Role of Biochar in Anaerobic Fermentation of Kitchen Waste

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Abstract. Food waste has high moisture content and organic matter content. If it is not treated in a timely manner, it will have a very terrible impact on people's living environment and health, and it is simple to breed pathogenic microbes, bacteria and other chemicals that are hazardous to the human body. Anaerobic fermentation of kitchen waste can produce methane while processing kitchen waste, realizing resource utilization. However, in the process of anaerobic fermentation, there is poor system stability, low efficiency, and prone to acid inhibition. The development of an efficient treatment method has become a current research hotspot. Due to its physicochemical properties, biochar can effectively alleviate the phenomenon of acid inhibition and improve the efficiency of anaerobic fermentation. As a result, adding biochar to an anaerobic fermentation system has a positive effect on system operation. In this research, effects of biochar as an additive on anaerobic fermentation systems and their mechanisms were analyzed from three aspects, including anaerobic fermentation system stability, interspecific direct electron transfer (DIET) and microbial community. And prospected the aspects to be further studied in the application of biochar for the anaerobic fermentation of kitchen waste.

Keywords: Food Waste, Anaerobic Fermentation, Biochar, Application.

1. Introduction

Food waste refers to food residues, food processing wastes and waste edible oils produced by restaurants, restaurants and canteens of enterprises and institutions, which is the main component of urban organic solid waste [1]. It is estimated that by 2025, the world will generate 220 million tons of food waste. As of 2020, the average amount of kitchen garbage produced annually in China is 158 million tons. And due to the country's fast urbanization, this amount is continually increasing. Traditional food waste disposal methods include sanitary landfill, incineration, feeding livestock and aerobic composting, etc. Compared with incineration or landfill, anaerobic fermentation has the advantages of less secondary pollution, low energy consumption, and recovery of methane and hydrogen with greater energy and economic effects. It is currently the most potential biological treatment method for kitchen waste.

However, there are still three key problems in the anaerobic fermentation process of food waste. Due to the system being overly acidic from the buildup of volatile fatty acids (VFAs) brought on by the quick decomposition of organic materials during anaerobic fermentation, the high concentration of ammonia caused by the decomposition of nitrogen-containing organic matter can inhibit the growth of methanogens' activity, thereby affecting the stability of the anaerobic fermentation system. The flora is more sensitive to the reaction conditions, and changes in external conditions can easily lead to low utilization of product substrates and instability of the system. The rate of methane is slow and the efficiency is low. Common measures to improve the performance of anaerobic fermentation mainly include mixed fermentation, pretreatment, adding trace elements and improving the reactor. In order to reduce the buildup of intermediate products during separate fermentation, such as ammonia nitrogen and VFAs, mixed fermentation involves mixing food waste with other substrates that have a high methane generation potential. Pretreatment uses physical, chemical and biological methods to destroy cells, increase organic matter inside and outside cells and increase methane production. The issue that the enzymatic reaction of anaerobic microbes and the activity of methanogens are suppressed due to the system's lack of trace elements can be resolved by adding trace elements. The improved reactor method is to carry out the acid production stage and the methane production stage in two reactors respectively, which promotes the development and reproduction of microorganisms at every stage and speeds up the system's reaction rate.

Through pyrolysis and carbonization, biomass macromolecules are broken down into small molecules under aerobic or anaerobic conditions, and carbonization results in the production of solid biochar with controllable structure. Biochar is widely used in agriculture and environmental science due to its high alkalinity, rich functional groups and pore structure, and large specific surface area [2]. For example, the application of biochar in carbon sequestration and emission reduction, plant growth conditioning, soil microbial control, water pollution control, etc. In addition, biochar can also be used in food waste anaerobic fermentation systems. Adding different biochar to the system can not only alleviate the phenomenon of acid inhibition and ammonia nitrogen inhibition, but also have an impact on the composition of microbial communities, enhance methanogen activity, and then increase the methane production rate and the system stability. At present, in the process of increasing the anaerobic fermentation of food waste's ability to produce methane, anaerobic fermentation can be made substantially more effective by using biochar as an ingredient. Relieve acid inhibition, high ammonia nitrogen concentration inhibition, increase methane production, etc. The main mode is to increase the anaerobic fermentation system's stability and to encourage species-to-species electron transfer, and affect the microbial community. In biochar-added anaerobic fermentation systems, microbial activity plays a key role in maintaining process stability and methanogenesis efficiency, and studying microbial communities is beneficial for dissecting microscopic processes of anaerobic fermentation [3]. Especially as biochar is one of the main measures to strengthen anaerobic fermentation, the research on its internal mechanism is worth in-depth discussion and summary.

