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Harnessing Poisonous Plants for Sustainable Energy: Biogas Production Optimization through Co-digestion of *Datura Stramonium* and *Ricinus Communis*

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Abstract

The aqueous leaf extracts of Datura stramonium and Ricinus communis were analyzed for phytoconstituents, including alkaloids, steroids, flavonoids, terpenoids, phenolic compounds, tannins, cardiac glycosides, anthraquinone glycosides, saponins, and triterpenes. The extracts revealed promising compounds in both plants. Given the toxicity of these compounds, it is recommended to eradicate these species or find beneficial uses for them. This research explores waste elimination and biogas generation through anaerobic digestion, providing a sustainable renewable energy option. Biogas production was evaluated via co-digestion of cow manure (CM) with Datura stramonium leaves (DSL) and Ricinus communis leaves (RCL) in three ratios (60% DSRC: 40% CM, 50% DSRC: 50% CM, 40% DSRC: 60% CM) under mesophilic conditions at 38ºCover 30 days. Physicochemical factors such as pH, total solids, volatile solids, organic carbon, nitrogen, and the carbon-to-nitrogen ratio were measured before and after digestion. Results showed significant increases in pH and total nitrogen, with notable decreases in volatile solids and organic carbon. Biogas yield was higher when substrates were co-digested with cow dung compared to leaves alone, with the 60% DSRC: 40% CM ratio yielding the most biogas. The study concludes that the co-digestion of cow manure with Ricinus communis and Datura stramonium leaves in a 60%:40% mix ratio significantly enhances biogas yield and reduces volatile and total solids.

Keywords: Anaerobic digestion, Cow dung, *Datura stramonium*, *Ricinus communis, Biogas.*

Introduction

Plants exhibit an inexhaustible capability to generate aromatic compounds, predominantly secondary metabolites like alkaloids, tannins, saponins, flavonoids, and phenolics. These compounds serve a defensive role, shielding plants from external threats such as nematodes, bacteria, viruses, and fungi (Mithraja et al., 2011). Datura, also known as thorn apple, belongs to the Solanaceae family and is inedible for animals. Various Datura species, including *Datura stramonium, Datura metaloides, Datura arborea*, and others, have been identified (Anadon et al., 2012). Datura contains tropane alkaloids like hyoscyamine, scopolamine, and atropine, which are hazardous to humans (Naude, 2007). *Ricinus communis* poisoning is linked to the toxic properties of castor beans, primarily found in temperate regions. The plant's oil is commonly used as a laxative and antihelmintic, devoid of harm due to the absence of ricin. Ricin 1, a potent phytotoxin in ricinus communis cake, is part of a group of lectins found in the plant, with ricin 2 being more toxic. Extensive research has been conducted on ricin lethal doses across various animal species, resulting in symptoms such as abdominal pain, diarrhea, bloody diarrhea, and vomiting (Greenfield et al., 2002; Albretsen et al., 2000). One of the most significant obstacles to global prosperity is the energy crisis, with approximately 1.5 billion people lacking electricity and around 2 billion lacking access to modern energy services (World Bank, 2006). Predictions indicate a severe energy shortage within the next fifty years, with natural gas estimated to deplete in 50 years and crude oil in 40 to 70 years (Courtney and Dorman, 2003). Anaerobic digestion emerges as a valuable technique for treating agricultural wastes, livestock manure, and other biodegradable substrates, addressing crucial factors like the carbon-to-nitrogen ratio (CN ratio), alkalinity, and biodegradability index (Koniuszewska et al., 2020; Bhatt and Tao et al., 2020). Microbes require nitrogen for cell structure development and carbon for energy, emphasizing the need for a balanced CN ratio (Tripathi et al., 2021; Zhou, 2017). Lignocellulosic material, primarily composed of lignin, hemicellulose, and cellulose, has a high CN ratio, resulting in less biogas production. Co-digestion of high and low CN ratio feedstocks can enhance biogas generation due to increased nutrient stability promoting microbial development (Mirmohamadsadeghi et al., 2021; Mshandete et al., 2004). Various factors, including temperature, CN ratio, pH, organic loading rate (OLR), hydraulic retention time (HRT), substrate particle size, total solids, and digester configuration, influence biogas yield (Naik et al., 2020; Budiyono et al., 2013; Rincon et al., 2009; Nalinga and Legonda, 2023; Liu et al., 2006). Utilizing waste for biogas yield aids in reducing greenhouse gas emissions, mitigating odors from organic waste decomposition, and generating power. The co-digestion process entails combining two or more substrates with an instantaneous digestion process, offering various advantages, including ecological, economic, and technological benefits compared to the digestion of a single substrate (Rughoonundun et al., 2012). In contrast, the process associated with the digestion of a single feedstock results in a decrease in biogas production compared to the rate observed in co-digestion. Factors such as higher lipid biodegradation and the carbon/nitrogen (C/N) ratio plays a crucial role in enhancing biogas and methane yield in co-digestion (Zhang et al., 2013). This research delves into the co-digestion process involving cow dung and a blend of *Datura stramonium* and *Ricinus communis* assessing the impacts of temperature, pH, and substrate mixing ratios on biogas production. The research contributes to renewable energy generation, environmental pollution reduction, and global efforts against climate change. It introduces the toxic plants *Datura stramonium* and *Ricinus communis* in the study area, emphasizing their potential harm to animals and human health. The innovative aspect lies in the co-digestion of cow dung with combined *Datura stramonium* and *Ricinus communis* leaves, offering a novel approach for environmentally friendly biogas production as renewable energy, addressing the growing demand for energy and climate concerns (Barua and Kalamdhad, 2019). The study aims to investigate biogas production from the mixed leaves of *Datura stramonium* and *Ricinus communis* combined with cow manure.

