Effect of Various Potential Additives on Hydrogen Fermentation During the Co-Digestion of Food Waste and Cow Dung

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Research

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Abstract

The effect of calcium peroxide (CaO₂), zinc oxide (ZnO), copper oxide (CuO), and calcium carbonate (CaCO₃) as additives during the anaerobic co-digestion of food waste and cow dung is experimentally investigated to enhance the hydrogen fermentation. The maximum concentration of hydrogen in the generated gas is found to be 26.43%, 21.67%, 17.64%, and 20.84% while the cumulative yield of hydrogen remains 114.1, 109.27, 104.87, and 107.38 mL g⁻¹ Total Solid (TS) with CaO₂, ZnO, CuO, and CaCO₃ respectively. The sample in which no additive is used (control) exhibits a maximum hydrogen concentration of 17% and a cumulative hydrogen generation of 101.57 mL g⁻¹ TS in the produced gas. Result reveals an enhancement in the hydrogen concentration up to 9.43% whereas the cumulative hydrogen yield is increased up to 11% with additives compared to the control sample. Overall the hydrogen fermentation can be significantly enhanced with the additives through the anaerobic co-digestion process.

1. Introduction

Food waste is generated at various stages of production, refining, processing, distribution, and consumption. The accumulation of food wastes at all stages is very huge and transforms into a global problem. Various surveys and analyses show that the amount of global loss of food and food wastes is one-third and half of all the food produced from different sources [1]. In the developing countries, most of the food waste is generated at the production stage due to lack of updated technologies whereas in developed countries the losses occur at the consumption stage. The data reflects that in developed countries about 100 kg per person per year of food is wasted at the consumption stage [1]. The accumulation of food waste generates various problems and creates many diseases along with the emissions of harmful gases. The problem of disposal makes it compulsory to find ways of reduction. The ancient methods of disposal like landfill and incineration are not so useful because of land availability and high energy consumption [2]. So, an effective method needs to be introduced which can solve the problems related to food waste. The various ways and methodologies have come up from the research fraternity and suggest that energy can be extracted from the food waste.

The effective conversion of food waste into energy might be a blessing to society rather than a problem. Biogas and Hydrogen generation is the best alternative that can be generated from food waste. Performances of biogas and hydrogen on a diesel engine in dual fuel mode were found to have a significant effect on reducing the emission for a clean and green environment [3]. Hydrogen serves as the most promising and very important carrier of energy which could play a magnificent role in greenhouse gas emissions [4]. Various mechanisms and biological pathways can be followed for the production of Hydrogen. Dark fermentation by bacteria becomes an accepted and easier method for the production of hydrogen. The compounds like Hydrogen (H₂), carbon dioxide (CO₂), and simple organic compounds like volatile fatty acids (VFAs) and alcohols are formed by the fermentation of carbohydrates by large groups of obligate or facultative anaerobic bacteria. Electrons responsible for the production and generation of
Hydrogen generally come from oxidation of substrate to pyruvate and the second electron comes from the conversion of pyruvate to volatile fatty acids and alcohols [5]. In the first stage, Nicotinamide Adenine Dinucleotide Hydrogen (NADH) is generated by the oxidation of the substrate to pyruvate. Pyruvate is converted to acetyl-CoA in the second stage. Formate is formed by the help of Pyruvate Formate Lyase (PFL) and reduced Ferredoxin is formed from Pyruvate Ferredoxin Oxidoreductase (PFOR). These depend on the organisms which produce Hydrogen (H\textsubscript{2}) where the reaction takes place. In the pathway of PFL, Formate Hydrogen Lyase is used by some organisms which contain hydrogenase, and formate is converted to H\textsubscript{2} and CO\textsubscript{2}. Reduced Ferredoxin is produced in the PFOR pathway [6]. The VFAs are generated when pyruvate enters the acidogenic pathway.