As a result, this research will discuss the effect of the added biochar on the methane production efficiency for food waste anaerobic fermentation. What's more, the impact mechanism of biochar on the anaerobic fermentation of food waste will be systematically analyzed, including anaerobic fermentation system stability, interspecific electron transfer and the change in microbial community. Finally, this research will prospect the application of biochar in the anaerobic fermentation of food waste.

2. Effect of biochar on anaerobic fermentation system

Biochar is a solid charcoal produced by incomplete combustion of biomass under anoxic or anaerobic conditions. It has low solubility, high hydroxy esterification and aromatization structure, and has a certain specific surface area, rich in a variety of trace elements and small molecular organic matter and other characteristics. The performance of the biochar can be affected by its surface functional groups, surface roughness and surface free energy. For example, surface functional groups affect the pH of materials, surface roughness and surface free energy affect the adhesion and colonization of biochar to microorganisms. Materials with rough surfaces can increase the contact area with microorganisms, while bacteria and archaea in anaerobic fermentation systems are more likely to colonize materials with low surface free energy. The particle size and pore structure of biochar also affect the colonization of functional microorganisms such as fermentation bacteria and methanogens on the surface of the material. Therefore, the physicochemical properties of biochar have an important impact on the anaerobic fermentation system, and the impact mechanism will be elucidated from three aspects: system stability, interspecific electron transfer, and microbial community (Figure 1)

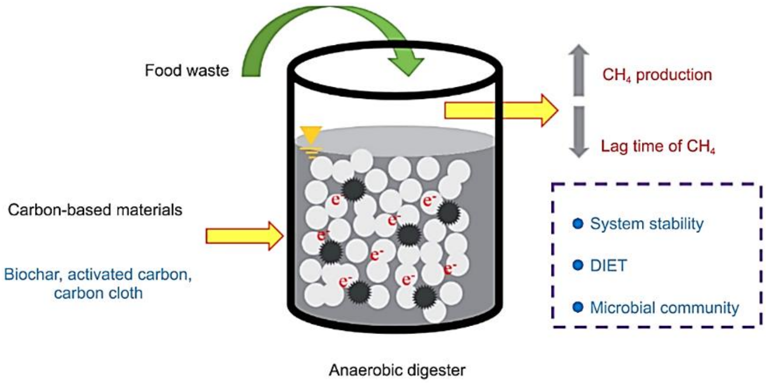


Figure 1. Effects of the added carbon materials on the anaerobic fermentation of food waste [1]

2.1. System stability

Increasing the organic load (volatile solid, VS) of anaerobic treatment can effectively improve the efficiency of food waste treatment, but the increase of organic load will directly lead to serious acidification of the system. As shown in Figure 2, the pH is an important factor affecting the microbial activity in anaerobic digestion system. Methanogen activity is constrained, and VFA consumption ceases, and acidogenic bacteria can still create acidic intermediates. VFAs accumulation occurs when the production of VFAs in the system exceeds the utilization capacity of methanogens. The added biochar can be used to change the pH at the initial stage of the reaction, which make the pH return to the range suitable for the growth of methanogens more quickly and promote the methanogenesis process of the system.

