Environmental Challenges 5 (2021) 100262

Contents lists available at ScienceDirect

Environmental Challenges

journal homepage: www.elsevier.com/locate/envc

Application of Biochar on methane production through organic solid waste and ammonia inhibition

Apourv Pant[a,*], J.P.N. Rai[b]

[a] Research Scholar, Department of Environmental Sciences, Govind Ballabh Pant University of Agriculture and Technology, GBPUAT Pantnagar, Pantnagar, Uttarakhand 263145, India

[b] Department of Environmental Sciences, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand 263145, India

ARTICLE INFO

Keywords:
Biochar amendments
Gompertz model
Methane enhancement
Ammonia inhibition
Anaerobic digestion

ABSTRACT

The present study discusses the role of biochar in enhancing methane (biogas) production from organic solid waste (OSW) employing a co-culture of *Pseudomonas aeruginosa* and *Methanosarcina mazei.* The high porosity, alkalinity, and high ion-exchange capacity of biochar make it an efficient support material for microbial cell growth and proliferation. Here the effect of different doses of biochar on biogas production parameters, i.e., cumulative methane production, maximum methane production rate, and lag phases, are studied. The synergistic effect of biochar for its supplemental methane production via ammonia mitigation potential is also studied. The results illustrate a maximum methane yield of 109±0.42 $mlCH_4gCarbo_{initial}$ with a maximum of 54.83±0.20% COD removal was achieved at 12.5 g/L biochar concentration. Also, there is a significant improvement in the lag phase from 13.2 ± 0.3 h at control (without biochar) to 8.8±.15 h at the same 12.5 g/L effective biochar concentration. The effect of ammonia addition revealed low methane production rates, which were subsequently reduced with the biochar amendment that conversely increased the methane production rates in each experimental batch. Thus the result showed that biochar addition could significantly affect methane production rates, ammonia inhibition potential and also showed increased volatile fatty acid generation.

1. Introduction

Energy is the essential requirement for all types of activities derived from the burning of fossil fuels or other non-renewable energy sources. These conventional energy sources cause much disturbance to the environment by releasing vast amounts of toxic gasses and chemicals into the ecosystem. These pollutants in converse cause global-scale phenomenon such as global warming, air and soil pollution, acid rain, and biodiversity loss. Therefore, there is a need for a cheap and eco-friendly alternative energy source. Biogas is a promising fuel that gives a high calorific value of 55 KJ/g compared to traditional fuels; liquefied petroleum gas, kerosene, wood, charcoal. Biogas is an environmentally friendly alternative that consists of 55–70% CH_4 and 30–50% CO_2, with a trace amount of other impurities and H_2S. Conventionally biogas enhancing methods include various pre-treatments (thermal, chemical, and biological) or solvent absorption, pressure/ temperature adsorption, and membrane separation (Bauer et al., 2013). Recently some newer technologies were used to increase process efficiency and production, such as metal and organic materials-based adsorption structure and pressure swing adsorption (Chaemchuen et al., 2013; Shen et al., 2015a). Biogas is often a readily available and commercialized form of energy in India, which found its application in daily household uses in most rural and urban towns. The primary method is the biogas plants (Gobar-gas plants) subsidized by India's government under the National Biogas and Manure Management Programme (NBMMP), 2014.

At present, 50–60% of methane is generated from anaerobic digestion processes only. Nowadays, several methane production concepts and technologies are used. The concept of the generation of methane from more potential methanogenic bacteria is gaining interest in recent years (Enzmann et al., 2018). A considerable number of likely microbial species have been isolated in recent years for their industrial-scale methane production applications, such as *Methanosarcina barkeri, Methanosarcina barkeri, Methanobacterium thermoautotrophicum,* and *Methanobacterium wolfei, Methanobrevibacter ruminantium, Methanoflorens stordalenmirensis,* etc. (Karrasch et al., 1990; Borner et al., 1991; Schmitz et al., 1992). This study uses a co-culture of facultative anaerobe *Pseudomonas aeruginosa* and *Methanosarcina mazei. P. aeruginosa* consumes the leftover oxygen present in the reaction mixture and helps create a perfect anaerobic environment for *Methanosarcina maezi.* This co-culture technique helps increase methane production by maintaining a strict anaerobic microenvironment inside the reaction bottles (Yeung et al., 2017; Pant and Rai, 2018). *Methanosarcina* spp. are a

* Corresponding author.
E-mail address: environmentalist90@gmail.com (A. Pant).

https://doi.org/10.1016/j.envc.2021.100262
Received 4 March 2021; Received in revised form 30 August 2021; Accepted 31 August 2021

diverse group that shows high growth rates (doubling time 1.1– 1.2 days) and high pH (0.8–10) tolerance, compared to other methanogens (Conklin et al., 2006; Liu et al., 2011; Shin et al., 2011). *Methanosarcina* can tolerate a high concentration of ammonium up to 7000 mg TAN $L-^{1}$ (total ammonia nitrogen) and can operate in low pH conditions such as pH 5.0 (Smith, 1966; Calli et al., 2005; Schnurer and Nordberg, 2008). *Methanosarcina* sp. has the advantage of utilizing both acetoclastic and the hydrogenotrophic methanogenesis pathway due to their specific tolerance towards inhibitors such as fluoroacetate and methyl fluoride (Thauer et al., 2008; Liu et al., 2011). Thus, *Methanosarcina* sp. is the better choice for anaerobic methane production studies. It helps achieve stable growth, higher organic loading rates, and high ammonia tolerance at low retention times (> 4 days). On the other hand, there are several studies on the use of *Pseudomonas aeruginosa* in methanogenic experiments. In an experiment by Potivichayanon et al. (2011) on bakery waste, *Pseudomonas* cells increased the methane production from 24.90 to 44.33%; also glycerol, which is essential for anaerobic digestion, showed an increase from 12.83 to 48.10%. *Pseudomonas* also helps in methane formation by forming biosurfactants that help in better degradation of fatty molecules in the anaerobic digester (Jadav et al., 2017).

Anaerobic digestion is an oxygen deficit biological process that requires the wet phase conditions to operate and produces valuable fuel gasses such as methane and hydrogen. Anaerobic digestion, in recent years, provided a promising and effective approach for organic waste reduction, generation of important fuel gasses, and excellent bio-manure for crops (Mata-Alvarez et al., 2000; Ariunbaatar et al., 2014). Several reactions in the anaerobic digestion process include acidogenesis, acetogenesis, hydrolysis, and methanogenesis (Weiland, 2010). Hydrolysis of organic solid wastes being the rate-limiting step in an anaerobic digestion process (Choi et al., 2006; Rittmann et al., 2008). There is also a problem of inhibition while dealing with high N-containing wastes by excess ammonia (Ward et al., 2008). There are specific methods reported such as ammonia stripping; struvite precipitation, and addition of zeolites that can reduce or scavenge the ammonia ions from the medium (Borja et al., 1993; Ho and Ho, 2012; Calli et al., 2005; Rajagopal et al., 2013), but they are all inorganic materials that add towards increased COD in the waste effluent. Therefore, there is a need to increase organic solubility and accelerate the biodegradation rate by providing more surface area for microbial action, thus reducing the sludge load (Khalid et al., 2011). Biochar amendment gives a suitable eco-friendly option in anaerobic digestion processes. Biochar has gained popularity in recent years due to its multidisciplinary application in the agriculture, environment, and energy sector (Chen et al., 2019) and proposed impacts on soil carbon and fertility (Ameloot et al., 2013). Biochar provides excellent support material for the growth of diverse microorganisms because of its structure and high organic content. Biochar thus increases the soil water retention property, conductivity, porosity, and nutrient retention property (Glaser et al., 2002; Lehmann and Rondon, 2006; Kookana, 2010). Biochar is an excellent additive in the anaerobic digestion process because it provides biofilm formation and mitigates ammonia and acid inhibition (Torri and Fabbri, 2014). A 6.7% dose of hydro-char, a type of biochar produced from hydrothermal carbonization, prevents mild ammonia inhibition and increased methane yield up to 32.0% (Mumme et al., 2014). Biochar also helps in reducing the methanogenic lag phase by 30.3% and increases methane production by 86.6% (Luo et al., 2015). Similarly, Inthapanya et al. (2012) found that 1% pyrolytic rick husk increases methane production by 31.0%, and further addition did not increase gas production, thus showing the concentration-dependent effect of biochar.