Materials and methods

Substrates

The leaves of *Ricinus communis*, *Datura stramonium*, and cow dung were obtained from the vicinity of Annamalai University in Tamil Nadu. After thorough cleaning with both running tap water and distilled water, the leaves were dried in the shade and finely pulverized into a powder. To prevent microbial degradation of the mixed substrates until their intended use, the powdered components were stored in airtight containers at 4°C.

Preparation of Aqueous extract for phytochemical screening

Soaking 50g of *Datura stramonium* and *Ricinus Communis* leaf powder separately in 500ml of water, the mixtures were subjected to an orbital shaker at room temperature for 48 hours. After this period, the mixture underwent filtration using fresh muslin cloth. The filtrate was further filtered through Whatmann No. 1 filter paper. The resulting extracts were concentrated and dried using a rotary evaporator at 37°C until a sticky mass formed. These dried extracts were stored at 4°C until required, post-evaporation (Ogu GI et al., 2012).

Analytical methods

The biogas production rate and pH were regularly monitored, and samples from both the influent and effluent were collected and analyzed following standard methods for pH, Total Solids (TS), Total Suspended Solids (TSS), Total Dissolved Solids (TDS), and COD (APHA 2015). Achieving a stable state condition, the hydraulic retention time (HRT) was determined based on measured COD effluent and biogas. The experimental data obtained during the steady state were utilized to evaluate the process performance of the batch reactor systems. The total biogas generated and its composition, determined through gas chromatography, were measured using the water displacement method.

Experiment

For the batch reactor studies illustrated in Fig. 1 (a&b), borosilicate glass containers with a capacity of 1L each were employed. To maintain anaerobic conditions within the reactor, the bottle opening was securely sealed with a rubber stopper. Two ports were provided for biogas collection and sampling. The water displacement unit was utilized to monitor the evolution of biogas in conjunction with the reactor.