Degradation of Substrates in AD ensures the interconversion of metabolites, as a result, the probability of getting reduced equivalents in the cell is increased. The transfer of electron through various steps to produce hydrogen in the fermentative process is shown in Fig. 1. The H\textsuperscript{+} released from redox mediators like Nicotinamide Adenine Dinucleotide Hydrogen (NADH) and Flavin Adenine Dinucleotide Hydrogen (FADH) is removed due to NADH-dehydrogenase which finally reduces the H\textsubscript{2} by the help of the hydrogenase (HydA enzyme) which is produced by the oxidized (Ferredoxin) Fd (co-factor). Electron transport through Q-pool is facilitated by the motile carrier (quinone) proteins and some membrane-bound complexes of proteins like NADH dehydrogenase and Cytochrome bc-1. The continuous interconversions take place from Q and H\textsuperscript{+} which is converted to QH\textsubscript{2} then QH\textsubscript{2} again converted to Q and H\textsuperscript{+} which transfers electrons e\textsuperscript{−} to the Cytochrome bc-1 complex and then Cytochrome aa3. The electron (e\textsuperscript{−}) is donated by the reduced Fd to the activated site of the HydA enzyme and this enzyme helps in the reduction of H\textsuperscript{+} with this electron (e\textsuperscript{−}) to produce molecular hydrogen [7].

Hydrogen produced by dark fermentation is satisfactory but the performance yields can be improved by following various methods. These methods include pre-treatment like heat-shock, acid or alkali treatment, etc. The addition of certain additives such as calcium oxide (CaO) and calcium peroxide (CaO\textsubscript{2}) results in a significant enhancement in hydrogen production during the fermentation of wheat straw [8]. Further, an enhancement in the rate of hydrolysis reaction due to the addition of CaO\textsubscript{2} during the anaerobic digestion (AD) of corn straw was identified by Fu et al. [9]. Similarly, Chen et al. [10] investigated the dewatering properties of waste sludge using CaO\textsubscript{2} and concluded that the filtration was improved by adding 20 mg g\textsuperscript{−1} total suspended solid due to the enhanced oxidation which was further deteriorated at a higher dosage due to the extensive release of polymeric substances such as protein. Wang and Li [11] observed substantial enhancement in the degradation efficiency by using the combination of CaO\textsubscript{2} and microwave pretreatment resulting in higher methane generation. However, none of the above works have used food waste and cow dung as the feedstock to investigate the effect of CaO\textsubscript{2} on hydrogen fermentation. Some efforts have also been given to use calcium carbonate (CaCO\textsubscript{3}) during the anaerobic digestion. Zhang and Wang [12] identified enhanced hydrogen fermentation with reduced lag phase by adding CaCO\textsubscript{3} enriched white mud during the fermentation of food waste. It was found that the stability and the alkalinity of the process were enormously optimized resulting in increased hydrogen generation. Liu et al.
[13] reported significant improvement in hydrogen and total fatty acid production by adding carbonate in the co-digestion of glucose and leachate maintained at alkaline conditions. The addition of carbonate also restrains the inhibitory effect of Ca$^{2+}$ upon hydrogenase resulting in enhanced hydrogen generation. The accumulation of volatile fatty acid was enhanced during the AD of food waste by adding lime mud (enriched with CaCO$_3$) due to the increased alkalinity along with the presence of several other compounds like calcium and magnesium which facilitates the microbial activity during the reaction [14]. The improved process stability and hydrogen generation were further confirmed by the presence of CaCO$_3$ and NaOH with the addition of 1–4 g lime mud during the hydrogen fermentation of food waste [15]. Moreover, the addition of CaCO$_3$ showed a promising effect on hydrogen fermentation but its use is limited with other forms such as lime mud rather than a primary additive. The addition of copper oxide (CuO) and zinc oxide (ZnO) also served as interesting additives for the researchers to investigate the hydrogen fermentation. Numerous experiments have been performed in this regard to improve hydrogen production. Chen et al. [16] observed that the performance and the microbial activity were enhanced by the supplementation of nano-carbon powder and nano-aluminium oxide whereas the use of nano-ZnO and nano-CuO appreciably reduce the microbial action in addition to the performance of the anaerobic fermentation process. Zheng et al. [17] observed a reduced performance in the AD process by using the ZnO nanoparticle but the performance was found identical to the control group when the combination of ZnO and TiO$_2$ was used as the multi engineered nanoparticle material. Zhang et al. [18] identified a higher accumulation of volatile fatty acid by adding ZnO nanomaterial compared to the control sample mainly due to the effect of Zn$^{2+}$ during the AD of waste activated sludge. Mu et al. [19] found that nano-ZnO inhibits the methane generation by suppressing the enzyme activity during sludge hydrolysis acidification and methane generation. Luna-del Risco et al. [20] revealed that the toxicity of the microorganism was regulated by the size of CuO and ZnO particles with considerable effect on biogas and methane generation. Most of the research on CuO and ZnO addition on anaerobic digestion is based on the nanoparticle rather than the bulk particle of these additives. However, it is suggested from the literature survey that the addition of bulk organic compounds as well as nanomaterials can improve the hydrogen fermentation during the co-digestion process.