This study investigates the role of different concentrations of biochar on cumulative methane yield and its ammonia mitigation potential. This includes the co-culture of *Pseudomonas aeruginosa* and *Methanosarcina mazei* on organic solid waste as a substrate in an anaerobic digestion system.

2. Material and methods

2.1. Feedstock

The organic waste was collected from the agriculture farm of Pantnagar University. It was composed of cow dung waste mixed with local household biological waste such as peels of vegetables, fruits, rice, bread, and paper. The organic compost is prepared by grinding and mixing the waste in smaller-sized particles (2 mm) and then further filtered and dewatered. The feedstock was collected in plastic zip lock bags and stored in the refrigerator at −4 °C to avoid biological degradation. The characteristics of OSW are mentioned in Table 1 (See supporting file).

2.2. Biochar

Biochar was procured online from Greenfield Eco Solutions Company, India. Biochar is manufactured through pyrolysis of woody biomass, i.e., heating the biomass to 400–500^{0}C in a low oxygen environment. Composition of biochar was surface area: 124 m^2g^-1; conductivity <1500 μS cm^{-1}, particle size: 5.4–20.6 mm 83.15% carbon, 8.23% oxygen, 4.21% hydrogen, 0.39% nitrogen, and 0.44% sulfur (dry weight basis).

2.3. Experimental design conditions

The experiment was conducted in the ecotechnology lab at the department of environmental sciences, GBPUAT, Pantnagar. The lab experiments were conducted using 500 ml Duran reagent bottles fitted with rubber screw caps, which were used as anaerobic fermenters; further, the bottles were connected to the mass-spectrometer with the help of rubber pipes to analyze gas samples. The gas flow was controlled using a second-hand quadrupole (fitted with a pump with a turbo and a rotary) to detect low methane levels (ppm) with excellent results. Each bottle was filled with 300 ml of organic solid waste (25 gm/l OSW); pure nitrogen gas was passed through each bottle for 5 min, sealed with a rubber cap, and covered with aluminum foil. All glassware and media were autoclaved at 125 °C and 15 psi pressure for about 35 min to avoid any type of contamination. Then each bottle was inoculated with 50 ml of inocula consisting of 25 ml of *Pseudomonas aeruginosa* and 25 ml *Methanosarcina mazei* (10^2 CFU/ml) using a sterile loop. *P. aeruginosa* creates a perfect anaerobic environment for *Methanosarcina mazei* by consuming the excess oxygen in the reaction mixture. Thus co-culture technique favors the increased methane production by maintaining the strict anaerobic microenvironment inside the bottles. Different biochar concentrations were supplemented to each bottle with the help of a sterile spatula, as mentioned in Table 1. Before being used in batch experiments, the samples were ground and sieved to a size fraction of 1.8–2 mm for uniform surface area. The pH of the biochar samples was maintained at 8.50 ± 0.14 (mixed with deionized water at 1:10, w/v). All were repeated in triplicates with control for each experiment prepared as blank in Duran reagent bottles, without the addition of biochar, besides the inocula. The bottles were then incubated at 35–40 °C in the Remi CS-2014 incubator, and pH was maintained at an optimum range of 6.8–7.8, using 1 N sodium hydroxide and 1 N hydrogen chloride solution. In this experimental set-up, in one batch, only ammonia was added. In another set-up, batches of biochar were supplemented in increasing concentration and ammonia, as depicted in Table 2. The amount of methane production was regularly monitored every 24 h. The effect of different biochar concentrations on ammonia mitigation is also estimated.

2.4. Analytical methods

Total Kjeldahl nitrogen (TKN), Total solids (TS), Chemical oxygen demand (COD), Volatile solids (VS), Lipids, and Ammonia were assessed by standard methods (APHA 2005). Phenol-sulfuric corrosive strategy

Table 1
Batch design conditions for determining the experimental effect of biochar on methane yield.

Batches	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
OSW(g/L)	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
Biochar concentration(g/L)	0	2.5	3.5	5	7.5	10	12.5	15	17.5	20	22.5	25	27.5	30	32.5	35

Table 2
Experimental design conditions for quantifying the effect of biochar on methane production profile and ammonia inhibition.

Batch No.	OSW (g/L)	Biochar conc. (g/L)	Ammonia conc. (g/L)
Ammonia with Nil biochar concentration			
1	20	Nil	0.5
2	20	Nil	1
3	20	Nil	1.5
4	20	Nil	2
5	20	Nil	2.5
6	20	Nil	3
7	20	Nil	3.5
8	20	Nil	4
9	20	Nil	4.5
10	20	Nil	5
11	20	Nil	5.5
12	20	Nil	6
13	20	Nil	6.5
14	20	Nil	7
15	20	Nil	7.5
16	20	Nil	8
Ammonia with 12.5 g/L Biochar concentration			
1	20	12.5	0.5
2	20	12.5	1
3	20	12.5	1.5
4	20	12.5	2
5	20	12.5	2.5
6	20	12.5	3
7	20	12.5	3.5
8	20	12.5	4
9	20	12.5	4.5
10	20	12.5	5
11	20	12.5	5.5
12	20	12.5	6
13	20	12.5	6.5
14	20	12.5	7
15	20	12.5	7.5
16	20	12.5	8

and colorimetric technique were used for quantifying carbohydrates and protein. Further, the samples were subjected to 0.45 μm Millipore filter paper which removes particulate COD and helps in calculating soluble chemical oxygen demand (SCOD). After that, SCOD was evaluated by the standard method given in APHA (2005). The following formula (Eq. (1)) is used for calculating particulate COD:

$$COD_{particulate} = COD_{Total} - COD_{soluble} \tag{1}$$

2.5. Methane and carbon dioxide (CH_4 and CO_2) analysis

Gas chromatography (GC-2014) Shimadzu is used for methane and carbon dioxide analysis. The column is equipped with a 1.2 m X 3-mm diameter capillary column (Porapak Q) and a thermal conductivity detector (TCD). Operating conditions temperature for injector, column, and detector were 120 °C, 100 °C, and 150 °C, respectively. Same GC conditions were also used for analyzing volatile fatty acids using a flame ionization detector (FID).

For evaluating methane potential and maximum methane production rate, a modified Gompertz equation was used (Eq. (2)):

$$G_m = P_x \exp\left\{ -\exp\left[\frac{R_m X e}{P}(\lambda - t) + 1 \right] \right\} \tag{2}$$

Where G_m= Methane production (ml) at particular reaction time.'

P_x= Total Methane production potential

R_m= Rate of maximum methane production (ml/h)

λ= Lag phase duration (h)

e = 2.7182

Inhibition coefficient calculated for specific inhibitor as follows (Eq. (3)):

$$Inhibition\,coefficient = 100 - \frac{Methane\ yield\ from\ OSW\ with\ inhibitor}{Methane\ yield\ from\ OSW\ without\ inhibitor} * 100 \tag{3}$$

2.6. Statistical analysis

The results were analyzed with Minitab® 17.1.0 statistical analysis software using a one-way ANOVA statistical test.