Fig.1 (a) An-aerobic batch reactors setup for various mixing proportions and pH

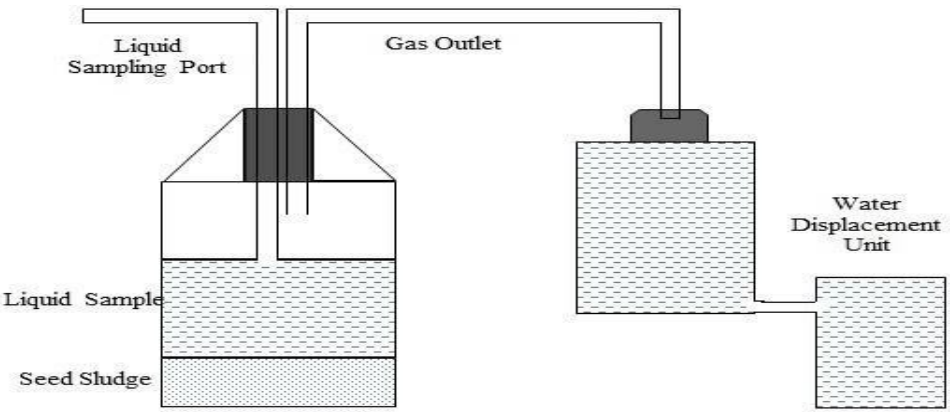

Fig.1 (b) schematic view of the batch reactor experimental setup

A gas collection chamber was established above the filled sludge and blended substrates by introducing 100 ml of pre-heated seed sludge and 600 ml of mixed leaves from Ricinus communis and Datura stramonium into the reactor. Nitrogen was employed to purge the batch reactor bottles, creating an anaerobic environment. The experiment was fine-tuned at 35°C for the mixing ratios and assessed under consistent pH conditions within the alkaline range for various hydraulic retention times (HRT). Daily monitoring was conducted for pH, organic carbon, organic nitrogen, and biogas parameters.

Results and Discussion

Characteristics of feedstock

Cow manure (CM), *Datura stramonium* (DS), and *Ricinus communis* (RC) leaves were utilized as feedstocks for the anaerobic digestion (AD) process to produce biogas. DSRCL and CM were sourced from the main campus of Annamalai University. To ensure uniformity in the blend, both DSRC and CM underwent drying and crushing using a versatile high-speed crushing machine. Total solids were quantified prior to AD by using equal portions (10g) of each dried fresh substrate. The two substrates were then mixed in varying ratios, specifically 60% DSRC: 40% CM, 50% DSRC: 50% CM, and 40% DSRC: 60% CM. To initiate AD, mixed substrates, the appropriate volume of distilled water, and 100ml of seed sludge were employed (Sutaryo et al., 2012). Seed sludge, obtained from the municipal wastewater treatment facility at Annamalai University, was filtered through a cloth with a 0.5 mm sieve diameter to remove solid components from the slurry. It was then carefully transferred to a plastic bottle. The substrates underwent a 30-day anaerobic digestion process in a 0.5 L digester at 35°C. The pH of the slurry was maintained within the optimal range for biogas production (around neutral) by adding sodium hydroxide and hydrochloric acid to the organic substrate, as recommended by (Yadvika et al., 2004).

Table. 1 Characteristics of *Ricinus communis*, *Datura stramonium* and cow dung in different mixing ratios.

Parameters	60DSRC:40CD	50DSRC:50CD	40DSRC:60CD
pH	5.20	4.86	4.85
COD	16800	16500	16700
TS	11300	11500	11600
TSS	4900	4800	4600
TDS	6400	6700	7000
MLSS	6800	6200	6500
MLVSS	5200	4700	4200

All Parameters are in mg/L except pH

Temperature and pH impacts

The initial pH values of the slurries were adjusted to align with or closely approach the optimal range recommended for Anaerobic Digestion (AD) in biogas production, typically falling between 6.8 and 7.2 for bacteria (Rajeshwari et al., 2000). According to Shanmugam and Horan (2009), the pH of 6.5, situated close to the neutral range, was identified as the point where the highest specific biogas production rate was observed. The pH range of the slurries utilized in this investigation was maintained between 6 and 7. As long as the substrates meet other nutrient requirements to support microbes, various groups of bacteria, particularly anaerobic bacteria, can operate effectively. Although the pH of the slurry containing 60DSRC:40CD was within the ideal range, it was higher than that of other mixing proportions. Consequently, the extreme pH levels of these two substrates were buffered to more neutral or nearly neutral values, a crucial requirement for the AD process in biogas formation. The ability to buffer pH and provide the necessary nutrients for normal bacterial function is considered a benefit of co-digestion of substrates (Hills and Roberts, 1981). As depicted in the figure 2, the pH values of all digesters significantly increased after anaerobic digestion. This rise in pH during Anaerobic Digestion (AD) could be attributed to the breakdown of organic compounds in the digester, leading to the generation of alkali chemicals such as ammonium ions (Gerardi, 2003).