It is clear from the literature study that the performance and the microbial activity during the anaerobic co-digestion process could be enhanced by the supplementation of various additives. Though numerous experiments have been conducted by using CaO$_2$, ZnO, CuO, and CaCO$_3$, there is a significant research gap to investigate the effect of these additives on the hydrogen fermentation of food waste and cow dung which has never been studied previously. To enhance the hydrogen production from the anaerobic co-digestion, a precise investigation is needed on the use of these additives during the hydrogen fermentation. Therefore in the present work, a novel investigation on the effect of CaO$_2$, ZnO, CuO, and CaCO$_3$ addition on the anaerobic fermentation of food waste, cow dung, and sludge solution was carried out and compared with the control sample in which no additives were added. The finding of the work engenders encouraging outcomes towards the enhanced hydrogen fermentation.
2. Materials And Methods

2.1. Substrate and inoculum

The food waste (FW) was accumulated from the university canteen of ITER, Odisha, India. The non-biodegradable substances such as bones, eggshells, and plastic present inside the FW were removed manually and then the FW was shredded using a home grinder by diluting with tap water to prepare the slurry. The cow dung (CD) was collected from a nearby dairy farm and the slurry was prepared by mixing with tap water. The substrate was a mixture of FW and CD slurries in equal proportion. The sludge solution (SS) was accumulated from the wastewater treatment plant (Bhubaneswar, India) located nearby. It was used as the inoculum during the experiment. The characteristics of food waste, cow dung, and sludge solution were presented in Table 1. The substrate and inoculum were used in the experiment without any pretreatment.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Food waste</th>
<th>Cow dung</th>
<th>Sludge solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total solid (%)</td>
<td>10.3 ± 0.06</td>
<td>67.37 ± 0.09</td>
<td>9.6 ± 0.04</td>
</tr>
<tr>
<td>Volatile solid (%)</td>
<td>8.7 ± 0.15</td>
<td>34.71 ± 0.34</td>
<td>7.3 ± 0.07</td>
</tr>
<tr>
<td>pH</td>
<td>4.4 ± 0.1</td>
<td>6.7 ± 0.3</td>
<td>7.2 ± 0.1</td>
</tr>
<tr>
<td>Carbon (%)</td>
<td>48.6 ± 0.31</td>
<td>41.54 ± 0.43</td>
<td>24.58 ± 0.18</td>
</tr>
<tr>
<td>Hydrogen (%)</td>
<td>6.5 ± 0.14</td>
<td>3.57 ± 0.36</td>
<td>3.29 ± 0.21</td>
</tr>
<tr>
<td>Oxygen (%)</td>
<td>32.46 ± 0.26</td>
<td>37.58 ± 0.41</td>
<td>49.64 ± 0.34</td>
</tr>
<tr>
<td>Nitrogen (%)</td>
<td>3.16 ± 0.17</td>
<td>2.71 ± 0.08</td>
<td>1.64 ± 0.10</td>
</tr>
<tr>
<td>Sulphur (%)</td>
<td>0.4 ± 0.04</td>
<td>0.09 ± 0.003</td>
<td>0.61 ± 0.03</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>5.29 ± 0.12</td>
<td>12.78 ± 0.23</td>
<td>46.4 ± 0.30</td>
</tr>
<tr>
<td>Moisture content (%)</td>
<td>82.6 ± 0.46</td>
<td>79.65 ± 0.52</td>
<td>98.5 ± 0.67</td>
</tr>
<tr>
<td>Fixed carbon (%)</td>
<td>12.29 ± 0.09</td>
<td>10.64 ± 0.11</td>
<td>8.73 ± 0.21</td>
</tr>
<tr>
<td>Cellulose (%)</td>
<td>0.84 ± 0.03</td>
<td>2.187 ± 0.07</td>
<td>ND</td>
</tr>
<tr>
<td>Hemicellulose (%)</td>
<td>0.38 ± 0.04</td>
<td>2.038 ± 0.06</td>
<td>ND</td>
</tr>
<tr>
<td>Lignin (%)</td>
<td>0.29 ± 0.07</td>
<td>3.273 ± 0.05</td>
<td>ND</td>
</tr>
</tbody>
</table>