3. Results and discussion

3.1. Effect of biochar amendments on methane production profile

Modified Gompertz model equation (Eq. (2)) was correlated with cumulative methane production data to obtain kinetic parameters using regression analysis. There is a significant impact on methane production rate with biochar amendment. An enhancement in cumulative methane production from 218±2.0 ml to 944.96±4.9 ml is observed when increasing the biochar concentration from nil to 15 g/L (Table 3). And also, there was subsequent volumetric methane production from 0.72 $LCH_4/L_{substrate}$ to 3.18 $LCH_4/L_{substrate}$.

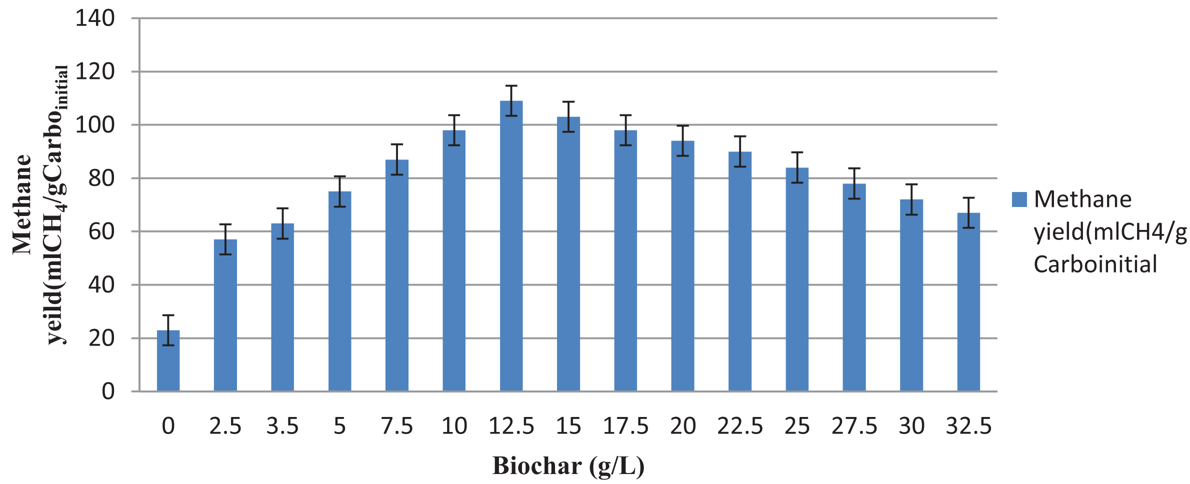

Fig. 1. Effect of different concentrations of biochar on methane yield ($mlCH_4/gCarbo_{initial}$) **(**Note: The P-value is < 0.001 at 95% significance, which shows results are highly significant (see supporting file).

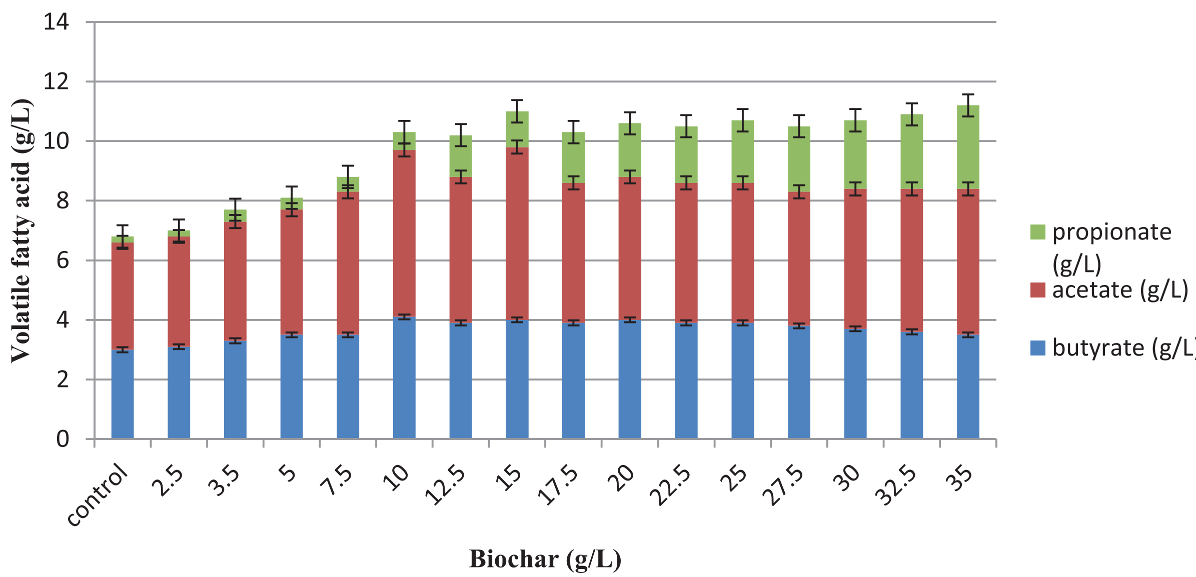

Fig. 2. *Biochar supplementation effect on volatile fatty acid profile* **(**Note: The P-value is < 0.001 at 95% significance, which shows results are highly significant (see supporting file).

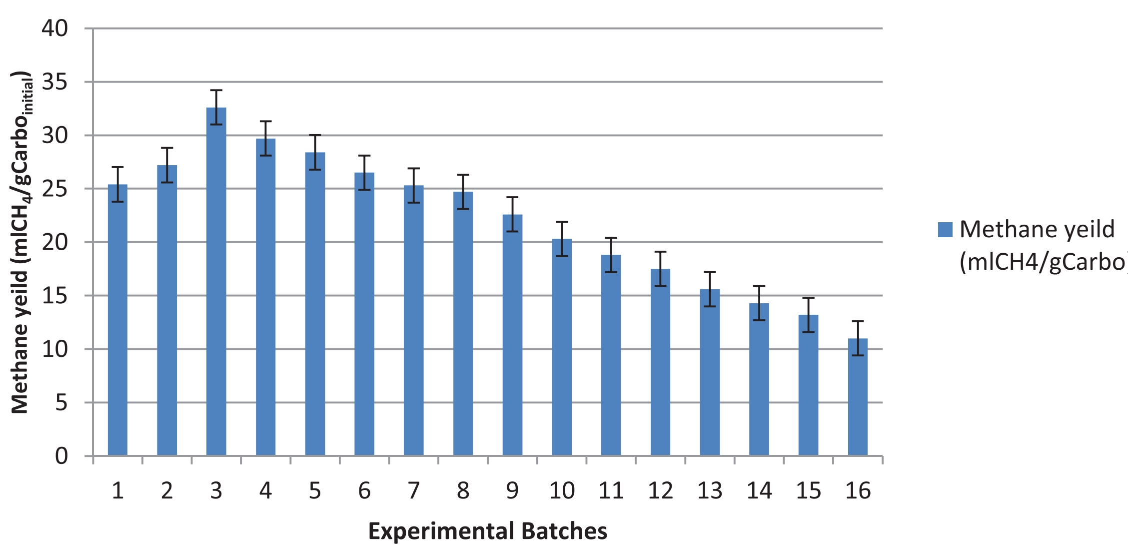

Fig. 3. *Ammonia supplementation affects methane yield* **(**Note: The P-value is < 0.001 at 95% significance, which shows that results are highly significant (see supporting file).

Table 3
Cumulative methane production (P), maximum methane production rate (R_{max}), and lag phase (λ) through different concentrations of biochars.