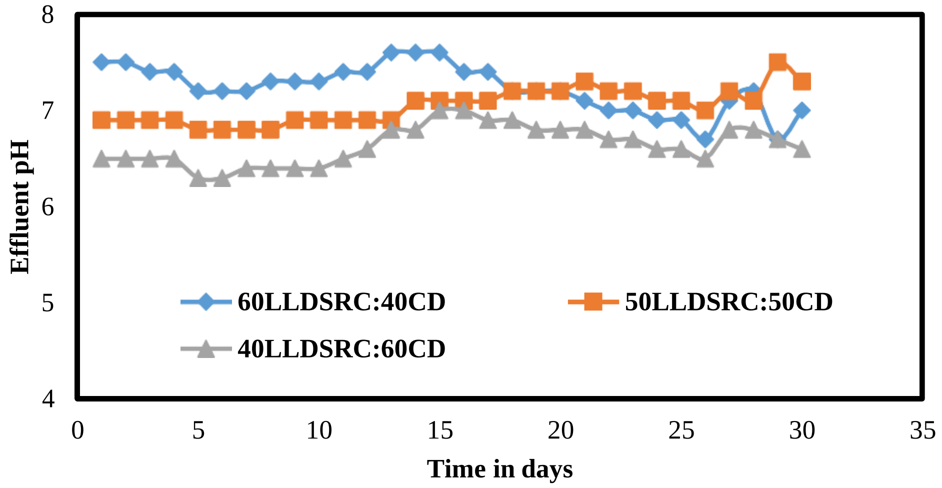

Fig.2 Effluent pH for 60%DSRC: 40%CD, 50%DSRC: 50%CD and 40%DSRC: 60%CD

Slurries containing a ratio of 60% dry (DSRC) to 40% cow dung (CD) exhibited notably higher levels of organic carbon and total nitrogen compared to mixtures with different proportions. This clearly indicates the presence of organic carbon and nitrogenous compounds in the total solids (TS) and volatile solids (VS) that microorganisms can utilize. No significant differences were observed in total nitrogen and the percentage of organic carbon among various treatments. However, after anaerobic digestion (AD) in all slurry treatments with different total solids, there was a consistent decrease in organic carbon and an increase in total nitrogen, as illustrated in Figure 3. According to (Abdel-Hadi and El-Azeem 2008), the decrease in the organic carbon percentage after anaerobic digestion (AD) can be ascribed to bacteria consuming organic debris, ultimately releasing biogas. This reduction signifies the degradation process during anaerobic digestion, as claimed by (Devlin et al., 2011). Notably, in digesters containing cow dung and DSRC in a 60:40% ratio, a significantly higher reduction in organic carbon and volatile solids was observed, irrespective of the initial total solids content. All digesters experienced an increase in total nitrogen values following anaerobic digestion, indicating the breakdown of proteins, amino acids, and other nitrogenous organic molecules, as suggested by Smith et al. (2007). The carbon-to-nitrogen ratio stands as a pivotal factor affecting anaerobic digestion, influencing both methane yield and the rate of biogas generation (Santosh et al., 2004). Researchers commonly advise maintaining a carbon/nitrogen ratio within the 20 to 30 range for optimal anaerobic bacterial growth in anaerobic digestion environments, aligning with the recommendations of (Li et al., 2011). In this study, the carbon-to-nitrogen ratio ranged from 20.09 to 28.79 before AD and 20.35 to 30.42 after AD, as depicted in Table 2. These values fall within the optimal range for biogas production, as suggested by (Marchaim 1992), who considers the ideal C:N ratio to range from 20:1 to 30:1. A higher C:N ratio indicates efficient utilization of nitrogen by methanogens without excess carbon for increased biogas production. On the flip side, a reduced C:N ratio results in a higher concentration of nitrogenous molecules like NH3, leading to an elevation in pH and hindering bacterial growth (Braun, 1982).