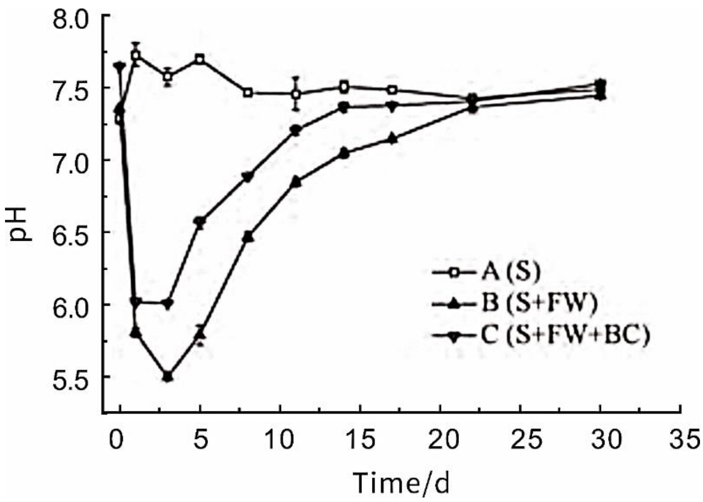
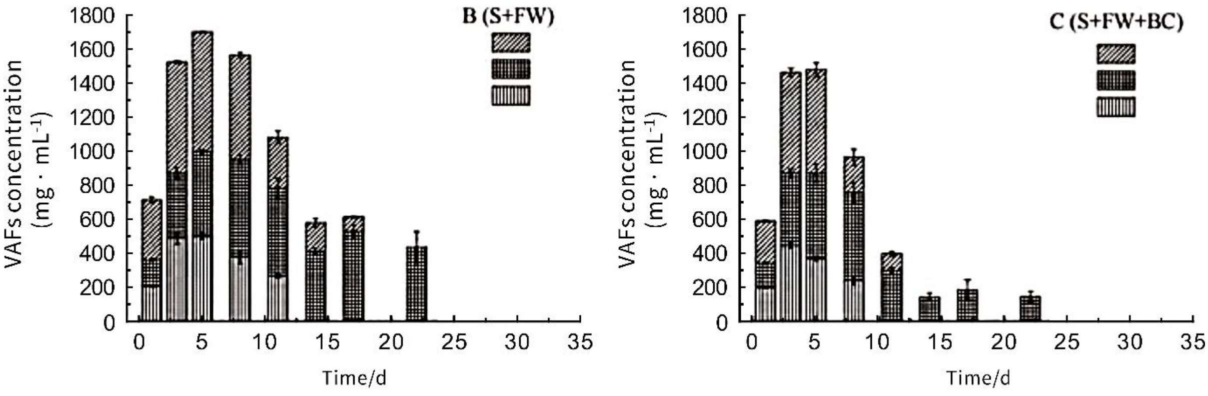


Figure 2. Variation of pH in each group [4]

The surface morphology of biochar is an influencing factor for the efficiency of anaerobic digestion and fermentation. Xu et al. [2] found that biochar enhanced the CH_4 yield during anaerobic fermentation of glucose and ethanol. The archaea and bacteria mainly grow on the rough outer surface of biochar and accelerate the consumption rate of VFAs produce more CH_4 (Figure 3), shorten the anaerobic fermentation to reach time required to produce maximum daily methane, and increase the maximum daily methane production. The physical and chemical properties of biochar have a great impact on anaerobic fermentation, and the pore structure of biochar might offer microorganisms a specific growth site and an ideal growing environment, thereby fostering their growth and metabolism and accelerating the consumption of VFAs. In addition, biochar's surface functional groups and metal ions can enhance the material's alkalinity and pH, thereby buffering the acid inhibition produced by anaerobic fermentation. Methanogens are encouraged to multiply and become more active. The existing functional groups the biochar surface may be effectively reflected using Fourier transform infrared spectroscopy.



(a) Changes of VAFs acid with time in group B (b) Changes of VAFs acid with time in group C

Figure 3. Changes of VAFs acid in different conditions [4]

In addition, anaerobic fermentation of food waste might be unstable depending on the ammonia nitrogen content, and the total ammonia nitrogen is composed of ammonium ions and free ammonia. For example, high concentrations of free ammonia caused by the decomposition of proteins and other organic nitrogenous substances are one of the reasons for the disturbance or even failure of the anaerobic fermentation process, and high concentrations of free ammonia can inhibit the growth of methanogens. Few researches have focused on how adding biochar affects the amount of ammonia nitrogen in anaerobic fermentation systems, and the added biochar can promote the methanation process under the inhibition of high concentration of ammonia. The microbial immobilization of biochar can reduce the inhibition of ammonia nitrogen in the anaerobic fermentation system, which is beneficial to the stability of the system. Compared with the biochar reaction performance in the absence and presence of biochar, Sasaki et al. found that biochar can be used for the stable proliferation of acetate-trophic methanogens by alleviating ammonia inhibition [3], which in turn improves the organic compounds degradation and methane production efficiency.

