms by which inhibition occurs.

2.1. Direct inhibition

As mentioned earlier, direct inhibition in AD results from a constituent of the organic substrate; this implies that the compound is readily available to the microbial cells, thus increasing the risk of AD process failure. The indirect inhibitors are not released until after hydrolysis-acidogenesis and thus they do not pose an immediate threat to the AD process. An example of direct inhibitors include limonene from citrus peel, furans hydrolysate from the chemical pre-treatment of lignocellulose materials, azo-dye from textile production, antibiotics and pesticides. Limonene occurs naturally in citrus peel and reports show that the compound can inhibit the AD process at concentrations of 65-88 g l⁻¹ (Mizuki et al., 1990). Even after the extraction of limonene prior to AD, studies have shown that inhibition of the AD process occurred, particularly when the organic loading rate (OLR) was increased from 3.67-5.10 gVS l⁻¹.
In addition, the co-digestion of orange peel and sewage sludge (70:30) resulted in a methane yield of 0.165 l gVS\(^{-1}\) \(\text{added}\) and the accumulation of volatile fatty acids when the OLR was above 1.6 gVS l\(^{-1}\) d\(^{-1}\) (Serrano et al., 2014). Likewise, furans (furfural, hydroxymethylfurfural (5-HMF)) are produced during the dehydration of pentose- and hexose-sugars locked within the lignin structure (Barakat et al., 2012). These are metabolites from the hydrolysis of lignin but because they are not produced because of the microbial interaction, they are considered to be directly inhibitory. There are indications that the furans are not inhibitory and can be utilised for methane production at a concentration of less than 25 mM (Rivard & Grohmann, 1991; Belay et al., 1997). According to Barakat et al. (2012), the 5-HMF is more inhibitory than the furfural compound because, after incubation of 1 g l\(^{-1}\) of the compounds with 2 g l\(^{-1}\) of xylose separately, methane values of 533 and 583 ml/g were recorded, respectively. Similarly, Monlau et al. (2013) observed that the AD process was severely inhibited at 5-HMF concentration, which was above 6 g l\(^{-1}\). Other direct inhibitors are antibiotics and pesticides, which are present in industrial and pharmaceutical wastewater (Lin, 1990; Ji et al., 2013). Antibiotics such as amoxicillin (0.16 g l\(^{-1}\)), trihydrate (0.06 g l\(^{-1}\)), oxytetracycline (0.12 g l\(^{-1}\)) and thiamphenicol (0.08 g l\(^{-1}\)) have been used to treat pigs and reports show partial inhibition to AD (Lallai et al., 2002). Ji et al. (2013) showed acute toxicity of four antibiotics in the order amoxicillin (0.39 g l\(^{-1}\)), lincomycin (0.43 g l\(^{-1}\)), kanamycin (0.51 g l\(^{-1}\)) and ciprofloxacin (0.56 g l\(^{-1}\)). A noticeable trend common to all direct inhibitors is the similarities in the mechanisms of inhibition. These compounds inhibit the growth of microbial cells as follows: (i) diffusing through the cell membrane; (ii) increasing the surface area of the cell membrane, and (iii) causing
leakage of the contents of the microbial cell (Sikkema et al., 1995; Griffin et al., 1999; Fisher & Phillips, 2008).

2.2. Indirect inhibition

Indirect inhibition is displayed when metabolic intermediates are produced at high concentrations during the AD thereby inhibiting microbial activity. They have been reported to suppress microbial activity and reduce methane production. An examples of indirect SII, their effects and counteracting measures. Metabolic intermediate products are generally produced during the AD process and they depend on the constituent of the substrate (Figure 1). Metabolic products such as acetic acid, hydrogen and carbon dioxide are essential to the AD process and are used to produce methane (Madsen et al., 2011). However, intermediates such as long chain fatty acid, ammonia (NH$_3$) and ammonium (NH$_4^+$) are examples of indirect inhibitors.

Researchers have shown that free ammonia is more toxic than ammonium nitrogen because of its ability to penetrate the cell membrane (Gallert & Winter, 1997; Sung & Liu, 2003). According to Zeshan et al. (2012), an increase in the C/N ratio of the feedstock can minimise the possible effect of high protein feedstock because the addition of carbon will reduce the concentration of nitrogen rich material and also provide alternative metabolic routes thereby reducing the production of NH$_4^+$. They recorded a 30% reduction in the NH$_3$ content of the digestate and 50-73% surplus energy when the C/N ratio of the feedstock was increased to 32. Yangin-Gomec and Ozturk (2013) achieved a 1.2 fold increase in the methane yield when maize silage was co-digested with chicken and cattle manure to suppress ammonia toxicity. As mentioned earlier, protein is essential for microbial growth but at a high concentration, it will increase the possibility of ammonia toxicity. Ammonia is beneficial to the
growth of anaerobic bacteria as long as it does not exceed a certain concentration that can be toxic to methanogenic activity (Angelidaki and Ahring, 1994). Similarly, a substrate high in lipid produces a higher concentration of long-chain fatty acids (LCFAs) and glycerol during hydrolysis. LCFAs (e.g. oleate, stearate and palmitate) can be converted into hydrogen and acetate through the $\beta$-oxidation pathway (Alves et al., 2009). According to Sousa et al. (2013), methanogens can be inhibited by LCFAs at concentrations between of 0.3 and 1 mM. Like LCFAs, the mechanisms of suppression of microbial activity during indirect inhibition are similar (i) diffusing through the cell membrane; (ii) inhibiting methane producing enzymes, and (iii) causing proton imbalance and potassium deficiency (Rinzema et al., 1994; Gallert & Winter, 1997; Chen et al., 2008; Rajagopal et al., 2013; Zonta et al., 2013).