Table 2 C:N ratios in the batch reactor before and after digesting process

	C:N Ratio Before AD			**C:N Ratio After AD**		
Days	60LLDSRC:40CD	50LLDSRC:50CD	40LLDSRC:60CD	60LLDSRC:40CD	50LLDSRC:50CD	40LLDSRC:60CD
0	0	0	0	0	0	0

1	24.05	28.23	20.51	26.10	22.88	21.19
2	24.76	29.1	21.51	25.66	24.66	21.06
3	24.04	30.21	18.28	25.75	22.66	20.29
4	25.66	30.2	17.61	22.81	27.79	20.64
5	25.75	29.43	18.41	21.23	24.15	21.58
6	22.81	30.45	20.01	20.71	20.58	18.94
7	21.23	29.56	19.26	23.88	20.74	20.21
8	20.71	28.9	18.06	25.66	24.97	20.41
9	23.88	30.24	19.90	23.66	21.32	20.47
10	25.66	31.8	21.19	28.79	22.29	20.63
11	23.66	30.49	21.06	25.15	23.49	19.44
12	28.79	29.59	20.29	21.58	21.20	18.41
13	25.15	28.54	20.64	30.49	24.54	21.77
14	21.58	28.6	21.58	29.59	19.35	22.68
15	21.74	27.43	18.94	28.54	22.66	23.50
16	25.97	26.42	20.21	28.60	20.86	24.04
17	22.32	27.48	20.41	27.43	20.34	25.66
18	23.29	28.5	20.47	26.42	24.61	25.75
19	24.49	29.9	20.63	27.48	19.64	22.81
20	22.20	30.42	19.44	28.50	20.58	21.23
21	25.54	28.7	18.41	29.90	17.94	20.71
22	20.35	29.3	21.77	30.42	19.21	23.88
23	23.66	30.21	22.68	22.20	19.41	25.66
24	21.86	26.39	23.50	25.54	19.47	23.66
25	21.34	27.73	23.75	20.35	19.63	28.79
26	25.61	26.46	23.72	23.66	18.44	25.15
27	24.28	25.3	26.18	21.86	17.41	21.58
28	20.97	26.49	25.21	21.34	26.00	21.74
29	21.01	27.38	25.68	25.61	25.00	25.97
30	20.09	25.5	24.31	24.28	24.00	22.32

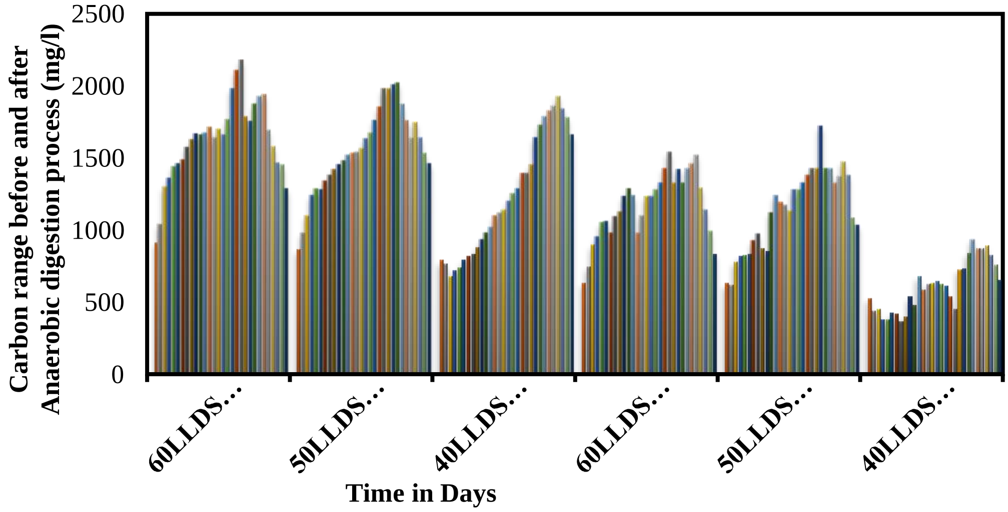

Fig.3 Organic carbon range in the batch reactor before and after digestion process