Note: “ND” signifies the parameter was not determined.

2.2. Experimental methodology
The experiment was performed in conical glass reactors with a volume of 1 L. The reactors were loaded with the substrate to inoculum ratio of 1:3 on a volume basis. Previously experiments were conducted at the various substrate to inoculum ratio from 1:1 to 1:5 to find the optimum ratio for maximum hydrogen generation. The consequence of substrate to inoculum proportion on hydrogen production was explained in our earlier work [21]. One of the reactors (control) did not receive any additive while the other reactors were added with CaO$_2$, ZnO, CuO, and CaCO$_3$ at a dosage of 2 mg L$^{-1}$ according to the earlier studies [22, 23]. After proper mixing, nitrogen gas was used for purging inside the reactors to confirm the anaerobic condition. The reactors were sealed immediately with rubber stoppers and placed on the magnetic stirrer as shown in Fig. 2. The temperature of the water was controlled by PT 100 thermostatic probe to maintain a constant temperature of 37 ± 1 $^{0}$C throughout the experimentation based on the earlier study [21]. Each of the experiments was conducted thrice and the average value obtained was used for better accuracy.

**2.3. Analytical method**

Total solids, volatile solids, pH, ash, moisture, and fixed carbon were measured by following the standard methods [24]. The elemental concentration of carbon, hydrogen, oxygen, nitrogen, and sulfur was measured by CHNS elemental interpreter (Nario EL III). Cellulose, hemicellulose, and lignin concentrations were evaluated followed by the standard methods [25]. Scanning electron microscopy (SEM) was carried out using a JSM-JEOL 6360 scanning electron microscope provided with an auto coater for coating which is automatically adjusted and varied according to the sample. The instrument was operated with an accelerating voltage of 15 kV. The gas composition was analyzed in a gas chromatograph (GC-2010, CIC, Baroda) provided with a stainless steel column (2 m × 3 mm) and a thermal conductivity detector. The gas sample was collected in a pressure lock airtight syringe (VICI, USA) and inserted into the chromatograph to analyze the composition. The carrier gas (nitrogen) was supplied with a flow rate of 20 mL min$^{-1}$. The composition of the gas was monitored daily throughout the experiment. The water displacement strategy was used for the measurement of gas volume produced during the process.