2.3. Acclimation of microbial cells to inhibition

The mechanisms of direct and indirect inhibition are not similar; a general model illustrating the various mechanisms of attack (cell membrane disorder, interference with fermentative pathway and intracellular swelling/leakage) of the microbial cell is represented in Figure 2. SII cannot be avoided during the operation of AD systems, but to some extent the ability of microorganisms to adapt to unfavourable conditions can alleviate the effects of SII. Acclimation is the adaptation of microbial populations to changes in conditions and can be achieved in different ways: (i) synthesis of specific enzymes which were absent prior to exposure to the inhibiting condition; (ii) emergence of new metabolic capabilities/pathway, and (iii) modification of the surface layer of the microbial cell membrane (Liebert et al., 1991; Ruiz & Flotats, 2014). An example of modification of the surface layer of a cell membrane was observed during the exposure of microbial cells to a high dose of limonene; this
resulted in increases in the concentration of unsaturated fatty acids in the cell membrane (Ruiz & Flotats, 2014). Another example has been reported where methanogens were exposed to 2 g l\(^{-1}\) of ammonia and, following a subsequent increase in the concentration of ammonia to 11 g l\(^{-1}\), no inhibition was recorded (Koster & Lettinga, 1988; Borja et al., 1996a). This implies that the microbial cells were able adapt to the unfavourable conditions and further suggests that AD operators should only inoculate their plant with inoculum from an active AD system using a similar substrate. Quintero et al. (2012) showed that the hydrolysis of lignocellulose was more efficient when the feedstock was inoculated with microflora from cattle rumens rather than pig manure. Likewise, Van Velsen (1979) showed that the microbial community in the pig manure inoculum acclimated to 2.4 g l\(^{-1}\) of NH\(_4^+\) while the digested sewage sludge acclimation rate was limited to 1.8 g l\(^{-1}\) of NH\(_4^+\).

