The Co-fermentation of Organic Substrates: A Review
Performance of Biogas Production under Different Salt Content

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Abstract. Sustainable development is the projected demand of all nations at present. Only 20 percent of world’s primary energy requirement is met by renewable sources like solar and wind energy, hydropower, biomass, municipal and agri-wastes. Biomass, can be utilized to produce biogas due to its high biodegradability, calorific value and nutritive value to microbes, which will reduce our dependency on fossil fuels so that These organic wastes require being managed in a sustainable way to avoid depletion of natural resources, minimize risk to human health, reduce environmental burdens and maintain an overall balance in the ecosystem. The annal of the National Office of Statistics (NOS) indicated that municipal solid waste overtook more and more million tons in 2016, almost 3.5 times the 1992 rates. About 40–70% of civilian solid waste CSW is composed of civilian biomass waste (CBW), such as kitchen waste (KW), fruit/vegetable remains (FVR) and waste water sewage sludge (WWSS). There was a need to find several ways to deal with such wastes. One of these methods is the anaerobic digestion process, as a waste management system, has risen due to it has the usefulness of lowering volume and getting back energy. Several physical and chemical factors influence the creation of methane, and inhibition of bio-methane efficiency by certain materials (salts, oils, ammonia and toxic substrates ) may take place when inhibitors levels appear in high concentrations. In addition, high salt in the organic substrates can inhibit the activity of fermentation microbes by impacting the osmotic pressure of the cell wall of bacteria in the anaerobic system. The purpose of this study was to discuss the effect the types of substrates and salt content on biogas generation in anaerobic digestion.

Keywords: Anaerobic digestion, Inhibition of methane, High saline content, Biogas

1. Introduction

International comfort and community expansion driven by pride and energy, which were especially as a basic requirement of humanity [1]. More than 77% of the world’s energy depletion stems from the combustion of traditional sources [2]. The rapid increase in population and the combustion of fuels (oil) have extremely shared to the rise in earth warming caused greenhouse gas releasing [3]. Sustainable (renewable) ability sources could be a applicable alternative to fossil energies. The energy of bio-waste has been widely enhanced without impacting the ecosystem and human performance [4], organic waste has become the main origin, of smelling, poisonous gas, and groundwater impurity, which decomposed major threat to the ecosystem if process improperly [5]. One such well renowned and exceedingly utilized manner for bio-conversion of trash into bio-fuel is anaerobic digestion (AD), which is consider as the modest technique because it’s very confined ecosystem effect [6, 7,8] and high energy recapture potential [6,7,9]. Although comprehensive research has been carried on bio-gas of wastes by AD system, moreover...
research is presently in go ahead in order to reinforce the clean energy production, get high bio-
degradation averages and decline the amount of eventual organic waste to be disposed [7,8,9,10].
Scientifically biogas is mixing from CH4 and CO2 in the ratio of 3:1, with confirmed contaminant leftover
of SO2, NO2 etc. So, bio-energy of organic waste avails both the targets: i. organic waste dropping ii
waste recovery, iii and biofuel production as substitutional and renewable source. The leftovers of waste
after AD procedure performances as superior muck that can be facilely applied in agricultural soils as a
replace for fertilizer [11]. AD system is conditional on specific microbial assembly for devolution of bio-
woaste out of main steps namely hydrolysis, acetogenesis and methanogenesis as shown in Fig 1.
Investigators have indicated that methanogenesis is the rate-limiting stage for easily degradable materials
whereas hydrolysis is the average shackling step for complex organic wastes due to formation of toxic by-
products like complex heterocyclic synthesises and un-likeable volatile fatty acids(VFAs). Biogas system
is greatly dependent on environmental parameters like pH, T, C / N ratio, C / P , dimension of particle,
inhibitors and type of substrate being applied to get back energy from it [12]. The anaerobic handling of
organic waste is not as common, as the aerobic process, fundamentally due to the long-term period
desired to realize stabilization [13]. The Interaction is also sensoble to raise levels of free ammonia, oils
(fatty acids, grease) conducting from anaerobic devolution of the nitrogen /protein ratios components [14]
. A considerable number of agents that inhibited the AD performance have been considerable reported,
such as ammonia, heavy minerals, oil composition and high salt content.