Batches	Modified Gompertz Kinetics			
	P(ml)	R_{max}(ml/h)	λ (h)	R^2
1(Control)	218±2.0	18.6 ± 1.1	13.2 ± 0.3	0.996
2	517.26±4.1	43.76±0.3	11.53±0.3	0.994
3	610±2.2	51.86±1.4	10.83±0.1	0.998
4	712.10±1.5	63.86±1.4	10.63±0.2	0.992
5	821.8 ± 1.6	72.13±0.3	9.9 ± 0.1	0.989
6	911.93±1.6	80±0.9	9.16±0.1	0.996
7	944.96±4.9	86.23±1.6	8.83±0.1	0.997
8	913.76±1.6	81.73±0.4	9.2 ± 0.3	0.997
9	882.3 ± 1.9	78.16±0.9	9.73±0.2	0.998
10	856.83±4.1	75.26±1.1	10.16±0.2	0.998
11	815.16±2.7	72.16±0.2	10.3 ± 0.1	0.999
12	780.56±1.9	69.03±0.2	10.72±0.1	0.996
13	739.23±1.2	65.46±0.8	10.83±0.1	0.994
14	703.5 ± 0.8	61.06±0.2	11.06±0.2	0.997
15	672.13±1.9	57.16±0.4	11.27±0.1	0.994
16	629.13±3.0	54.16±0.7	11.53±0.20	0.998

Note: The *P*-value is < 0.001 at 95% significance, which shows that results are highly significant (see supporting file). Further, all the R^2 values above are close to 1, which explains the variability of methane production rate can easily be predicted and recorded using the modified Gompertz equations.

Similar observations were reported by Sunyoto et al. (2016). Increasing the biochar concentration above 33 g L^{-1} inhibits the further methane yield; the positive effect of biochar is directly related to the biochar's electron-donating capacity (EDC) (Viggi et al., 2017). Significantly few scientists reported the full-scale study of the effect of biochar on methane yield in an anaerobic system like Meyer-Kohlstock et al. (2016) observed the increasing trend of cumulative methane production from 5 to 10% while increasing the biochar concentration from 5% (dry weight of organic waste) to 10%. Biochar composition plays an important role; for example, in its in-situ experiments, Linville et al. (2017) observed that fine shell biochar increases the methane yield by 77.5%–98.1% CH_4 compared to coarse shell biochar of 78.9% CH_4. Similarly, an on-field experiment conducted by Wang et al. (2019) observed a 0.5–37.5% increase in CH_4 when N-fertilizer is amended with biochar. The fine quality or surface area of biochar is crucial for optimum methanogenic activity; the high surface area of biochar results in higher methane activity (>90%) by enhancing the methane yield, bio methanation rate constant, and maximum methane production rate by up to 7.0%, 8.1%, and 27.6%, respectively (Shen et al., 2015b). Torri and Fabbri (2014) studied the biochar effect on APL (Aqueous pyrolysis liquid), increasing the theoretical methane yield by 65±5%.

The higher surface area of biochar helps in biofilm formation that accommodates a wide range of microorganisms, thus enhancing microbial activity and shortening the lag phase, and boosting the exponential phase, i.e., Enhance methane production (Cooney et al., 2016). Biochar supports microbial metabolism and growth by acting as a good electron exchanger (APHA 2015). Similarly, in Gompertz, kinetic parameters, i.e., cumulative methane potential (p), lag phase (l), and maximum methane production rate (R_{max}), we're dependent on effective biochar concentration (Table 3). Here maximum methane production rate (Rmax) increased from 18.6 ± 1.1 ml/ h at control (0 g/L) to 86.23 ± 1.6 ml/h at 12.5 g/L concentration of biochar. Subsequent biochar amendment decreases the Lag phase from 13.2 ± 0.3 h at control to 8.83 ± 0.1 h at 12.5 g/L concentration of biochar. Similar results were reported by Sunyoto et al. (2016), which showed a decrease in lag phase by 41–45% and an increased maximum production rate by 23.0– 41.6% and CH_4 production potential by 1.9–9.6% of CH_4. The Syntrophic association of Pseudomonas aeruginosa and Methanosarcina mazei increases the cumulative methane yield, while Methanosarcina's high metabolic capability easily increases its biomass while digesting different complex carbon sources (Xu et al., 2015). A further decrease in methane production was observed when biochar concentration exceeded the 12.5 g/L limits. Viggi et al. (2017) reported a gradual decrease to nil lag phases in all biochar-amended bottles before the onset of VFAs degradation compared to the unamended control bottles where the lag phase of almost 10days recorded (Fig. 1).

This Figure depicts the effects of various concentrations of biochar on methane yield ($mlCH_4/gCarbo_{initial}$) based on initial carbohydrate. From the figure, it was concluded that at 12.5 g/L biochar concentration, a maximum methane yield was achieved, i.e., 23.45 ± 2.1 $mlCH_4/gCarbo_{initial}$ in control to 109.66 ± 2.5 $mlCH_4/gCarbo_{initial}$. However, Mumme et al. (2014), have found no effect on methane yield even at 8.3 g/L pyrochar concentration. This behavior is attributed to complex biochar–microbe interactions and the function of continuously fed anaerobic digesters that need to be studied. The biological methane content of 46.4 ± 0.7 to 78.0 ± 0.5% is reported in Table 4. Further, the system reports the only CO_2, with no hydrogen detected in samples. Therefore, it may be concluded that biochar supplementation helps in enhanced methane production by supplementing anaerobic bacterial growth (Viggi et al., 2017; Cai et al., 2016).

3.2. Effect of biochar amendment on direct cod removal

In anaerobic processes, the organic waste is readily metabolized, leading to increase COD removal and methane production rate (Hutnan et al., 2013). Table 4 showed that the biochar supplementation increases the COD removal rate in each experiment significantly than the control. This depicts the role of biochar amendment in improving microbial activities while increasing the biochar concentration from nil to 12.5 g/L also increases the COD removal efficiency from 32.7 ± 0.3% to 54.8 ± 0.2%. However, after increasing the biochar concentration above 12.5 g/L, the COD removal efficiency started decreasing, with a minimum of 41.2 ± 1.0% biochar concentration of 35 g/L.

Biochar application helps in reducing NH_3 emission and subsequently increases COD removal, corresponding to high methane production (Maurer et al., 2017). Different fractions of OSW such as lipid, carbohydrate, and protein were estimated related to the COD removal. In contrast, carbohydrates contribute the maximum towards methane production, followed by lipids and proteins, respectively (Table 4). The percentage contribution of carbohydrates, proteins and lipids towards COD removal was observed as 69.1 ± 0.2% to 81.1 ± 0.3%, 4.6 ± 0.1 to 14.2 ± 0.7% and 8.9 ± 0.2 to 28.1 ± 0.2%, respectively. Nielfa et al. (2015) and Labatut et al. (2011) have compared the COD and BMP method for determining the methane yield with lower error; he found that COD methods showed well for co-digestion while BMP showed good results in terms of complex substrate dairy manure or corn silage. Nielfa et al. (2015) observed that lipids contribute more towards biogas production (1m^3 per kg of volatile solids) than proteins and carbohydrates. Effective carbohydrates, lipid, and protein (40:40:20) are important for methane production as they can balance the acidification and methanation in the system (Johnson and Johnson, 1995; Xue et al., 2019). The carbon fraction of carbohydrates are easily digested compared to the lignin–associated cellulosic components that result in increased methane production and more effective substrate utilization (Mulat et al., 2018).