3. **Nutrient loss and environmental pollution**

In order to keep up with the increasing demand for food production, soil fertility is maintained by adding fertilizers (Qin et al., 2015). The spreading of anaerobic digestate and compost material on farmland has increased and has become a method of complimenting or replacing synthetic fertilizer usage. In addition, this is driven by changes in agricultural practices and policies that focus on reducing climate change and improving soil quality (Qin et al., 2015; Stoate, 2009; Riding et al., 2015). Anaerobic digestate is rich in minerals, biomass, nitrogen, phosphorus and carbon which are essential for maintaining the soil ecosystem and sustaining increased plant growth (Montemurro et al., 2010; Tambone et al., 2010). In a study carried out by Alburquerque et al. (2012), the effect of digestate on horticulture crop production showed that the application of digestate provided a short term source of phosphorus.
and nitrogen and the microbial biomass and enzyme activities were relatively higher than the non-amended soil. Despite the benefits of utilizing digestate, the risk of atmospheric and water pollution following the application of digestate to land are high (Tiwary et al., 2015). This problem is particular to digestate because of the fast release of nutrients, which is often beyond the utilization rate of the plants and soil microorganisms, thus making leaching and nutrient loss unavoidable. Unlike the digestate, the nutrient content of the inorganic fertilizer is slowly released into the environment, thus reducing the possibility of leaching in relation to organic fertilizers (Basso & Ritchie, 2005; Kim et al., 2014). Digestates with high concentrations of inorganic N are of particular concern due to the high potential for volatilization of NH₃ (Fernandes et al., 2012). Reports have shown that N losses are also significant during the processing of digestate with up to 85% of the NH₄⁺ content emitted as NH₃ gas (ApSimon et al., 1987; Rehl and Müller, 2011). NH₃ is recognised as a major contributor to nitrous oxide (N₂O) production, a biological process carried out by ammonia-oxidizing bacteria (Law et al., 2013). The N₂O is formed as an intermediate product between nitrification and de-nitrification. The microorganisms first convert NH₃ into hydroxylamine (NH₂OH), then into nitrite (NO₂⁻) and finally into N₂O. N₂O is an important atmospheric gas but at high concentrations it contributes to the formation of acid rain and thinning of the ozone layer (Badr & Probert, 1993). Tiwary et al. (2015) reported that the emission of NH₃ may be reduced by 85% if the digestate is introduced into the subsoil but the emission of N₂O is inevitable and it was found to be 2% higher than the other assays because of the contribution of the subsurface denitrifying microorganisms. Another route for nutrient loss from digestate applied to soil is diffuse pollution. Nutrient leaching from the digestate can result in the transfer of N and P to water bodies causing eutrophication (Anthonisen et al., 1976; Soaresa et
Eutrophication itself is a process whereby an ecosystem is transformed through nutrient enrichment from an external source (Conley et al., 2009). Following the increase in nutrients, the growth of certain organisms such as algae, photosynthetic and heterotrophic bacteria increases, thus raising demand for resources which were present during the influx of the external enrichment resources (O'Sullivan, 1995). Accelerated eutrophication of aquatic ecosystems owing to nitrogen and phosphorus enrichment has been reported to have a negative impact on the aquatic life. Firstly, light penetration into the littoral zone is limited thus inhibiting the growth of plant and predators that depend on light for survival; dissolved inorganic carbon is depleted and the alkalinity of the water increases (Lansing et al., 2008). Secondly, after depletion of the nutrients, the algal boom dies and microbial decomposition of the algal biomass depletes the dissolved oxygen, thus creating an anoxic or dead zone (Nagamani & Ramasamy, 1999). In addition, the proliferation of pathogens such as *Ribeiroia ondatrae*, which infects birds, snails and amphibian lava causing limb deformation has also been reported in the literature (Johnson et al., 2007). Apart from nutrients, digestate may also contain metals, particularly heavy metals (Ni, Zn, Cu, Pb, Cr, Cd, and Hg) in varying concentrations (Demirel et al., 2013). Digested sewage sludge is an example of feedstock with high heavy metal concentrations; this places a limitation on its land application (Wang et al., 2005). In Guangzhou, China, the concentrations of heavy metals in wet sludge samples were 4567±143, 81.2±2.8, 148±6, 121±4, 785±32 and 5.99±0.18 mg·kg\(^{-1}\) DM for Cu, Pb, Ni, Cr, Zn and Cd, respectively (Liu & Sun, 2013). Comparing these values with the PAS 110 upper limit standards, which were set at 200, 200, 50, 100, 400 and 1.5 mg·kg\(^{-1}\) DM, only the concentrations of Pb and Zn were below the standard thresholds. German sewage sludge recorded 202, 5, 131, 349, 53 and 1446 mg kg\(^{-1}\) DM for Pb, Cd, Cr, Cu, Ni, Zn and only copper and
nickel were below the standard thresholds (Benckiser & Simarmata, 1994). Amongst the prevalent heavy metals in sewage sludge, Cr, Ni, Cd and Pb have been considered as the most toxic elements in the environment (Lei et al., 2010). When applied to farmland, high levels of these metals in soil can lead to phytotoxicity, which ultimately ends up in the human diet through crop uptake (Islam et al., 2014). The ingestion of heavy metals is associated with health risks and reports show that countries like Bangladesh have high levels of Pb and As in their cereals and pulses (Islam et al., 2014). However, in developed countries, such as the UK, PAS 110 sets a threshold standard for heavy metal concentration in digestate and for operators who cannot meet this standard the digestate resource cannot be spread on farmland.

4. Optimizing the AD process: the use of adsorbent

As mentioned earlier, inhibition in AD has been counteracted with numerous approaches ranging from the acclimation of bacterial cells, adopting thermophilic operating conditions and reducing the concentration of the inhibitors either by dilution or co-digestion with other substrates (Table 1). These approaches do not remove the inhibitor from the process, which can result in accumulation of the inhibitor and the eventual destabilization of the AD system. It is beneficial to look for methods that remove, reduce the mobility or bioavailability the inhibitor within the digestion process (Chen et al., 2008). An example of a technique that can be used to remove potential inhibitors is the steam distillation of citrus peel to remove limonene prior to AD (Martin et al., 2010). Air stripping and chemical precipitation have also been used to remove NH$_3$ and toxic heavy metals, respectively (Chen et al., 2008). There is the possibility that carbonaceous sorbents could also be used to remove contaminants or toxic compounds. This approach is currently employed by industries involved in food,
beverage and textile production and by water companies (Borja et al., 1996b; Palatsi et al., 2012). The use of adsorbents such as bentonite, activated carbon and zeolites in AD has been investigated and the removal of inhibitors has been observed (Angelidaki & Ahring, 1992; Milan et al., 2003; Bertin et al., 2004; Mumme et al., 2014).

Adsorbents are chemically inert materials with adhesive properties that cause the accumulation of atoms, ions or molecules on their surface. This is a surface based interaction between a solid and a fluid; the rate of sorption depends on the adsorbent (the material used as the adsorbing phase) and the adsorbate (the material being adsorbed). There are different types of adsorbent with a variety of applications; some are synthetic whilst others are made from agricultural residues or modified plant and animal material (Angelidaki & Ahring, 1992; Milan et al., 2003; Bertin et al., 2004; Mumme et al., 2014). Biochar is an example of adsorbent made from agricultural residues and because it relatively cheaper to adsorbents like activated carbon, zeolite, and its application is gradually increasing. The subsequent subheading will be focusing on different adsorption mechanisms of the biochar material.