**3. Results And Discussion**

**3.1. Microstructural characterization of the additives**

The SEM image of CaO$_2$ represents a uniform particle size distribution of as shown in Fig. 3a. The structure and dispersion of particles show higher uniformity compared to the other additives which facilitate the hydrogen fermentation due to increased surface contact of the microorganism with the feedstock. On the other hand, a closely packed microstructure as well as a non-uniform distribution can be observed from the SEM image of CuO (Fig. 3b) due to the lower solubility which results in reduced microbial growth during the reaction. Similarly, the SEM image of ZnO (Fig. 3c) shows an unseparated and closed structure but it is slightly dispersed compared to CuO. The particle size distribution of both CuO and ZnO represents that the particles are highly agglomerated and incompletely separated promoting a less encouraging effect on the hydrogenase microbial action with the feedstock during the
initial stages of the co-digestion. The crystallographic structure of CaCO$_3$ (Fig. 3d) shows rhombohedral crystals with dense and grouped particles. The crystals are also undefined with agglomeration which deteriorates the performance of the system causing a reduced hydrogen fermentation during co-digestion. Moreover, the crystallographic characterization signifies that CaO$_2$ leads to the enhanced hydrogen fermentation compared to the other additives. Similar results on the microstructural configuration of the additives have also been reported previously in the literature [26–29].

### 3.2. Effect of the additives on hydrogen concentration

The hydrogen concentration of the five reactors is shown in Fig. 4. It was observed that the hydrogen concentration in the control sample remains within the range of 17% whereas a higher concentration of hydrogen was found by supplementing the additives inside the reactors. The maximum hydrogen concentration after the addition of CaO$_2$, ZnO, CuO, and CaCO$_3$ was found to be 26.43%, 21.67%, 17.64%, and 20.84% respectively. The highest concentration of hydrogen was observed by adding CaO$_2$ while the lowest concentration was observed by adding CuO as an additive with the feedstock. The hydrogen concentration gradually increases in all the reactors up to the fourth or fifth day when it reached the peak after that the concentration decreases significantly throughout the anaerobic fermentation. It was identified from the above results that additives could enhance the hydrogen concentration during the fermentation especially when the feedstock was supplied with CaO$_2$. The addition of ZnO and CaCO$_3$ has a moderate effect whereas CuO did not show any significant impact on the hydrogen concentration throughout the anaerobic fermentation process.

The fermentation process of hydrogen was significantly enhanced by adding CaO$_2$ compared to the other additives. There are two main reasons involved in this observation. Firstly the dissociation of CaO$_2$ results in the generation of OH$^-$ and ·O$_2$ that facilitate the hydrolysis of protein along with carbohydrate due to the increased breakdown of the microorganisms and the release of the amino acids [30]. Secondly, the release of OH$^-$ and H$_2$O$_2$ as the intermediate products due to the reaction between CaO$_2$ and water leads to an increased reaction rate during the initial stages of AD [31]. These two factors are primarily responsible for the enhanced hydrogen generation during the fermentation process. The dissociation of ZnO and CuO results in the formation of Zn$^{2+}$ and Cu$^{2+}$ ions that lead to an increase in the toxicity of the medium and inhibit the microbial growth during the AD [32]. Also, the toxic nature of the ions was responsible for the lower volatile fatty acid generation caused by the decreased hydrolysis of protein [18]. The addition of CaCO$_3$ causes less ion conductivity and lower pH which results in a lower concentration of hydrogen during the acidification [33]. Also the reduced ion activity of Ca$^{2+}$ and CO$_3^{2-}$ leads to the lower rate of reaction during the hydrolysis. Moreover, the combined effect of lower pH and higher toxicity results in lower hydrogen formation during the reaction. Baldi et al. [34] observed a maximum hydrogen percentage of 22.9% during the AD of activated sludge with food waste. A similar result was also reported by Yeshanew et al. [35] through the anaerobic fermentation of food dissipate.