2.2. Electron transfer between species

Food waste has a high organic content. Macromolecular organics, such as starch, protein and lipid, are first decomposed into small molecular substances by hydrolyzing bacteria during the anaerobic fermentation process, and it is further degraded into VFAs. The rate and pathway of electron transfer between microorganisms determine the efficiency of anaerobic fermentation, and there is a balanced complementary relationship between acetogenic and methanogenesis. The hydrogen partial pressure of the system can be maintained at a low level only when the hydrogen produced by the acetogens is effectively utilized by the methanogens in the anaerobic system, where propionic acid and butyric acid are two VFAs whose breakdown can only occur naturally. Therefore, the mutualistic relationship between acetogens as electron donors and electron acceptor methanogens is a necessary process to convert macromolecular organic matter to CH_4 and CO_2. Interspecies electron transfer (IET) is crucial for the interaction of microorganisms between organizations. It is also a crucial phase in anaerobic fermentation for the formation of methane. Compared to indirect interspecies electron transfer (IIET), the direct interspecies electron transfer (DIET) is more stable and faster with H_2 or HCOOH as carrier, to maintain a stable and efficient mutual business relationship between acetogenic bacteria and methanogenic bacteria. DIET was originally discovered in a co-culture system of *Geobacter* species such as *Geobacter metallireducens* capable of transferring electrons from ethanol to *Geobacter sulfurreducens* through conductive pili. Studies have found that DIET can occur in a co-culture system of *Geobacter* and acetotroph methanogens (such as *Methanosaeta* and *Methanosa Rcina*) using ethanol as a substrate. Rotaru et al. have confirmed that *Methanosaeta harundinacea* can directly accept electrons from *Geobacter metallirecucens* for methane production [5]. DIET mainly includes two pathways: direct contact between cells and mediated through conductive materials. The former means that electrons released by electron donor microorganisms pass through C-type cytochromes and conductive fimbria--nanowires are directly transferred to electron acceptor microorganisms.

Biochar is conductive and can act as a carrier to store and transfer electrons to provide better interspecies electronic connections for microorganisms, speedier electron transport through carbon-based materials from electron providers (fermentation bacteria) to electron acceptors (methanogens), which encourages methane synthesis (Figure 4). The biochar can form a non-biological conductive network to promote DIET between microorganisms without conductive pili and C-type cytochromes, thereby promoting mutual metabolism between microorganisms. And also, the added biochar can be used to increase the conductance of the leachate, and in combination with the methanogenesis situation, biochar may further improve the methanogenesis process by promoting DIET between anaerobic microorganisms.

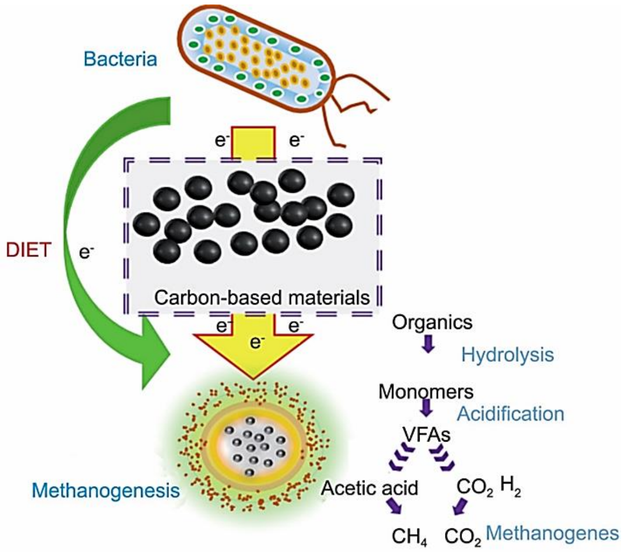


Figure 4. Mechanism of carbon-based materials mediating DIET [1]

2.3. Microbial community

In the study of anaerobic fermentation of food waste by biochar, microbial community analysis is to understand the dominant flora in the system and to study the interaction between microbial species and the mechanism of electron transfer between microbial species. Biochar can affect the diversity, dominant flora and abundance changes of microbial communities during anaerobic fermentation, indicating that methane production during anaerobic fermentation is related to microbial metabolism.

On the one hand, biochar has microbial physical fixation, microbial cells colonize the surface of its stable solid material, and maintain a higher biomass than in suspension cell systems. The physical fixation of biochar can also increase the biomass residence time. Microorganisms can colonize the surface of biochar materials and form biofilms to promote microbial metabolism [6]. The porous structure of some carbon-based materials can reduce internal biomass scour and shorten the distance between mutualistic bacteria and methanogens, which is conducive to VFAs degradation and H2 generation. On the other hand, the rich pore structure can provide a suitable microbial environment for microbial growth, promote microbial metabolism, increase the degradation rate of VFAs, and increase the methane production rate. The added biochar may change the anaerobic fermentation system's physicochemical characteristics and have an impact on the microbial environment. The promotion of methanogen activity is related to the change of redox potential of anaerobic fermentation system. Salvador et al. found that the addition of multi-walled carbon nanotubes can make the redox potential in the pure culture system tend to change in the range (200-400 mV) that is favorable for the activity of methanogens [7]. The addition of conductive biochar can also affect the redox potential in the system, so that the transmission of the electrons liberated through oxidation of organic matter to the electron acceptor, and the production of methane is promoted. The addition of carbon-based conductive materials will also lead to changes in the conductivity of the system. The study discovered that the addition of biochar boosted the system's conductivity. This may be because bacteria involved in direct electron transfer between species expressed their conductive pili to a greater extent. In addition, biochar is also able to release cations to change the system conductivity.