4.1. Mechanisms of biochar adsorption

Adsorption is a dynamic process where the adsorbate associates with the surface of the adsorbent until an equilibrium state is achieved. The process of adsorption can be achieved by (i) adsorbate settling on the surface of the adsorbent (physical adsorption), (ii) adsorbate forming layers on the surface of the adsorbent (surface precipitation and complexation), (iii) condensation of the adsorbate into the pores of the adsorbent (pore filling), hydrogen bonding, electrostatic attraction, ion exchange and hydrophobic effect (Pignatello, 2011). This process occurs in stages: the clean zone (no adsorption), the mass transfer zone (adsorption in progress) and the
exhausted zone (equilibrium), (De Ridder, 2012). Furthermore, the saturated and clean
zones will increase and decrease respectively but the mass transfer zone will remain
unchanged unless the concentration of the adsorbate is increased. When the material is
passed through the adsorbent, it associates with the first section of the adsorbent
before moving to another section. This trend continues until the adsorbent is nearly
saturated; the near saturation point is called the breakthrough point (Moreno-Castilla,
2004).

Figure 3 shows the mechanisms of adsorption of organic and metal adsorbates.
For metals adsorption largely occurs through electrostatic attraction, ion-exchange and
precipitation onto the surface of the adsorbent. For organic molecules, important
mechanisms are hydrophobic interactions and hydrogen bonding (Figure 3). Another
mode of adsorption that is common for organic compounds is the van de Waals force
of attraction. This form of adsorption is induced by the surface chemistry of the
biochar. Brennan et al. (2001) showed evidence of different functional groups such as
nitro, chloro, hydroxyl, amine, carbonyl, and carboxylic on the surface of biochar.
This form of sorption can be described as the electron donor-acceptor mechanism
(Mattson et al., 1969). The uneven distribution of electrons between the adsorbent
functional group and the organic compound creates an electron donor-acceptor
relationship. However, for complex organic compounds with substituent groups
(nitro- and chloro-) the electron density of the interaction between the compound and
the adsorbent is greatly reduced and this increases the electrostatic interaction between
them (Cozzi et al., 1993). This is because the substituent group in the compound is a
stronger electron acceptor (Dubinin, 1960; Liu et al., 2010).

4.2. Controls on biochar adsorption processes
The factors that influence the performance of adsorbent during adsorption have been
extensively reported in literature. These parameters include the contact time, operating
temperature, adsorbent and adsorbate dosage, particle size and pore distribution,
surface chemistry and pH (Li et al., 2014; Hadi et al., 2015; Yargicoglu et al., 2015).

4.2.1. Structure and pore size
Adsorbent materials contain pores of various sizes, which have been categorised into
micropores, mesopores, and macropores. Based on the size of the various pores, the
sorption rate of the adsorbate is expected to increase in this order: macropores >
mesopores > micropores, although this also depends on the size of the adsorbate
(Zabaniotou et al., 2008). Biochar material has been reported to have an abundance of
micropores, which have a high capacity for adsorbate and water uptake (Zabaniotou et
al., 2008). As mentioned earlier, the size of the adsorbate also has some effect on the
rate of sorption (Duku et al., 2011). For example, if the size of the adsorbate is
relatively large or there are fewer sites for diffusion, this might be affected by steric
hinderance (Liu et al., 2010). Further, large adsorbate size can cause exclusion or
blockage of smaller sorption sites (Duku et al., 2011). Studies have shown that smaller
particle sizes reduce the mass transfer limitation and increase the van der Waal or
electrostatic force for penetration of the adsorbate inside the adsorbent (Daifullah &

4.2.2. Surface chemistry and pH
The functional groups on the surface of the biochar will influence the adsorption rate.
For instance, biochars derived from sewage sludge and poultry manure have higher
amounts of nitrogen and sulphur functional groups than woody biomass materials
Brennan et al. (2001) reported the presence of different functional groups on the surface and pores of biochar, including hydroxyl, amine and carboxylic groups. The surface chemistry of a carbonaceous sorbent can change, particularly when it is immersed in water; these changes are attributed to the chemical characteristics of the adsorbent (functional groups or ionic compound present in water) and the pH of the solution (Moreno-Castilla, 2004). As illustrated in Figure 4, at higher pHs, phenolic and carboxylic groups release protons and obtain a negative charge, while at low pH basic functional group, such as amine, take up a proton and obtain a positive charge (Schwarzenbach et al., 2005). This implies that the adsorption behaviour of asorbent is a function of the pH of the medium. Changes in the pH can have significant impacts on the ability of a material to adsorb certain compounds. For example, soluble mercury species can be easily adsorbed at higher pHs, whereas lowering the pH increases the solubility of mercuric ions (Eligwe et al., 1999).

Changes in pH may also result in reductions in the electrostatic force between the adsorbate ions and the adsorbent (Rao et al., 2009).