### 3.3. Effect of the additives on cumulative hydrogen yield
Figure 5 shows the cumulative hydrogen yield of all the reactors. It was observed to be lowest in the control sample while the highest cumulative hydrogen yield was obtained by adding CaO2 with the feedstock. The cumulative hydrogen yield of all the reactors after 15 d of fermentation was found to be 101.57 mL g⁻¹ TS (control), 114.1 mL g⁻¹ TS (CaO₂), 109.27 mL g⁻¹ TS (ZnO), 104.87 mL g⁻¹ TS (CuO), and 107.38 mL g⁻¹ TS (CaCO₃) respectively. It was found to follow the order CaO₂ > ZnO > CaCO₃ > CuO > control. The hydrogen production was accelerated by the additives compared to the control samples during the reaction. Hydrogen generation was enhanced up to 11% by adding CaO₂ with the feedstock compared to the control sample in which no additives were added during the fermentation process. The increase in hydrogen generation by adding ZnO, CuO, and CaCO₃ was found to be 7%, 3%, and 5.4% respectively compared to the control one. It was clear from the observation that additives could increase hydrogen production significantly during the anaerobic fermentation of food waste.

The cumulative hydrogen yield was significantly enhanced by adding CaO₂ due to the release of alkali, ·OH, and ·O₂⁻ from the decomposition of CaO₂ which facilitate the hydrolysis of the feedstock producing more amount of hydrogen during the early stages of AD [31]. Also, CaO₂ was more prone towards the inhibition of hydrogen consuming bacteria rather than the hydrogenase which is also responsible for the higher hydrogen generation during the reaction [36]. The higher toxicity of ZnO and CuO restrict the growth of hydrogen fermentative bacteria during the earlier stages of AD which is the main reason for lower hydrogen generation when compared to CaO₂ [20]. However, the cumulative hydrogen yield by adding ZnO and CuO was found to be higher compared to the control sample due to the enhanced hydrolysis of the feedstock during the initial period of digestion. The lower pH of the feedstock along with the lower ion activity of Ca²⁺ and CO₃²⁻ results in lower hydrogen generation by adding CaCO₃ as an additive during the hydrogen fermentation [33]. Nevertheless, an increase in hydrogen generation compared to the control sample was observed by adding CaCO₃ due to the stimulated growth of hydrogenase caused by the release of ion during the acidification of the feedstock. The cumulative hydrogen production of 60.23 mL g⁻¹ VS was detected during the anaerobic fermentation of food waste by Li et al. [37]. A comparable outcome was also reported by other authors during the AD of peanut shells [38].

### 3.4. Effect of the additives on carbon dioxide concentration

The effect of different additives on carbon dioxide concentration compared to the control sample is presented in Fig. 6. A significant increase in carbon dioxide proportion was observed through the initial period (7 to 8 d) of AD in all the cases. After the attainment of the peak hydrogen concentration it gradually decreases during the remaining days of digestion. The maximum carbon dioxide concentration in the control, CaO₂, ZnO, CuO, and CaCO₃ reactors was found to be 57.63%, 54.62%, 58.37%, 59.2%, and 58.4% respectively. The carbon dioxide concentration of CaO₂ was lower than the control sample but ZnO, CuO, and CaCO₃ result in higher carbon dioxide concentration compared to the control sample. The addition of CaO₂ compared to the other additives significantly reduces the carbon dioxide formation which leads to improved hydrogen formation during the acidogenic stages of the reaction. However, the
addition of ZnO, CuO, and CaCO₃ reduced the hydrogen generation compared to CaO₂ which was the consequence of higher carbon dioxide concentration during the AD.

Lower carbon dioxide concentration in the CaO₂ reactor results in the higher hydrogen concentration compared to the other reactors. This may be due to the formation of alkali, ·OH, and ·O₂⁻ which accelerate the hydrogen fermentation and suppress the generation of carbon dioxide during the reaction [31]. The higher carbon dioxide concentration by adding ZnO, CuO, and CaCO₃ was due to the increased toxicity of the medium caused by the ionic activity of Zn²⁺, Cu²⁺, and Ca²⁺ respectively [39]. The acidification of the feedstock during the initial period of digestion was enhanced by the ions while the hydrogenase microbial growth was restricted due to the toxicity inside the reactor. A similar observation on carbon dioxide concentration was reported by the other researchers through the anaerobic fermentation of food residue [40, 41]. The results showed a significant variation in the carbon dioxide concentration by using the additives compared to the control sample. The addition of CaO₂ exhibited a positive effect by reducing the carbon dioxide concentration whereas ZnO, CuO, and CaCO₃ addition increase the concentration of carbon dioxide which is undesirable during the hydrogen fermentation.