Figure 1: Mechanism of anaerobic digestion[15]

2. Anaerobic digestion of bio waste:

2.1. Anaerobic digestion for mono substrates

With the preamble of both commercial and anaerobic digestion station projections through the early
1990s, AD of bio-waste has received worldwide [16]. Practicability, by which nearly any kitchen waste
(KW) can be biologically transformed into another portion, in the deprivation of oxygen. The diverse
microbial communities degrade organic waste, which outcomes in the generation of biogas and other
energy-rich bio-compounds as limit products [17]. An AD is usable for a wide range of single substance
including civilian, agricultural and manufacturing wastes, and plant scraps. Moreover, this operation has
some features over aerobic system due to a low energy demand for operation and a low biomass output [18], and it is considered a fertile technique renewable energy [19]. The AD (with single substrates) has some side effects such as long retention times and low abstraction performance of organic compositions. A considerable raise, in ammonia concentration and salt inhibition, occurs due to fermentation of wastes alone. This problem turns into especially serious when reactors are fed with single wastes rich in protein and salt content without dilution, also didn't enhance carbon to nitrogen ratios [9].

2.2. Anaerobic co-digestion co-substrates.

Bio-waste composed of tiny-nutrients like carbohydrates, proteins, oils, organic wastes are convenient for bio-methane. Oils are the main benefactor of biogas with long-term retention time due to retarded biodecomposition, whilst proteins and carbohydrates show quicker transference rates. Co-fermentation also was known as anaerobic co-digestion (ACoD) that raises the capacity of mixed organic materials and speed up the degradation of macro-molecules in substrates by bio-energizing by mix-up substances with one another in various ratios and proportion keeping the C/N (carbon: nitrogen) ratio within the accepted range of 25 – 30. Particularly mix-up organic wastes have indicated in the acceptable range of 20 – 30 [20, 21]. Higher yield of biogas by co-digestion is related to the synergistic influence of the bacteria (microorganisms). Hence, co-digestion is preferred than AD of waste with mono substrates because of its various benefits [22, 23] like reduction of toxic synthesis present in any one of the organic waste has high correlating toxicity, gets better carbon to nitrogen (C/N) ratio of a waste, it also leads to the reduction of the salt content of organic waste and sewage because salt is one of the inhibitors, sludge adjustment, PH enhancement and moisture enhancement content compared to AD with mono substrate [24]. It has been spotted that co-digestion of multi-materials confirm the feed to the bioreactor, thereby improving the C/N ratio and decreasing the concentration of nitrogen, salt [22]. The use of a co-digestion system with a low nitrogen and oil import, waste increases the generation of gas because integral features of both kinds of bio-waste, thus reducing problems related with the piling up of moderate, volatile synthesis, high salt, and ammonia concentrations [25]. Various studies have indicated that blends of agrarian, domestic and industrial wastes can be digested totally and efficiently with one another. In conclusion, the anaerobic system (AD) of individual substrates presents some problems related to substrate features as municipal sewage Sludge (MSS), described by low organic capacities, the municipal solid waste (MSW) could have contained inappropriate substances as well as high levels of heavy metals and salts , therefore The anaerobic co-digestion (multi substrates ) is the at same time AD of multi substrates is a convenient option to overcome the inhibitions of mono-digestion[26,27] as shown in Table 1.
| Substrate               | Co-substrate            | Biogas production rate (l/d), methane production rate (l/d) | Comments                                                                 | References |
|------------------------|-------------------------|---------------------------------------------------------------|---------------------------------------------------------------------------|------------|
| Kine excreta           | Mill waste              | 1.10, 179                                                     | The co-fermentation system generated more than 300% higher biogas than that of excreta single | [28]       |
| Cattle muck            | Agricultural waste      | 2.70, 620                                                     | the increment in biogas creation from the co-digestion was spotted        | [30]       |
| Vegetable waste        | Bucher's wastewater     | 2.53, 611                                                     | The addition of Bucher wastewater to the feedstock rose biogas yield up to more than 50% | [31]       |
| Domestic solid waste   | Fly ash                 | 6.50, 222                                                     | Utilizing of fly ash significantly promoted biogas creation yields of the domestic solid waste | [32]       |
| Civilian biowastes     | Fat, oil, grease (FOG)  | 13.6, 350                                                     | Co-fermentation outputted in a rise of 72% in biogas creation and 46% methane generation yield in comparison with municipal solid waste | [27]       |
| Animal manure          | Fish bio-waste          | 16.4, 620                                                     | Maximum biogas generation rate was got by a blend of wastes               | [33]       |
| Residual potato waste  | Sugar beet              | 1.63, 680                                                     | Co-fermentation enhanced methane creation up to 60% compared to the digestion of single waste | [34]       |
| Slaughterhouse waste   | Sewage sludge           | 8.60, 500                                                     | Biogas creation of the co-fermentation systems more than digestion of Slaughterhouse waste alone | [22]       |
3. **Effect the salt content on co-digestion performance**:

3.1. *Effecting of salt content on biogas volume*

The biogas creation is affected by various concentration of salt through the digestion period. A massive of factors that causing anaerobic system failure has been formally recorded, such as ammonia, thick minerals, oils and saline content salinity fundamentally consisted of several cations, which could limit the performance of the AD [35]. In past study found ramifications of rising NaCl concentrations. Where a decrease in the biogas volume rate and an increase in the length of the fermentation period, pointed according to microbial activity. A previous study has shown that when adding different concentrations of salt to common precipitation, gas production will be significantly affected by these concentrations. Where salt and gas concentrations were respectively (0, 16, 30, 60) gm NaCl, (45, 21, 5, 2) ml/d from gas rate. Through the chart as shown in Fig 2, we can observe the rate of the gas reached the high level with a concentration of NaCl zero and became small or almost non-zero concentration with 60 g NaCl [36]. This height saline could lead cell osmotic fatigue imponderables, causing in dissolution of cytoplasm and/or loss of effectiveness of cells and inhibition biogas rate yield [37]. A comparatively high NaCl concentration of 3,100–5,100 mg/L would lead to average inhibition, but at 9,000 mg/L, it would lead to a terrible inhibitory in the gas output [34]. The effect of organic matter in seawater has a negative impact on the performance of gas production because the organic matter in seawater has a high salt content of more than 10 g/NaCl [38]. The volume of the gas was measured by Equation (1) [39].

\[
V_{biogas} = \Delta P \cdot \frac{V_{head} \cdot c}{R \cdot T}
\]  

- Wheresoever \( V_{biogas} \) means for diurnal biogas volume (L)
- \( \Delta P \) means absolute compressing various
- \( V_{head} \) indicates to the volume of the top vacuum (L).

**Figure 2**: Biogas generation rate through criterion periods with various NaCl concentrations [36].
3.2. Effecting of salt content on methane generation