4.2.3. Hydrophobicity

The presence and number of O- and N-containing functional groups determine the hydrophobic nature of biochars. Biochars with less O- and N-containing functional groups are typically less hydrophobic (Moreno-Castilla, 2004). Hydrophobic interactions are believed to contribute to the sorption of insoluble adsorbates (Moreno-Castilla, 2004). In aqueous solutions, the adsorbate with the least solubility has the tendency to be adsorbed and retained in the pore of the adsorbent. According to Li et
al. (2003), removal of adsorbates, such as 2-propanol, is higher with β-zeolite than dealuminated β-zeolite because the latter is less hydrophobic. Equally, Li et al. (2002) showed that hydrophobic activated carbon is more effective in the removal of relatively polar methyl tertiary-butyl ether (MTBE) and relatively nonpolar trichloroethene (TCE). The hydrophilic adsorbents are less effective because of the sorption of water, which in turn reduces the available sites for the adsorbate-adsorbent interaction (Li et al., 2002).

4.3. Mechanisms of desorption or regeneration

Adsorbents are useful for separation applications, especially in the purification of wastewater and gaseous compounds. However, the progressive accumulation of adsorbate on the surface of the adsorbent will reduce its sorption capacity until the breakthrough point and finally equilibrium (Salvador et al., 2015). However, the regeneration of the adsorbent gives it an economical advantage over other separation methods and numerous regeneration methods have been developed (Lu et al., 2011; Martin & Ng, 1987; Salvador et al., 2015). Regeneration pathways involve the removal of the adsorbate from the adsorbent. These have been demonstrated using chemical reagents, water, hot gases, ozone, superficial fluid, electric current and microorganisms (Salvador et al., 2015).

In AD the application of water in regeneration is not efficient because water is not a good solvent of organic material and in the process of regeneration the water is polluted with the contaminant. Chemical regeneration employs the use of reagents such as NaOH to remove contaminants, or to change the pH of the adsorbent so that non-reactive chemicals like aniline and dye can be desorbed (Leng & Pinto, 1996). However, chemical agents are expensive and the chances of environmental pollution
are often high. Supercritical fluid regeneration employs a combination of pressurised 
CO$_2$ and water at 125-250 bar to desorb benzene, naphthalene and phenol from 
activated carbon (Chihara et al., 1997; Tan & Liou, 1989). However, this approach is 
energy intensive. Another approach called ozone (O$_3$) regeneration employs the O$_3$ in 
direct oxidation of the contaminant. The hydroxyl and oxygen radicals are very 
reactive and able to oxidize the contaminant. There are indications of moderate 
efficiency of 80-90% when O$_3$ is used because some of the oxidative product might 
block the pore sites (Valdés et al., 2002). Unlike the other regeneration methods 
mentioned earlier, the biological approach is the most economical and 
environmentally friendly because it employs the activities of microorganisms in the 
regeneration of the adsorbent. For instance, the biological activated carbon added to 
activate sludge in wastewater treatment improves the simultaneous sorption and 
biological degradation of the contaminant under aerated conditions (Xiaojian et al., 
1991). Another approach to the microbial regeneration of an adsorbent is the 
inoculation of an exhausted adsorbent with microorganisms. This approach has been 
reported to be less effective because of the eventual blockage of the pore entrance by 
colonies of microorganisms. (Hutchinson & Robinson, 1990; Toh et al., 2013). 
Perhaps the application of water solvent as a backwash can be used to supplement the 
microbial regeneration of exhausted adsorbent. Considering that the level of 
contamination from SII is relatively lower and less recalcitrant when compared to 
wastewater industries, biological regeneration could be easily achieved but this needs 
to be optimized with solvent backwash.

5. The role of biochar in anaerobic digestion

5.1. Biochar
Biochar is a soil additive produced from the thermal degradation of organic material in the presence of little or no amount of oxygen, a process known as pyrolysis (Shafizadeh, 1982). During pyrolysis the volatilization of the organic matter increases, the pore sizes enlarge and the structure of the biomass is rearranged Lua et al. (2004). Factors such as biomass retention time, the properties of the biomass and the operational parameters can influence the final structure of the biochar (Lua & Guo, 2000). Novakov (1984) describes biochar (or black carbon) as “combustion produced black particulate carbon having graphitic microstructure”. Biochar is a carbonaceous, porous and carbon stable material but it is distinctly different because it is produced at a lower temperature (< 700 °C) without any form of activation (Schulz & Glaser, 2012). This makes the surface area of the biochar less efficient than that of the activated carbon but in terms of production cost, biochar is cheaper (Lehmann & Joseph, 2012). Biochar material is attracting attention as a means of improving plant growth and cleaning contaminated water and land (Tan et al., 2015). Apart from the direct benefits of plant growth and the cleaning-up of polluted ecosystems, biochar can serve as carbon storage, thus contributing to the mitigation of climate change (Montanarella & Lugato, 2013). Biochar material is stable and like other carbon capture technologies it can ensure long-term storage of carbon and reduced CO₂ emission (Woolf et al., 2010). The use of biochar as an adsorbent in AD has not been fully investigated as yet, but there is potential for it to have a positive impact both on the operational stability of the AD process and the quality of the digestate produced (Mumme et al., 2014). The continuous addition of biochar during AD can be suggested to reduce SII and increase process stability in three ways: (i) through the sorption of inhibitors, (ii) by increasing the buffering capacity of the system, and (iii) through immobilization of bacterial cells. In addition, the application of biochar can
be extended to the improvement of digestate quality. The addition of biochar to digestate can contribute to nutrient retention, increase the carbon to nitrogen ratio and reduce nutrient leaching after land application of the digestate mixture (Figure 6).