3.3. Biochar amendment effects on volatile fatty acid generation rate

Volatile fatty acids generation is directly correlated with the methane production rate in an anaerobic digestion process. Therefore, quantifying its concentration and distribution is indicative of methane production and monitoring. An enhanced concentration of volatile fatty acids reported in all the batched with biochar supplementation, propionate, acetate, and butyrate, was detected in all the batches (Fig. 2).

Table 4
Biochar supplemented effect on methane production profile and COD removal.

Batches	CH_4 (%)	CO_2 (%)	H_2 (%)	COD removal (%)	Carbohydrate removal (%)	Protein removal (%)	Lipid removal (%)
1	46.4 ± 0.7	54.4 ± 0.4	Nil	32.7 ± 0.3	77.4 ± 0.4	4.6 ± 0.1	20.5 ± 0.1
2	53.7 ± 0.2	47.7 ± 0.4	Nil	39.7 ± 0.4	76.1 ± 0.1	4.6 ± 0.2	21.4 ± 0.2
3	58.8 ± 0.2	42.9 ± 0.7	Nil	43.9 ± 0.3	73.6 ± 0.4	6.9 ± 0.2	22.8 ± 0.3
4	68.5 ± 0.6	31.5 ± 0.4	Nil	48.9 ± 0.1	78.8 ± 0.2	6.2 ± 0.6	17.5 ± 0.1
5	74.9 ± 0.4	28.1 ± 0.2	Nil	52.8 ± 0.4	69.1 ± 0.2	5.0 ± 0.2	28.1 ± 0.2
6	76.2 ± 0.3	27.1 ± 0.3	Nil	53.6 ± 0.2	74.9 ± 0.1	8.9 ± 0.3	19.1 ± 0.2
7	78.0 ± 0.5	26.7 ± 0.3	Nil	54.8 ± 0.2	79.1 ± 0.2	13.4 ± 0.3	14.5 ± 0.8
8	76.5 ± 0.4	25.4 ± 0.2	Nil	50.3 ± 0.6	76.4 ± 0.3	9.4 ± 0.4	20.7 ± 0.5
9	77.5 ± 0.3	26.4 ± 0.2	Nil	49.4 ± 0.3	81.1 ± 0.3	10.9 ± 0.2	15.1 ± 0.2
10	76.1 ± 0.2	27.4 ± 0.1	Nil	49±0.1	73.7 ± 0.6	10.3 ± 0.1	22.4 ± 0.3
11	74.9 ± 0.1	29.1 ± 0.2	Nil	47.2 ± 0.3	71.0 ± 0.3	11.4 ± 0.2	24.5 ± 0.4
12	71.4 ± 0.2	31.4 ± 0.4	Nil	45.2 ± 0.4	77.8 ± 0.8	9.4 ± 0.3	19.9 ± 0.1
13	67.9 ± 0.3	35.4 ± 0.4	Nil	43.2 ± 0.2	73.4 ± 0.2	8.3 ± 0.2	14.7 ± 0.3
14	62.9 ± 0.1	39.2 ± 0.5	Nil	43.5 ± 0.2	79.6 ± 0.8	10±0.3	12.8 ± 0.2
15	59.3 ± 0.4	42.4 ± 0.4	Nil	41.1 ± 0.2	80.9 ± 0.7	14.2 ± 0.7	8.9 ± 0.2
16	57.3 ± 0.5	45.6 ± 0.3	Nil	40.0 ± 0.1	80.3 ± 0.5	13.8 ± 0.1	12.2 ± 0.3

Note: Note: The P-value is < 0.001 at 95% significance, which shows results are highly significant (see supporting file).

Experimental batches recorded a lower propionate concentration followed by higher butyrate and acetate when a lower amount of biochar, i.e., < 12.5 g/L, was applied. But as the concentration of biochar increased (> 12.5 g/L), a subsequent increase in propionate production with a relative reduction in butyrate and acetate concentration was observed. This indicates that the butyrate metabolic pathway is favored at lower biochar concentrations, while organic overloading (higher biochar concentration) shifts towards the propionate pathway. A probable reason for this type of metabolic shift is due to cis-unsaturated fatty acids type inhibition that inhibits the methane production while increasing the propionic acid production when we increase the biochar concentration >12.5 g/L (Demeyer and Henderickx, 1967; Pereira et al., 2004). Awasthi et al. (2018) also found that an 8–10% biochar amendment increased methane production by reducing the VFAs profiles. Consequently, a higher dosage of biochar (> 8%) provides higher buffer capacity, increased porosity, and optimum moisture level of OSW that results in optimized pH and temperature profile throughout the anaerobic treatment duration that conversely enhance the rate of composting and its end quality products, i.e., methane (Awasthi et al., 2017). Further, increasing the contact time/incubation period up to four weeks, there is a subsequent reduction in VFAs profiling (> 95%) with an increase in methane yield (Amani et al., 2011).

3.4. Biochar amended effect on ammonia inhibition rate4

Increased ammonia production directly inhibits the methane production rate. Table 5 depicts the enhancement effect on cumulative methane production and maximum methane production rate and lag phase parameters by suitable ammonia addition. A 1.889 g/L ammonia concentration observed a slight increase in methane production and methane yield (Fig. 3). Subsequent higher methane production of 281.8 ± 1.1 ml was obtained at 1.889 g/L ammonia concentration compared to 226.5 ± 0.8 ml methane at control. Similarly, there is an increased methane yield from 25.6 $mlCH_4/gCarbo_{initial}$ in control to 34.6 $mlCH_4/gCarbo_{initial}$ at 1.889 g/L ammonia concentration (Fig. 3). At the same time, a fluctuation of maximum methane production rate and lag phase is observed at a lower concentration of ammonia (< 1.889 g/L) (Table 5). With the subsequent increase in ammonia concentration, there is an apparent cut decrease in methane production (Table 5) and methane yield (Fig. 3). Biochar causes the available ammonia to get absorbed and reduces its inhibition property by converting free ammonia to bioavailable nitrogen (Tu et al., 2020). Taghizadeh-Toosi et al. (2012) in an N^{15} tracer study, observed that the biochar amendment increases the ammonia bio-availability to plants. In a similar study, Mumme et al. (2014) has reported a mild ammonia inhibition via physical adsorption by pyrochar as 2.1 g TAN kg^{-1}. This shows that ammonia supports microbial growth at lower concentrations, followed by a slight increase in methane production at a lower concentration. However, a higher ammonia concentration interrupts anaerobic digestion by restricting microbial growth and its metabolic activities. A comprehensive review by many scientists also suggests that ammonia inhibition is an important parameter when it comes to anaerobic digestion (AD) processes and methanogenesis (Rajagopal et al., 2013; Yenigün and Demirel, 2013). NH_3/NH_4

Table 5
Combined effects of ammonia concentrations and biochar addition on cumulative methane production, maximum methane production rate, and lag phase.