In the anaerobic fermentation system, biochar can significantly improve the bacterial community structure. The colonization of a variety of dominant microorganisms in the micropore and mesoporous structure of the biochar also enables the system to withstand higher organic loads, keeping the anaerobic fermentation system stable as a result. According to the existing research, the added biochar can lead to a reduction in the diversity of the bacterial community in the anaerobic fermentation system of food waste, but biochar addition significantly promoted methane production. This may be due to the fact that the addition of biochar stimulates the growth of some major microorganisms, contributing to VFAs degradation and methanogenesis activities [8]. In addition, the addition of biochar was able to change the dominant population and abundance in the bacterial community, contributing to the enrichment of functional microorganisms. For example, biochar can encourage the growth of *Anaerolineaceae* (*Chloroflexi*) in the anaerobic co-fermentation system of food waste, where Anaerolineaceae's relative abundance increased from 5.47% in the control group to 30.08% [9]. And it has been proven that they are able to transfer extracellular electrons to electron acceptors, thereby encouraging DIET [10].

The community structure of archaea in anaerobic fermentation system is relatively simple, *Methanosarcina*, *Methanosaeta*, *Methanobacterium*, *Methanolinea* and *Methanospirillum* are the main methanogens. Among them, *Methanosarcina* belongs to mixed trophic methanogens, and *Methanosaeta* is an acetotrophic methanogen and can accept electrons through DIET to reduce CO_2 to methane. *Methanolinea* and *Methanospirillum* are considered to be hydrogenotrophic methanogens capable of converting CO_2 and H_2 to CH_4. Li et al. compared the microbial community structure of the co-fermentation system of food waste and residual activated sludge with and without the addition of biochar [11]. They discovered that there was a considerable increase in the relative abundances of both *Methanosaeta* and *Methanosarcina*. Wang et al. found that in the co-fermentation system, the abundance of *Methanosaeta* in the biochar treatment group increased by 43.8% compared with the control group [8]. Numerous studies have shown that *Methanosarcina* is significantly enriched when activated carbon and carbon cloth are added [12]. *Methanosarcin*a and *Methanosaeta* were shown to be two methanogens involved in DIET,so biochar and activated carbon may promote system stability and improve methanogenesis efficiency by selectively enriching methanogens involved in DIET.

3. Conclusions

With the increasing production of food waste around the world, how to efficiently treat food waste has become a major issue. Food waste can be transformed into bioenergy for use by anaerobic fermentation, which can also help minimize the quantity of solid waste, but this clean treatment method is accompanied by system instability and low efficiency. Adding biochar to anaerobic fermentation systems can increase system efficiency and stability, but economic viability is one of the key factors in adding biochar to treat food waste. Therefore, to lower the cost of using biochar and reduce the requirement for regeneration, the separation and recovery of biochar in the anaerobic fermentation system should be taken into consideration. The efficiency of anaerobic fermentation of food waste is related to the type of biochar, addition amount, surface structure, etc. As a result, the type of biochar and the most suitable addition amount according to actual conditions should be analyzed. For example, investigating how novel nanomaterials with high electrical conductivity, such graphene and carbon nanotubes, affect the anaerobic fermentation of food waste. In view of the fact that the anaerobic fermentation of food waste is prone to system instability, the impact of biochar addition under high oil, salt and high load stress can be paid attention to in the future. In addition, while most current research solely focuses on the change in microbial community structure, microbial activity also plays a key role in the anaerobic fermentation process of food waste. Future research can analyze the interspecific interactions of microorganisms through metabolomics, and carefully examine the microbial metabolic pathways that biochar uses to increase the effectiveness of anaerobic food waste fermentation. In addition to more in-depth microbial analysis, investigation into direct interspecies electron transmission is possible. There are many ways that microorganisms can carry out direct interspecies electron transfer. They can transfer electrons through microbial nanowires or cytochrome C, and can also transfer electrons by using conductive substances. Therefore, the strategy of microbial selection of electron transfer method will also be a research direction in the future.

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