5.2. Adsorption of inhibitors

Inhibitors, such as LCFA, ammonia, limonene, heavy metals and phenols, are either degraded or transformed into other metabolites and these metabolites can be as inhibitory as their precursors (Duetz et al., 2003). There is the opportunity for microbial acclimation to inhibitory compounds, but for most commercial operators there are cost implications of waiting for the whole consortia of cells to acclimate. The application of an adsorbent such as biochar creates an alternative route for removing and reducing the effect of SII during AD. This is because there are indications that biochar can sorb heavy metals and other organic compounds like pesticides, furfural and limonene (Kılıç et al., 2013; Taha et al., 2014; Hale et al., 2015). According to Komnitsas et al. (2015), 10 g l\(^{-1}\) biochar produced after pyrolysis at 550 °C was able to remove 0.015 g l\(^{-1}\) of Cu and Pb with almost 100% removal efficiency. Likewise, biochar has been shown to sorb organic compounds. For instance, in the amendment of polycyclic aromatic hydrocarbons in sewage sludge, when compared to the expensive activated carbon material, biochar does not have a greater effect with regard to sorption (Oleszczuk et al., 2012). Taghizadeh-Toosi et al. (2012) showed that biochar can adsorb NH\(_4^+\) and remain stable in ambient air but on exposure to the soil the NH\(_4^+\) is made bioavailable for plant uptake. Lü et al. (2016) equally reported that the application of biochar alleviate NH\(_4^+\) inhibition during anaerobic digestion of 6 g l\(^{-1}\) of glucose solution at an NH\(_4^+\) concentration of 7 g l\(^{-1}\). In addition, a recent report by Chen et al. (2015) showed that biochar can also be deployed to contaminated fields
because of its affinity for polycyclic aromatic hydrocarbons. The sorption capacity of biochar with different organic and inorganic materials has been extensively reported in the literature but with regard to most inhibitory compounds during AD it has not been well documented (Mohan et al., 2014). This may be attributed to the uncertainty surrounding the addition of biochar to AD systems. Adsorbents like biochar are not selective during sorption; hence, there is the possibility that some of the nutrients or useful metabolites will be adsorbed during the AD process (Mumme et al., 2014). This may not pose a major issue as a proportion of the material trapped within the pores of the adsorbent can be metabolised by the microorganisms attached to the adsorbent surface. In order to avoid nutrient or metabolite fouling of the biochar pores, the organic substrate can be pre-treated with the biochar before AD. However, this approach might limit the benefits of applying biochar with regard to the removal of only direct forms of SII.

5.3. Increasing buffering capacity

Alkalinity is a measure of the reactor’s liquid capacity to neutralise acids, i.e. absorb hydrogen ions without a significant pH change. Alkalinity is produced in AD through the degradation of some feedstocks and alkalinity is lost through the production and accumulation of VFAs. The accumulation of acid is an expected occurrence during AD, but in the event of high organic overloading and microbial inhibition, the accumulation of VFA can reduce the buffering capacity of the system (Chen et al., 2008; Rétfalvi et al., 2011). Nonetheless, the buffering capacity of an AD process can be increased or maintained by adding some alkali compounds or by controlling the OLR (Ward et al., 2008). A biochar material can be alkaline depending on the biomass source (Gul et al., 2015). Yuan et al. (2011) showed that the alkalinity of a
biochar increases with an increase in the pyrolysis operating temperature. The application of biochar for the purpose of increasing the buffering capacity is not well known, but this could be recognised as one of the benefits of adding biochar to the AD process. For instance, most operators usually add lime to the AD system to combat acidification. However, the continuous addition of alkaline biochar could increase the buffering capacity of the system (Cao et al., 2012; Zhang et al., 2014). A study by Luo et al. (2015), which compared biochar and non-biochar incubation using glucose as a substrate, showed that the biochar containing incubation increased the methane yield by 86.6% and reduced acidification. Likewise, Sunyoto et al. (2016) reported that the application of biochar not only support microbial metabolism and growth but buffered pH during bio-hydrogen production.