Studies have shown that joint digestion enhances the production of biogas, but this enhancement is affected by several factors, including the saline effect inhibited by the proportion of methane produced after fermentation. One of the past research pointed the effect of salt on co-digestion from food waste and sewage sludge with the concentrations of salt 0.8-4.5 g/L NaCl would raise the methane generation while 7 and 12 g/L NaCl recorded in a lessening of methane gas generation by 31% and 45%, respectively[38]. The formalization of methane gas from acetate would be discouragement by 12, 55 and 95 % respectively at salt concentrations of 6.5, 14.2 and 22 g/L [40]. Also, research indicated on how the salinity would influence the biomethane creation has been depiction rising interest. It was pointed that a saline concentration extend from 3 to 7 g/L would reasonably inhibit the biomethane activity, whilst, exceeding 7 g/L would highly dampen methanogenesis creation [41]. Decreasing 55 % of bio-methane performance with uninterrupted insinuation to salt in co-digestion reactors was spotted when sodium concentrations were more than 25 gL−1, but in another side, these inhibition levels were in the zone 7-18g from the salt ion therefore anaerobic co-fermentation is not contemplated proper, for food waste with salt at high condensations [42]. Bacteria of methanogenesis initiate to be influenced at a NaCl concentration of 6 g/L however bacteria of acidogenesis was hardly impacted only at NaCl level exceeding more than 20g/L as shown in Fig 3. The effect of salt on digestion is not limited to methane production but also to the efficiency of determinants such as pH. In same this study in the same study, the researchers pointed the increment of the addition of NaCl influenced on the pH value. The value in all reactors diminished with an rising salt content, with the ultimate, pH of 7.44, 7.40, 7.38 and 7.31 for the blank, L, M and H reactors respectively as shown in Fig 4, referencing that increasing NaCl could cause a higher VFAa and a lower pH [43].
Figure 4: pH variation at various concentrations of saline [44]

High concentration of salt will activate carbon dioxide and inhibit methane [41]. Some previous researchers pointed that decreasing salinity was advantageous for the growth bacteria of methane with value (0-0.85 g/L NaCl) and decrease CO₂, while 9-14 g/L NaCl would cause a stumbling block for methane creation and values more than 25 g/L and improving CO₂ generation as in Fig 5.

Figure 5: Comparing between carbon dioxide and methane under different salt concentrations [42]
Five anaerobic tanks (1-5) were investigated the different levels of salt (0-15 gm/NaCl) on methane creation from the co-fermentation of multi-organic matter. The outcome of bio-methane creation in the lack of NaCl (0 gm) caused mostly increase from methane generation through time period through 23 days. However, the proportion of methane has decreased significantly with the increase in salt doses between 10-15 g/salt as in Fig 6. The existence of Na+ and cl- at the high level would cause a negative influence on the production of bio-methane from multi-organic anaerobic co-digestion. Bio-methane creation of organic substrates reduced with the level of NaCl rose. For example, salt levels 0, 11 and 36 g/l, methane created were 84.0, 43.7 and 13.4 milliliters of methane g/l, respectively. Methane was zero at all when the salt level was above 65 gL-1. Several previous studies have confirmed that sodium levels average from 6.6 to 7.9gL-1 inhibited 50 percent methane effectiveness. High methane inhibitor level of sodium (13.0 gl-l) was indicated in the anaerobic treatment of salty municipal sludge in water remediation. The discrepancy can be referred to multi-factors, including inverse effects, synergies, and acclimation [45,46].

**Figure 6**: The proportion of methane has decreased significantly with the increase in salt doses between 10-15 g/salt [47].

![Cumulative methane production](image)

The effect of salts on the performance of total volatile fatty acid (TV) to total alkaline (TA) was studied. In a previous study, 9 tanks containing a mixture of biomaterials were used where the salt concentrations were from 0-8 g/l tank 1- tank 4, while concentration 10-17 g/l tank 5- tank 9. The creation and allocation of VFAs/methanol at each salt concentration. The effluent from the methane fermentation tank fundamentally contained acetate, ethanol, butyrate, and propionate. With the excess of the sodium salt concentration, there was a slow increase of the total V.F.A/methan (TV) from tank 1-tank4, and a heavy accumulation from tank 5- tank9. The critical, TV was found in tank 9 (which was 3541.8 mg/L), and the TV piling up matched well with the methane diminution. The TV concentration of >8 g/L could demoralize the gas generation CH4. TA plays a serious part in buffering the reduce pH of the anaerobic co-fermentation because of rapid hydrolysis and acidification of the food waste (FW). The rate of TV/TA could be utilized to estimate the system steadiness, with the excess of the ratio; the system steadiness will
be inhibited. So that, in the current study, the TV/TA the rate was also examined and is summed up in Table 2. It can be seen that the TV/TA rates of tank 1–tank4 were <0.1111 which agreed well to the highly efficient and steady reactors while the TV/TA rates of tank 5–tank 9 were all above 0.111, which was in matching with the unsettled methane creation procedures. On the fundamental of the outcomes, it can be suggested that when the TV/TA rate is above 0.1111, attentiveness should be driven to avoiding unsteady through the AD of KW.(kitchen wastes) [48,49].