Experimental Batches	Gompertz kinetics model			
	P (ml)	R_{max} (ml/h)	λ (h)	R^2
Ammonia addition + Nil biochar				
1	226.5 ± 0.8	20.0 ± 0.3	13.1 ± 0.2	0.998
2	248.4 ± 0.6	22.2 ± 0.3	12.5 ± 0.4	0.999
3	281.8 ± 1.1	26.0 ± 0.3	11.8 ± 0.2	0.996
4	268.1 ± 0.2	24.5 ± 0.4	12.0 ± 0.2	0.997
5	249.5 ± 0.8	23.5 ± 0.2	12.3 ± 0.3	0.995
6	238±0.4	22.3 ± 0.3	12.4 ± 0.3	0.998
7	220±0.7	21.2 ± 0.3	12.6 ± 0.2	0.994
8	210.5 ± 0.7	20.4 ± 0.3	12.8 ± 0.3	0.999
9	199.4 ± 0.7	19.6 ± 0.7	13.1 ± 0.2	0.998
10	188.5 ± 0.5	18.4 ± 0.2	13.2 ± 0.4	0.994
11	175.7 ± 0.7	17.6 ± 0.3	13.5 ± 0.2	0.989
12	163.4 ± 0.5	16.5 ± 0.3	13.7 ± 0.2	0.996
13	143.6 ± 0.5	15.5 ± 0.3	13.8 ± 0.1	0.997
14	130.1 ± 1.2	14.4 ± 0.3	14.3 ± 0.3	0.995
15	110.3 ± 0.6	13.5 ± 0.4	15.0 ± 0.2	0.998
16	102.4 ± 0.7	12.4 ± 0.3	15.5 ± 0.5	0.999
Ammonia addition + biochar				
1	889.3 ± 0.8	77.0 ± 0.4	10.1 ± 0.2	0.998
2	909.6 ± 0.7	77.4 ± 0.3	10.6 ± 0.1	0.999
3	917.8 ± 0.9	77.9 ± 0.3	9.8 ± 0.4	0.996
4	887.6 ± 0.5	80.6 ± 0.2	10.2 ± 0.2	0.986
5	865.5 ± 0.5	78.6 ± 0.4	10.7 ± 0.2	0.997
6	830.2 ± 0.7	75.6 ± 0.4	11.0 ± 0.1	0.998
7	808.2 ± 0.6	73.7 ± 0.5	11.3 ± 0.3	0.995
8	776.1 ± 1.0	69.6 ± 0.4	11.7 ± 0.1	0.998
9	766.1 ± 0.4	68.4 ± 0.1	12.1 ± 0.2	0.994
10	725.4 ± 0.8	64.7 ± 0.4	12.3 ± 0.1	0.998
11	701.0 ± 1.0	62.3 ± 0.7	13.1 ± 0.3	0.989
12	667.1 ± 1.0	59.8 ± 0.2	13.3 ± 0.3	0.996
13	634.5 ± 0.9	53.6 ± 0.3	13.5 ± 0.3	0.994
14	603.6 ± 1.5	49.9 ± 0.1	13.8 ± 0.2	0.997
15	577.7 ± 1.5	46.3 ± 0.4	14.2 ± 0.2	0.998
16	546.5 ± 0.6	44.0 ± 0.2	14.6 ± 0.1	0.997

Note: Note: The P-value is < 0.001 at 95% significance, which shows results are highly significant (see supporting file).

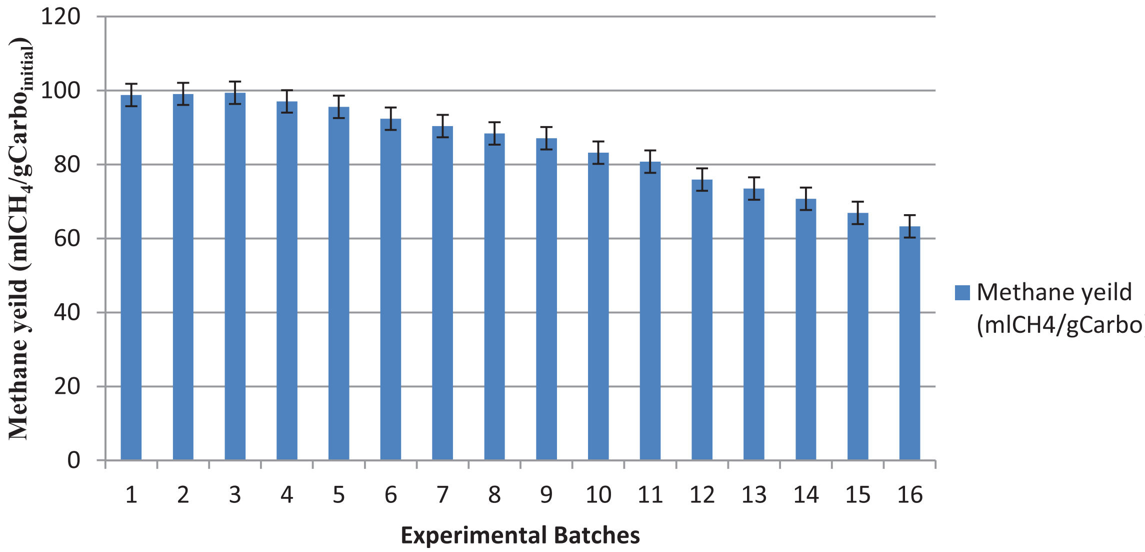

Fig. 4. *Combined effects of ammonia and biochar supplementation on methane yield* (Note: The P-value is < 0.001 at 95% significance, which shows that results are highly significant (see supporting file).

ratio disturbs the pH change by absorbing protons (H^+) and subsequently causes inhibition of specific enzyme reactions and increases the cell system's maintenance energy (Muller et al., 2004; Wittmann et al., 1995).

Results depicted in Table 5 indicate lower ammonia toxicity when using biochar. It also shows increased methane production and yields experimental batches supplemented with biochar and ammonia compared to batches without ammonia. Experimental batches 1, 2, and 3 with biochar showed increased cumulative methane production to 889.3 ± 0.8, 909.6 ± 0.7, and 917.8 ± 0.9 ml with ammonia compared with experimental batches only ammonium concentration of 0.889 g/L without biochar. This supports the observation that lower ammonia concentration is suitable for microbial growth, i.e., good methane yield and vice versa. Further, increasing the ammonia concentration > 1.889 g/L and constant biochar concentration of 12.5 g/L, there is a decline of cumulative methane production compared with the batched having biochar supplemented with ammonia. The ammonia addition on methane yields indifferent experimental batches supplemented with/without biochar addition is shown in Figs. 3 and 4, respectively. Thus results indicate the effectiveness of biochar in effective ammonia mitigation and increasing the methane yield.

Similarly, several researchers concluded that ammonia is one of the inhibitory substances in anaerobic processes (Luz et al., 2018; Mumme et al., 2014), where biochar is a promising solution for providing reaction surface area for anaerobic growth and mitigating the ammonia inhibition, thereby increasing the methane yield (Li et al., 2019; Malińska et al., 2014). However, ammonia mitigation depends upon the biochar and its particle size (surface area), pH, temperature and contact time, etc. (Taghizadeh-Toosi et al., 2012). So, these factors and their optimization for better ammonia inhibition and, subsequently, methane enhancement are required in a future study.

4. Conclusion

Therefore, the biochar amendments in anaerobic digestion enhance methane production rate but also helps in ammonia mitigation. Here. The results showed a maximum methane production of 944.96 ± 4.9 ml at an optimum biochar concentration of 12.5 g/L. They corresponded at the same biochar concentration higher methane yield and COD removal efficiency were also recorded (109 ± 0.42 mlCH$_4$/gCarbo$_{initial}$ and 54.8 ± 0.2%). Further biochar helps in reducing the lag phase from 13.2 ± 0.3 h to 8.83±0.1 h in control and 12.5 g/L biochar experimental batches.

Further, statistical analysis of the results using the one-way ANOVA test indicates the model's suitability. Results with p-values come out to be less than 0.001 at a 95% level of significance. Thus the effective concentration of biochar for all the Gompertz parameters found out to be 12.5 g/L, above which there is the onset of inhibition. It can also be concluded that biochar proves to treat the ammonia inhibition that directly affects methane production effectively. As a biochar supplement of a concentration up to 12.5 g/L, there is a significant increase in ammonia inhibition and methane production.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The author would like to acknowledge the financial and instrumental support provided by the Department of Environmental Sciences, Ecotoxicology lab, College of Basic Sciences and Humanities, Govind Ballabh Pant University of Agriculture and Technology Pantnagar, Uttarakhand, India.