5.4. Immobilization of microbial cell

Immobilization refers to the colonization of microbial cells on the surface of a solid material. The conventional methods for the immobilization of microbial cells are the use of entrapments such as gels, and physical adsorption to a solid surface, but this approach is limiting because of poor mass transfer (Hori et al., 2015). The discovery of naturally occurring immobilized cells called biofilms has received more attention because it allows the colonization of microbial cells on polymerised surfaces (Cheng et al., 2010). The immobilization of microbial communities in AD is important, particularly for the methanogens because it facilitates electron transfer between interspecies (Lü et al., 2014). One of the benefits of cell immobilization is to reduce biomass washout, an occurrence that is common with wastewater treatment. Anaerobic digesters such as fixed and fluidised beds have been designed with support media to increase and retain the growth of microbial cells (Fernandez et al., 2007).
Another advantage of using an immobilized cell is the acclimation rate of the microbial cell during SII (Chen et al., 2008; Montalvo et al., 2012). Sawayama et al. (2004) compared dispersed and immobilised cells, and observed that the biomass and methane production rate of the immobilised cells were higher even at an ammonium concentration of less than 6 g l$^{-1}$. Furthermore, immobilization of microbial cells has also been reported to reduce the distance between syntrophic bacteria and methanogens (Zhao et al., 2015). It has been reported that a distance of less than 1 µm is essential for the oxidation of volatile fatty acids and hydrogen production (Stams, 1994; Schink, 1997). Cell immobilization is often achieved when a bacterial cell is able to attach or grow on a support material. Support materials such as zeolite, clay, activated carbon and other plastic materials have been used to support microbial attachment and growth (Borja et al., 1993; Sawayama et al., 2004; Chauhan & Ogram, 2005; Bertin et al., 2010). The macropores aid the attachment of bacterial cells (Laine et al., 1991). Although, the application of biochar for cell immobilization is not as extensive as most other adsorbents, there is an indication that the macropores enhance the attachment of bacterial cells (Watanabe & Tanaka, 1999). Luo et al. (2015) observed the colonization of *Methanosarina* on biochar material during the AD of glucose solution and when compared to the non-biochar study, methane production was higher by 86.6%. There are several reports that shows that the addition of biochar increases microbial metabolism and growth, because the material provides favourable support (Cai et al., 2016; Lü et al., 2016; Sunyoto et al., 2016).

**5.5. Nutrient retention**

The management of digestate is attracting attention currently because it contains useful amounts of micronutrients, ammonium, phosphate, metal and organic material,
hence making it a good soil conditioner (Sapp et al., 2015). Using a circular economic approach where food waste is returned to land as a resource reduces the dependency on inorganic fertilizer, improves the soil ecosystem and provides an alternative source of phosphorous, which is currently limited (Hendrix, 2012; Zeshan & Visvanathan, 2014). Depending on the characteristics of the organic substrate and the stability of the AD process, the nutrient content of the digestate will vary. However, as mentioned earlier, a major problem with spreading digestate on land is leaching as this causes diffuse pollution to watercourses or the emission of residual CH$_4$ and NH$_3$ gas into the environment (Menardo et al., 2011). In order to reduce diffuse pollution resulting from digestate application to land, the C/N ratio of the digestate must be adjusted and the season of application must be considered (Zeshan & Visvanathan, 2014). However, these approaches are not solely effective because of the slow rate of microbial processes in soil thus extending the chances for nutrient loss from applied digestate via leaching or changes in the soil conditions (Alburquerque et al., 2012). The addition of biochar during or after AD can potentially improve nutrient retention and reduce leaching of digestate nutrient.

Studies examining the interactions between biochar and digestate have shown that the addition of biochar to digestate before land application increases the retention period of the nutrients for plant and bacterial uptake (Marchetti & Castelli, 2013; Eykelbosh et al., 2014). Biochar material was found to allow the sorption of organic matter and inorganic nutrients (Lehmann & Joseph, 2012). The surface of biochar is complex with pores containing metallic and organic compounds; this property is essential in the sorption behaviour of biochar. Research has shown that biochar can adsorb organic substrates, phosphate, nitrate, nitrite, ammonium, metals and carbon dioxide (Bagreev et al., 2001). According to Koukouzas et al. (2007) some biochar
material may contain metal oxides (MgO, CrO, CaO and Fe₂O₃) on its surface or pores and this induces the adsorption of NH₄⁺, thus reducing leaching and diffuse pollution (DeLuca et al., 2006). Le Leuch and Bandosz (2007) showed that the sorption of ammonium by biochar immobilizes the ammonium concentration in soil thus reducing the volatilization of ammonium to ammonia under alkaline conditions and during temperature changes within the soil. DeLuca et al. (2006), observed that ammonification reduction was higher in soil containing biochar and this would only have been possible due to the slow release of the ammonium compound. The advantage of this behaviour to the soil is that it immobilizes organic nitrogen within the pores and reduces nutrient loss during leaching thus making nutrients readily available over a longer term. An additional environmental benefit of nutrient sorption by biochar is the potential to mitigate the microbial production of N₂O following digestate application. Dicke et al. (2015) studied the effect of biochar material and digestate on N₂O fluxes under field conditions and showed that the addition of biochar reduced N₂O emissions, although the emission of N₂O was mostly influenced by temperature and precipitation. It could be argued that the higher specific surface area of the activated carbon is better than the biochar material thus making it a more reliable resource for microbial cell immobilization and the sorption of contaminants (Wang & Han, 2012). However, because biochar is cheaper to make there is no need to recover the material after the AD process and this will increase the value of the digestate.