Figure 4 presents the results of the analysis on the average daily and cumulative biogas generation resulting from the sole and co-digestion of substrates with various mixing ratios. Gas generation commenced on the first day of incubation, showing variations across treatments as the incubation period extended. Generally, gas production remained low during the initial three days, gradually increased, and reached its peak between six to ten days. Subsequently, starting from the 27th day, gas production exhibited a decline, eventually coming to a complete stop. The diminishing trend in biogas yield after the 11th day of incubation is attributed to the depletion of readily decomposable substrate with the prolonged incubation period (Ahn et al., 2009). The alkaline pH values observed after anaerobic digestion (AD) (Hansen et al., 1998) suggest a potential correlation with the pH rise during the incubation period. Cumulative yield over the incubation period was calculated based on mean daily biogas yields. Results indicate that a digester with a mix ratio of 60% dry sugarcane residue (DSRC) to 40% cattle dung (CD) produced the highest biogas output. This may be attributed to the presence of balanced nutrients, facilitating the most effective flourishing of microorganisms (Corral et al., 2008). Positive synergism between the digestions of various substrate types has been demonstrated in the digester (Li et al., 2009; Danqi, 2010; Jianzheng et al., 2011). Comparing mix ratios, slurries with 60% DSRC: 40% CD yielded higher biogas (6209 ml) compared to slurries with 40% DSRC: 60% CD (4922 ml) when 50% DSRC: 50% CD were examined. This observation aligns with the findings of Igoni et al. (2008), who emphasized that the biogas volume produced corresponds to the overall solid amount. Additionally, the batch fermentation system demonstrated a marginal increase in biogas volume for each percentage point increase in total solids (Lijuan et al., 2009).

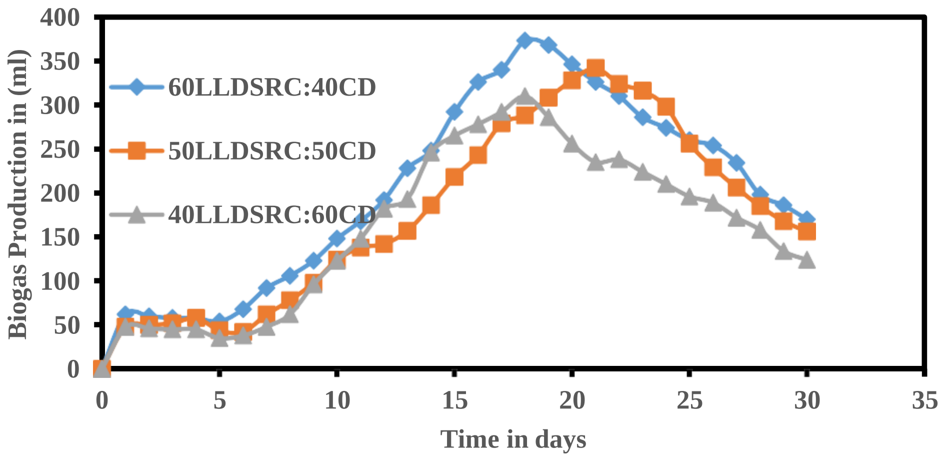

Fig.4 Biogas production for 60%DSRC: 40%CD, 50%DSRC: 50%CD and 40%DSRC: 60%CD

Conclusions

In conclusion, this research investigated the potential of harnessing poisonous plants, specifically *Datura Stramonium* and *Ricinus Communis*, for sustainable energy production through the optimization of biogas generation. The study initially characterized the phytoconstituents of these plants, revealing promising compounds such as alkaloids, steroids, flavonoids, terpenoids, phenolic compounds, tannins, saponins, and triterpenes. To address the presence of toxic phytocompounds in these plants, the study proposed a dual approach of eradication and utilization in a beneficial manner. The main emphasis was on co-digesting these plant materials alongside cow manure to generate biogas through anaerobic digestion, presenting an environmentally friendly alternative for renewable energy. The experimental outcomes showcased that the co-digestion of cow manure was observed with a mixture of *Datura Stramonium* and *Ricinus Communis* significantly enhanced biogas yield. The study analyzed various mixing ratios and found that a mix ratio of 60% dry (DSRC) to 40% cow dung (CD) resulted in the highest biogas production. The analysis of physico-chemical factors before and after anaerobic digestion revealed changes in pH, total solids, volatile solids, organic carbon, nitrogen, and the carbon-to-nitrogen ratio. The results indicated a significant increase in pH and total nitrogen after anaerobic digestion, while volatile solids and organic carbon exhibited a notable decrease. Overall, this research contributes to the understanding of utilizing poisonous plants for sustainable energy production. The co-digestion approach presented in this study not only addresses environmental concerns associated with toxic plants but also offers a practical solution for renewable energy generation through biogas production. The findings emphasize the potential of integrating toxic plant materials into the anaerobic digestion process plays a role in meeting the increasing need for clean and sustainable energy.

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