References

Amani, T., Nosrati, M., Mousavi, S.M., Kermanshahi, R.K., 2011. Study of syntrophic anaerobic digestion of volatile fatty acids using enriched cultures at mesophilic conditions. Int. J. Environ. Sci. Technol. 8 (1), 83–96.

Ameloot, N., Graber, E.R., Verheijen, F.G., De Neve, S., 2013. Interactions between biochar stability and soil organisms: review and research needs. Eur. J. Soil Sci. 64 (4), 379–390.

APHA, 2005. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington, DC, USA.

APHA, 2015. In: Lehmann, J., Joseph, S. (Eds.), Biochar For Environmental management: science, Technology and Implementation. Routledge.

Ariunbaatar, J., Panico, A., Esposito, G., Pirozzi, F., Lens, P.N., 2014. Pre-treatment methods to enhance anaerobic digestion of organic solid waste. Appl. Energy 123, 143–156.

Awasthi, M.K., Wang, M., Chen, H., Wang, Q., Zhao, J., Ren, X., … Zhang, Z., 2017. Heterogeneity of biochar amendment to improve the carbon and nitrogen sequestration though reducing the greenhouse gas emissions during sewage sludge composting. Bioresour. Technol. 224, 428–438.

Awasthi, M.K., Awasthi, S.K., Wang, Q., Wang, Z., Lahori, A.H., Ren, X., … Zhang, Z., 2018. Influence of biochar on volatile fatty acids accumulation and microbial community succession during biosolids composting. Bioresour. Technol. 251, 158–164.