6. Conclusions.

The application of biochar has the potential to improve AD process by counteracting SII, improve digestate quality through nutrient retention, contributing to the buffering
capacity of the system and create a surface area for the colonization of microbial cell. Comparatively, these functions can be achieved by another adsorbent like activated carbon with higher efficiency. However, the production of biochar is cost effective hence AD operators can afford to use the material without any need for recovery and this will further encourage the spreading of biochar and digestate on land. Biochar was not primarily designed for AD, hence future research in the interaction between biochar and AD microbes, buffering capacity of biochar during AD and sorption effect of biochar material on the AD using a continuous-fed digestion process should be investigated.

Acknowledgements

This work was supported by the Centre for Global Eco-innovation (X02646PR) and Stopford Energy and Environment.


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Table

Table 1: Inhibitors, their functions, effects and exiting counteracting methods

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Function</th>
<th>Inhibition</th>
<th>Counteracting methods</th>
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<tr>
<td><strong>Direct inhibitor</strong></td>
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<tr>
<td><strong>Heavy metals (Cu^{2+}, Zn^{2+}, Cr^{3+}, Cd, Ni, Pb^{4+} &amp; Hg^{2+})</strong></td>
<td>Part of essential enzymes and drives anaerobic enzymatic reactions</td>
<td>The order of inhibition to the acetogens (Cu&gt;Zn&gt;Cd&gt;Ni&gt;Pb) and methanogens (Cd&gt;Cu&gt;Cr&gt;Zn&gt;Pb&gt;Ni) (Lin, 1993)</td>
<td>Production of hydrogen sulphide to precipitate as metal sulphide (Gadd &amp; Griffiths, 1977) Co-digestion with another substrate (Pahl et al., 2008) Retention of metal on the cell wall (Jankowska et al., 2006) Lowering permeability of the cell membrane (Jankowska et al., 2006)</td>
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<tr>
<td><strong>Light metals (Na(^+), K(^+), Mg(^{2+}), Ca(^{2+}), and Al(^{3+}))</strong></td>
<td>Required for microbial growth Enhances bacterial cell immobilization (Ca) (Thiele et al., 1990; van Langerak et al., 1998) Formation of adenosine phosphate (ADH) (Na(^+))(Dimroth &amp; Thomer, 1989)</td>
<td>Restrict production of double cells (Mg(^{2+})) Neutralize cell membrane potential (K(^+))(Jarrell et al., 1987) Inhibit acetoclastic methanogens (Na(^+)) Precipitation of carbonates and phosphates thus destabilizing the buffering system (Ca(^{2+})) (van Langerak et al., 1998) Competition with adsorption of other metals (Al(^{3+})) (Cabirol et al., 2003)</td>
<td>Acclimation of bacterial cell (Chen et al., 2008) Na(^+), Mg(^{2+}) and NH(^4) mitigate potassium toxicity(Chen et al., 2008)</td>
</tr>
<tr>
<td><strong>Chlorophenols and Halogenated aliphatic</strong></td>
<td>Reduction of pathogens Interference with cellular energy transduction Disruptions of proton gradient through the cell membrane (Chen et al., 2008) Methanogens are greatly inhibited (Chen et al., 2014)</td>
<td></td>
<td>Removal of contaminant using activated carbon (Liu et al., 2010)</td>
</tr>
<tr>
<td><strong>Pesticides and antibiotic</strong></td>
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<tr>
<td></td>
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<td>Removal of contaminant using biochar (Yao et al., 2013)</td>
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<tr>
<td><strong>Lignocellulose</strong></td>
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<td></td>
<td>Acclimation of the bacterial cell (Palmqvist &amp; Hahn-)</td>
</tr>
<tr>
<td>compound</td>
<td>indirect inhibitor</td>
<td>Volatile fatty acids (VFAs)</td>
<td>Methane production</td>
</tr>
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\[
SO_4^{2-} + 4H_2 = H_2S + 4H_2O + 2OH^{-}
\]

\[
SO_4^{2-} + CH_3COOH = H_2S + 2HCO_3^{-}
\]

\[
NH_4^+ + OH^- \approx HCO_3^- + H_2O
\]

\[
CO_2^+ + H_2O + OH^- = HCO_3^- + H_2O
\]
Figure captions

Figure 1 Schematic representation of the anaerobic digestion process (Amaya et al., 2013)

Figure 2 A model of mechanisms of a chemical attack on the bacterial cell (Ibraheem & Ndima, 2013).

Figure 3 Summary of proposed mechanisms for adsorption on biochars (Adapted from; Tan et al., 2015)

Figure 4 Macroscopic representation of the features of carbon surface chemistry (Radovic et al., 2001)

Figure 5 The potential benefits of biochar in enhancing anaerobic digestion and digestate quality
Figure 1
Figure 2
Figure 3
Figure 4
Figure 5