3.5. Effect of the additives on maximum hydrogen and carbon dioxide concentration

The maximum hydrogen concentration was inversely proportional to the carbon dioxide concentration as shown in Fig. 7. The highest hydrogen concentration was detected in the CaO₂ sample with the lowest carbon dioxide concentration in comparison with other reactors. As the hydrogen concentration increases with CaO₂ compared to the control the carbon dioxide concentration was reduced simultaneously. Further decrease in the hydrogen concentration increases carbon dioxide concentration. Among the additives, the lowest hydrogen concentration was observed to be 17.64% in the CuO reactor that leads to the highest carbon dioxide concentration of 59.2%. The maximum hydrogen concentration of 26.34% was observed in the CaO₂ reactor with the lowest carbon dioxide concentration of 54.62% among all the reactors. It was noted that the addition of CaO₂ significantly promote the hydrogen generation by reducing the carbon dioxide concentration while the addition of ZnO, CuO, and CaCO₃ has a less significant effect on hydrogen generation but still the additives have a positive effect on hydrogen and carbon dioxide concentration compared to the control reactor.

The disintegration of CaO₂ leads to the generation of alkali, ·OH, and ·O₂⁻ those are primarily responsible for the increased hydrogen production by increasing the microbial disintegration during the reaction [42]. The enhancement of hydrogen generation simultaneously results in the reduction of carbon dioxide formed during the acidification. Also, the decomposition of CaO₂ increased the alkali generation during hydrolysis that facilitates the higher bacterial activity inside the feedstock [43]. As the hydrogen fermentation increases by adding CaO₂ the microbial activity could not shift towards the carbon dioxide fermentation which results in the lower carbon dioxide concentration compared to the other reactors. Moreover, the formation of Ca²⁺ due to the decomposition of CaO₂ did not show a significant effect on
the reduced growth of hydrogenase compared to the combined effect of alkali, ·OH, and ·O₂ during the reaction [44]. Lower alkali generation in ZnO, CuO, and CaCO₃ reactors inhibited the hydrogenase bacterial growth during the acidification resulting in the lower hydrogen generation compared to the CaO₂ reactor. The carbon dioxide fermentation was enhanced due to the inhibition of hydrogenase bacterial growth during the initial period of digestion. Also, the lower hydrogen concentration was the upshot of higher toxicity inside the reactors due to the formation of Zn²⁺, Cu²⁺, and Ca²⁺ during the decomposition of ZnO, CuO, and CaCO₃ respectively [45]. The increase in hydrogen generation with a simultaneous decrease in carbon dioxide concentration was reported by Algapani et al. [46] in which a maximum hydrogen concentration of 64% was observed with a reduced carbon dioxide concentration of 32% through the multi-stage digestate recirculated anaerobic fermentation of food residue.

### 3.6. Effect of the additives on hydrogen production

The cumulative hydrogen yield and the hydrogen concentration of all the reactors are presented in Fig. 8. The cumulative hydrogen yield was observed to be directly proportional to the corresponding hydrogen concentration of the reactor where the maximum hydrogen concentration and the cumulative hydrogen yield were observed to be 26.34% and 114.1 mL g⁻¹ TS respectively in the CaO₂ reactor. The lowest value of the hydrogen concentration and the cumulative hydrogen yield among all the reactors were observed to be 17% and 101.57 mL g⁻¹ TS respectively in the control reactor. It is worth noting that the additives have more or less increased hydrogen generation in comparison with the control sample during the anaerobic fermentation. The lowest hydrogen production among the additives was observed with CuO while the highest production was found with the CaO₂ sample. The maximum hydrogen concentration in the ZnO, CuO, and CaCO₃ reactors was found to be 21.67%, 17.64%, and 20.84% respectively while the cumulative hydrogen yield was identified to be 109.27 mL g⁻¹ TS, 104.87 mL g⁻¹ TS, and 107.38 mL g⁻¹ TS respectively from the same reactors. The cumulative hydrogen production demonstrated a direct link among the hydrogen concentration when the additives are used with the feedstock through the anaerobic co-fermentation.