Bauer, F., Persson, T., Hulteberg, C., Tamm, D., 2013. Biogas upgrading–technology overview, comparison, and perspectives for the future. Biofuels Bioprod. Biorefin. 7 (5), 499–511.
Borja, R., Sánchez, E., Weiland, P., Travieso, L., 1993. Effect of ionic exchanger addition on the anaerobic digestion of cow manure. Environ. Technol. 14 (9), 891–896.
Borner, G., Karrasch, M., Thauer, R.K., 1991. Molybdopterin adenine dinucleotide and molybdopterin hypoxanthine dinucleotide in formylmethanofuran dehydrogenase from Methanobacterium thermoautotrophicum (Marburg). FEBS Lett. 290 (1–2), 31–34.
Cai, J., He, P., Wang, Y., Shao, L., Lü, F., 2016. Effects and optimization of the use of biochar in anaerobic digestion of food wastes. Waste Manag. Res. 34 (5), 409–416.
Calli, B., Mertoglu, B., Inanc, B., Yenigun, O., 2005. Community changes during start-up in methanogenic bioreactors exposed to increasing levels of ammonia. Environ. Technol. 26 (1), 85–91.
Chaemchuen, S., Kabir, N.A., Zhou, K., Verpoort, F., 2013. Metal-organic frameworks for upgrading biogas via CO_2 adsorption to biogas green energy. Chem. Soc. Rev. 42 (24), 9304–9332.
Chen, W., Meng, J., Han, X., Lan, Y., Zhang, W., 2019. Past, present, and future of biochar. Biochar 1 (1), 75–87.
Choi, H., Jeong, S.W., Chung, Y.J., 2006. Enhanced anaerobic gas production of waste activated sludge pretreated by pulse power technique. Bioresour. Technol. 97 (2), 198–203.
Conklin, A., Stensel, H.D., Ferguson, J., 2006. Growth kinetics and competition between Methanosarcina and Methanosaeta in mesophilic anaerobic digestion. Water Environ. Res. 78 (5), 486–496.
Cooney, M.J., Lewis, K., Harris, K., Zhang, Q., Yan, T., 2016. Start-up performance of biochar packed bed anaerobic digesters. J. Water Process Eng. 9, e7–e13.
Demeyer, D.I., Henderickx, H.K., 1967. The effect of C18 unsaturated fatty acids on methane production *in vitro* by mixed rumen bacteria. Biochim. Biophys. Acta (BBA) Lipids Lipid Metab. 137 (3), 484–497.
Enzmann, F., Mayer, F., Rother, M., Holtmann, D., 2018. Methanogens: biochemical background and biotechnological applications. AMB Express 8 (1), 1.
Glaser, B., Lehmann, J., Zech, W., 2002. Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal–a review. Biol. Fertil. Soils 35 (4), 219–230.
Ho, L., Ho, G., 2012. Mitigating ammonia inhibition of thermophilic anaerobic treatment of digested piggery wastewater: use of pH reduction, zeolite, biomass, and humic acid. Water Res. 46 (14), 4339–4350.
Hutnan, M., Kolesárová, N., Bodík, I., Czölderová, M., 2013. Long-term monodigestion of crude glycerol in a UASB reactor. Bioresour. Technol. 130, 88–96.
Inthapanya, S., Preston, T.R., Leng, R.A., 2012. Biochar increases biogas production in a batch digester charged with cattle manure. Livest. Res. Rural Dev 24.
Jadav, S., Sakthipriya, N., Doble, M., Sangwai, J.S., 2017. Effect of biosurfactants produced by Bacillus subtilis and Pseudomonas aeruginosa on the formation kinetics of methane hydrates. J. Nat. Gas Sci. Eng. 43, 156–166.
Johnson, K.A., Johnson, D.E., 1995. Methane emissions from cattle. J. Anim. Sci. 73 (8), 2483–2492.
Karrasch, M., Borner, G., Enssle, M., Thauer, R.K., 1990. The molybdoenzyme formylmethanofuran dehydrogenase from Methanosarcina barkeri contains a pterin cofactor. FEBS J. 194 (2), 367–372.
Khalid, A., Arshad, M., Anjum, M., Mahmood, T., Dawson, L., 2011. The anaerobic digestion of solid organic waste. Waste Manag. 31 (8), 1737–1744.
Kookana, R.S., 2010. The role of biochar in modifying the environmental fate, bioavailability, and efficacy of pesticides in soils: a review. Soil Res. 48 (7), 627–637.
Labatut, R.A., Angenent, L.T., Scott, N.R., 2011. Biochemical methane potential and biodegradability of complex organic substrates. Bioresour. Technol. 102 (3), 2255–2264.
Lehmann, J., Rondon, M., 2006. Bio-char soil management on highly weathered soils in the humid tropics. Biol. Approaches Sustain. Soil Syst. 113 (517), e530.
Li, J., Zhang, M., Ye, Z., Yang, C., 2019. Effect of manganese oxide-modified biochar addition on methane production and heavy metal speciation during the anaerobic digestion of sewage sludge. J. Environ. Sci. 76, 267–277.
Linville, J.L., Shen, Y., Ignacio-de Leon, P.A., Schoene, R.P., Urgun-Demirtas, M., 2017. In--situ biogas upgrading during anaerobic digestion of food waste amended with walnut shell biochar at bench scale. Waste Manag. Res. 35 (6), 669–679.
Liu, Y., Zhang, Y., Quan, X., Zhang, J., Zhao, H., Chen, S., 2011. Effects of an electric field and zero valent iron on anaerobic treatment of azo dye wastewater and microbial community structures. Bioresour. Technol. 102 (3), 2578–2584.
Luo, C., Lü, F., Shao, L., He, P., 2015. Application of eco-compatible biochar in anaerobic digestion to relieve acid stress and promote the selective colonization of functional microbes. Water Res. 68, 710–718.
Luz, F.C., Cordiner, S., Manni, A., Mulone, V., Rocco, V., 2018. Biochar characteristics and early applications in anaerobic digestion-a review. J. Environ. Chem. Eng. 6 (2), 2892–2909.
Malińska, K., Zabochnicka-Świątek, M., Dach, J., 2014. Effects of biochar amendment on ammonia emission during composting of sewage sludge. Ecol. Eng. 71, 474–478.
Mata-Alvarez, J., Mace, S., Llabres, P., 2000. Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. Bioresour. Technol. 74 (1), 3–16.
Maurer, D., Koziel, J., Kalus, K., Andersen, D., Opalinski, S., 2017. Pilot-scale testing of non-activated biochar for swine manure treatment and mitigation of ammonia, hydrogen sulfide, odorous volatile organic compounds (VOCs), and greenhouse gas emissions. Sustainability 9 (6), 929.
Meyer-Kohlstock, D., Haupt, T., Heldt, E., Heldt, N., Kraft, E., 2016. Biochar as additive in biogas-production from bio-waste. Energies 9 (4), 247.
Mulat, D.G., Dibdiakova, J., Horn, S.J., 2018. Microbial biogas production from hydrolysis lignin: insight into lignin structural changes. Biotechnol. Biofuels 11 (1), 61.
Muller, W.R., Frommert, I., Jörg, R., 2004. Standardized methods for anaerobic biodegradability testing. Rev. Environ. Sci. Biotechnol. 3 (2), 141–158.
Mumme, J., Srocke, F., Heeg, K., Werner, M., 2014. Use of biochars in anaerobic digestion. Bioresour. Technol. 164, 189–197.
Nielfa, A., Cano, R., Fdz-Polanco, M., 2015. Theoretical methane production generated by the co-digestion of organic fraction municipal solid waste and biological sludge. Biotechnol. Rep. 5, 14–21.
Pant, A., Rai, J.P.N., 2018. Impact of chlorpyrifos, TCP and N-substituted aromatic compounds on methane production from organic solid waste (OSW) using co-culture of Pseudomonas aeruginosa and Methanosarcina mazei. Biofuels 1–9.
Pereira, M.A., Sousa, D.Z., Mota, M., Alves, M.M., 2004. Mineralization of LCFA associated with anaerobic sludge: kinetics, enhancement of methanogenic activity, and effect of VFA. Biotechnol. Bioeng. 88 (4), 502–511.
Potivichayanon, S., Sungmon, T., Chaikongmao, W., Kamvanin, S., 2011. Enhancement of biogas production from bakery waste by Pseudomonas aeruginosa. World Acad. Sci. Eng. Technol. 56, 529–532.
Rajagopal, R., Massé, D.I., Singh, G., 2013. A critical review on inhibition of anaerobic digestion process by excess ammonia. Bioresour. Technol. 143, 632–641.
Rittmann, B.E., Lee, H.S., Zhang, H., Alder, J., Banaszak, J.E., Lopez, R., 2008. Full-scale application of focused-pulsed pre-treatment for improving biosolids digestion and conversion to methane. Water Sci. Technol. 58 (10), 1895–1901.
Schmitz, R.A., Albracht, S.P., Thauer, R.K., 1992. A molybdenum and a tungsten isoenzyme of formylmethanofuran dehydrogenase in the thermophilic archaeon Methanobacterium wolfei. FEBS J. 209 (3), 1013–1018.
Schnurer, A., Nordberg, Å., 2008. Ammonia, a selective agent for methane production by syntrophic acetate oxidation at mesophilic temperature. Water Sci. Technol. 57 (5), 735–740.
Shen, Y., Linville, J.L., Urgun-Demirtas, M., Schoene, R.P., Snyder, S.W., 2015b. Producing pipeline-quality biomethane via anaerobic digestion of sludge amended with corn stover biochar with in-situ CO_2 removal. Appl. Energy 158, 300–309.
Shen, Y., Linville, J.L., Urgun-Demirtas, M., Mintz, M.M., & Snyder, S.W. (2015a). An overview of biogas production and utilization at full-scale wastewater treatment plants (WWTPs) in the United States: challenges and opportunities towards energy-neutral WWTPs. Renew. Sustain. Energy Rev., 50, 346–362.
Shin, S.G., Zhou, B.W., Lee, S., Kim, W., Hwang, S., 2011. Variations in methanogenic population structure under overloading of pre-acidified high-strength organic wastewaters. Process Biochem. 46 (4), 1035–1038.
Smith, P.H., 1966. The microbial ecology of sludge methanogenesis. Dev. Ind. Microbiol. 7, 156–161.
Sunyoto, N.M., Zhu, M., Zhang, Z., Zhang, D., 2016. Effect of biochar addition on hydrogen and methane production in two-phase anaerobic digestion of aqueous carbohydrates food waste. Bioresour. Technol. 219, 29–36.
Taghizadeh-Toosi, A., Clough, T.J., Sherlock, R.R., Condron, L.M., 2012. Biochar adsorbed ammonia is bioavailable. Plant Soil 350 (1–2), 57–69.
Thauer, R.K., Kaster, A.K., Seedorf, H., Buckel, W., Hedderich, R., 2008. Methanogenic archaea: ecologically relevant differences in energy conservation. Nat. Rev. Microbiol. 6 (8), 579.
Torri, C., Fabbri, D., 2014. Biochar enables anaerobic digestion of aqueous phase from intermediate pyrolysis of biomass. Bioresour. Technol. 172, 335–341.
Tu, C., Wei, J., Guan, F., Liu, Y., Sun, Y., Luo, Y., 2020. Biochar and bacteria inoculated biochar enhanced Cd and Cu immobilization and enzymatic activity in a polluted soil. Environ. Int. 137, 105576.
Viggi, C.C., Simonetti, S., Palma, E., Pagliaccia, P., Braguglia, C., Fazi, S., … Harnisch, F., 2017. Enhancing methane production from food waste fermentate using biochar: the added value of electrochemical testing in pre-selecting the most effective type of biochar. Biotechnol. Biofuels 10 (1), 303.
Wang, S., Ma, S., Shan, J., Xia, Y., Lin, J., Yan, X., 2019. A 2-year study on the effect of biochar on methane and nitrous oxide emissions in an intensive rice–wheat cropping system. Biochar 1 (2), 177–186.
Ward, A.J., Hobbs, P.J., Holliman, P.J., Jones, D.L., 2008. Optimisation of the anaerobic digestion of agricultural resources. Bioresour. Technol. 99 (17), 7928–7940.
Weiland, P., 2010. Biogas production: current state and perspectives. Appl. Microbiol. Biotechnol. 85 (4), 849–860.
Wittmann, C., Zeng, A.P., Deckwer, W.D., 1995. Growth inhibition by ammonia and use of a pH-controlled feeding strategy for the effective cultivation of Mycobacterium chlorophenolicum. Appl. Microbiol. Biotechnol. 44 (3–4), 519–525.
Xu, S., He, C., Luo, L., Lü, F., He, P., Cui, L., 2015. Comparing activated carbon of different particle sizes on enhancing methane generation in upflow anaerobic digester. Bioresour. Technol. 196, 606–612.
Xue, S., Zhao, N., Song, J., Wang, X., 2019. Interactive effects of chemical composition of food waste during anaerobic co-digestion under thermophilic temperature. Sustainability 11 (10), 2933.
Yenigün, O., Demirel, B., 2013. Ammonia inhibition in anaerobic digestion: a review. Process Biochem. 48 (5–6), 901–911.
Yeung, T., Kwan, M., Adler, L., Mills, T.J., Neilan, B.A., Conibeer, G., Patterson, R., 2017. Increased methane production in cyanobacteria and methanogenic microbe co-cultures. Bioresour. Technol. 243, 686–692.