Table 2: Features of effluents during fermentation with salt content NaCl [50, 51, 52].

| Tanks   | Salt conc. | pH  | Total Alkalinity (TA, g/l) | Total VFA/methanol (TV) | TV/TA ratio |
|---------|------------|-----|---------------------------|-------------------------|-------------|
| Tank 1  | 0          | 7.56| 6,661 ± 657               | 155 7.5                | 0.022       |
| Tank 2  | 4.5        | 7.43| 6,779 ± 665               | 206.1 ± 15.3           | 0.029       |
| Tank 3  | 5.8        | 7.42| 7,121 ± 732               | 245.0 ± 17.2           | 0.033       |
| Tank 4  | 8          | 7.41| 6,341 ± 715               | 349.1 ± 23.7           | 0.049       |
| Tank 5  | 10.2       | 7.39| 6,677 ± 684               | 761.2 ± 52.0           | 0.119       |
| Tank 6  | 12.1       | 7.33| 5,889 ± 597               | 1357.4 ± 83.5          | 0.212       |
| Tank 7  | 14.0       | 7.31| 6,001 ± 598               | 1876.2 ± 126.0         | 0.311       |
| Tank 8  | 15.4       | 7.25| 5,677 ± 576               | 2788.0 ± 174.8         | 0.477       |
| Tank 9  | 17         | 7.08| 6,342 ± 705               | 3541.8 ± 203.0         | 0.543       |

Previous studies have indicated that the cumulative methane rate and methane inhibition rate can be measured according to the equations below [53]. Biomethane outputs (MOs) out of the complete AD process, was applied according to the Equation 2.

\[
B = B_0 \cdot exp \left( -exp \left( \frac{\mu m e}{B_0} \right) (\lambda - 1) + 1 \right)
\]  

(2)

- where \( B \) means the methane yield (MY) (mL/g-VSadded)
- \( B_0 \) is the methane yield potency (MYP) (mL/g-VSadded)
- \( \mu \) means the superior methane output rate (mL/(g·VSadded))
- \( \lambda \) means to the lateness step period (in days)
- \( e \) is a constant that equals 2.72 and \( t \) means the incubation period (in days)

The inhibition point was estimated by Equation 3 [54].

\[
Inhibition degree \% = \frac{(A - B)}{A} \cdot 100\%
\]

(3)

- Where \( A \) is the heaped-up methane output without adding inhibitor into any tank
• B is the heaped-up methane output after adding a certain condensation of inhibitor in any tank.

4. Conclusion

Organic substrates can be considered as a resource due to its utilization to enhance sustainable and eco-friendly renewable outputs like biogas using anaerobic co-fermentation. Mechanism investigation showed that the digested of co-substrates enhanced the digestion parameters such as (PH, TS, VS, C/N) and enhanced the consumption of carbohydrate thereby elevating the rate of "hydrolysis, acidification, and methanogenesis" as compared with the mono food waste anaerobic digestion. But, some of the organic wastes have a massive amount of salt, which would inhibit fermentation system high salinity extremely leads inhibition and also stopped in the anaerobic system. The conclusion of this research is that most researchers have found inhibitors of methane generation from the anaerobic co-substrate of food waste under sodium chloride. More than 65 g/l sodium chloride, no bio-methane was created at all. Also in 11gl-1 sodium chloride, only half of the gas was created compared to salt minus organic waste. Low levels of NaCl increased the hydrolysis, and acidification processes but inhibited methanogenesis while both methanogenesis and acidification processes were seriously inhibited by high salt concentrations. Therefore high salinity materials in forms (sulfurite, sulfide, calcium chloride, sodium chloride, etc.) contained in anaerobic reactors should be less than 65g/l for control of methane inhibitors and poor reactor performance.

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