The increased hydrogen generation in the CaO₂ reactor corresponds to the increased hydrogenase growth during the acidogenesis in comparison with the other samples. The higher pH in the control sample results in lower volatile fatty acid accumulation during the acidogenic stage of AD which was the main reason for lower hydrogen generation [47]. Also, the microbial degradation of the feedstock was not significantly enhanced as the control reactor did not receive any additive that also results in lower hydrogen production. The lower hydrogen production in the ZnO, CuO, and CaCO₃ reactors mainly resulted due to the increased toxicity of the medium as well as the lower alkali formation which restricts the hydrogenase microbial growth and volatile fatty acid consumption during the initial stages of AD [45, 48]. A similar trend of hydrogen generation was identified by Wainaina et al. [49] during the AD of food waste where the organic loading rate serves as the base for the comparison of hydrogen generation.

### 4. Conclusion
The effect of CaO₂, ZnO, CuO, and CaCO₃ as additives during the hydrogen fermentation of food waste and cow dung was experimentally investigated. The additives showed encouraging outcomes on the enhancement of hydrogen generated during the process. The hydrogenase microbial growth was accelerated by using the additives resulting in an enhanced hydrogen concentration up to 9.43% whereas the cumulative hydrogen yield was increased up to 11% compared to the control sample which did not receive any additive. The transfer of electron by the reduced ferredoxin co-factor was significantly promoted by the additives towards the activated site of the HydA enzyme that helps in the reduction of H⁺ with this electron to produce hydrogen during the reaction. Among the additives, CaO₂ represented superior performance due to higher alkali accumulation along with the generation of OH⁻ and ·O₂⁻ that facilitate the enhanced growth of the HydA enzyme to decompose the protein and carbohydrate present inside the feedstock. The release of Zn²⁺ and Cu²⁺ ions from ZnO and CuO respectively increase the toxicity within the feedstock that leads to the inhibition of the microbial growth resulting in lower hydrogen formation. The reduced ion activity of Ca²⁺ and CO₃²⁻ liberated from CaCO₃ during the reaction resulted in similar observations based on the hydrogen fermentation. Moreover, the additives showed a significant improvement in the hydrogen fermentation process compared to the control sample during the AD of food waste. However, more research is required to reduce the carbon dioxide concentration during the AD process along with the morphological changes in the structure of the additives after long term use. The present work serves as a future scope for the large scale hydrogen production that can be used in the practical field as a renewable source of energy.

Declarations

Availability of data and materials

All experimental data generated and used in the manuscript are with authors and will be provided on request to authors.

Competing interests

The authors declare they have no competing interests.

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Authors' contributions

Chinmay Deheri done the formal analysis, data curation, investigation, methodology, and writing- original draft preparation. Saroj Kumar Acharya done the conceptualization, supervision, visualization, and validation. All authors read and approved the final manuscript.
Acknowledgements

Not applicable.

References


**Figures**
Figure 1

Electron transfer mechanism during the hydrogen fermentation.
Figure 2

Schematic representation of the experimental arrangement.
Figure 3

SEM images of (a) CaO2, (b) CuO, (c) ZnO, and (d) CaCO3.
Figure 4

Effect of the additives on hydrogen concentration.
Figure 5

Effect of the additives on cumulative hydrogen yield.
Figure 6

Effect of the additives on carbon dioxide concentration.
Figure 7

Comparison of hydrogen and carbon dioxide concentration.
Figure 8

Effect of the additives on hydrogen production.