Sustainability of *Ageratum conyzoides* (billy goat weed) for bioethanol and recycling of residues for gaseous fuel production

Shivam Pandey¹ | Vinod Kumar¹ | Mikhail S. Vlaskin² | Manisha Nanda³

¹Algal Research and Bioenergy Lab, Department of Chemistry, Uttaranchal University, Dehradun, 248007, India
²Joint Institute for High Temperatures of the Russian Academy of Sciences, 13/2 Izhorskaya St, Moscow, 125412, Russia
³Department Of Biotechnology, Dolphin (PG) Institute of Biomedical and Natural Sciences, Dehradun, India

**Correspondence**
Vinod Kumar, Algal Research and Bioenergy Lab, Department of Chemistry, Uttaranchal University, Dehradun-248007, India.
Email: vinodkdhatwalia@gmail.com, vinodbiochem@uttaranchaluniversity.ac.in

**Abstract**
*Ageratum conyzoides*, an herb found throughout the year, is generally considered as a weed: it causes reduction in soil productivity and leads to health hazards for cattle and humans. However, its biomass can easily represent a cost-effective source, which can be used for lignocellulosic biofuel production. The conversion of lignocellulosic biomass to ethanol has drawn much attention in recent times due to abundance of biomass. In the present study, the cellulose and hemicellulose biomass of the leaf and stem of *A. conyzoides* was converted to sugars using acid hydrolysis. 146.01 ± 02 mg/g of fermentable sugar was obtained from *A. conyzoides*. The maximum ethanol concentration 11.89 g/L was obtained after 7 days. Scanning electron microscopy was used to characterize the surface morphology after acid hydrolysis of biomass. In the current study, the residues of acid hydrolysis and fermented wastewater was used for biogas production through anaerobic digestion. The yield of biogas from the residues of acid hydrolysis and fermented wastewater was 204 L kg⁻¹ VS. The results obtained indicate that *A. conyzoides* may be considered as a promising feedstock for bioethanol and biogas production.

**KEYWORDS**
*Ageratum conyzoides*, bioethanol, biogas, biomass, weed

**1 INTRODUCTION**

Ever-increasing demands of energy, emission of greenhouse gases and depletion of fossil fuels have increased the research interest in value of bio-sourced lignocellulosic biomass. Biofuels produced from bio-based materials are in great demand as a renewable source of energy. Production of biomass from weeds comprising of lignocellulosic waste is inexpensive, possess short life, and is easily available. A lignocellulosic material generally consists of three main components, that is, cellulose, hemicellulose, and lignin. Lignin is a polymer of phenolic monomers, while hemicellulose and cellulose are made up of sugar units. Cellulose and hemicellulose can be easily broken into sugar units which can be further used for biofuel production.¹,² Sugarcane, corn, wheat, rice straw, sweet potato etc. which are mainly consumed as source of food are also a rich source of sugar which can be used for biofuel production. Researchers have now started focusing on weeds...
and other waste materials for producing biofuels. Generally, a weed is a plant which produces large numbers of seeds, inhibits the growth/kill other plants and is harmful for animals. *Ageratum conyzoides* is one of the most harmful exotic weeds and is also known as “billy goat weed” or “goat weed.”* Ageratum conyzoides* is a native plant of Central America but is now found throughout the world. It has a peculiar odor like Australian male goat and hence was named “goat weed” or “billy goat weed”. It is found as a weed in more than 36 crops in 46 different countries. *Ageratum conyzoides* weed reduces the yield of rice (25%-47%) and wheat (13%-38%) in India and is also reported to cause decline in the medicinal plant’s population in Shivalik hills of Himachal Pradesh, India.

*Genus Ageratum* is a rapidly spreading plant and has become a major problem for farmers and scientists. It produces a large number of fruits (8000-10 000/plant) that attract pollinators for cross-pollination and remain viable for a year. It is an erect soft hairy annual plant growing up to a height of 2.5 feet with pale blue or whitish flower heads. It is also a host plant of various pathogens and nematodes, which are responsible for infection in crops. Its essential oil is rich in ageratochromene and β-caryophyllene and is widely used in traditional medicine systems. The pungent oils released from it are reported to cause giddiness, nausea, and sometimes allergic reactions in humans. Weed Risk Assessment for *Ageratum conyzoides* (Billy goat plant) confirmed that it has strong ability to establish and spread a number of disease and contain pyrrolizidine alkaloids, which are toxic for humans and livestock. Veno-occlusive disease, a fatal disease reported in 2001 is associated with toxins from *A. conyzoides* and is characterized by chronic liver disease, fever, jaundice, etc.

Weed biomass can be used as a renewable energy source as they are nonconventional crop. Bhattacharjee et al performed pyrolysis of *A. conyzoides* (goat weed) and reported that the pyrolysis-gas has a gross calorific value of 5.32 MJ m⁻³ and can be utilized as an alternative gaseous energy source. Devi et al performed vermin-composting of *A. conyzoides* using earthworm species and reported that vermin composting can be an alternative environment friendly option for the management of *A. conyzoides*. Mandal et al prepared the biochar from *A. conyzoides* and reported the increase in the productivity of soil.

Omotoso showed potent gastroprotective activity of *A. conyzoides* which is closely associated with the anti-oxidant properties of their compounds. *A. conyzoides* biomass has a high heating value (HHV) of 14.60 MJ kg⁻¹ due to better H/C molar ratio of 1.70, but lower than the high heating value of woody biomass. The *A. conyzoides* biomass has a higher O/C molar ratio of 1.30, which is more than the O/C molar ratio of woody biomass (1.2) due to its greater concentration of hemicellulose and cellulose content and lower concentration of lignin. Amadi et al and Odeleye et al reported the presence of high concentration of alkaloids and low concentrations of leucoanthocyanins and steroids in the aerial parts extract of billy goat weed. Moreira et al found that methoxyflavone isolated from hexane extract of leaves of *A. conyzoides* has insecticidal activity. In addition to these chemical families, steroids, tannins and phenolic compounds are reported by Dash and Murthy.

To the best of our knowledge, no one till now has reported the use of *A. conyzoides* as a source of biomass for bioethanol and gaseous fuel production. Therefore, the present study is mainly aimed to investigate the feasibility of bioethanol and biogas production from *A. conyzoides*, and to optimize the conditions of sacharification process.

## 2 MATERIALS AND METHODS

### 2.1 Plant collection and physicochemical analysis

*Ageratum conyzoides*, was collected from Hamirpur, Himachal Pradesh, India in month of November (2016). Fresh samples were then placed under sunlight for 10 days. The sun-dried samples were further dried overnight at 50°C in the hot air oven. Finally, desiccated materials were grounded to powder form that could pass through 1 mm mesh by a high-speed blender and was stored in desiccator for further analysis. Overall scheme of this study is given in Figure 1.

### 2.2 Physical analysis

For physical analysis, moisture content, volatile matter, ash content and fixed solid were determined according to Sluiter et al method. The composition of the sample (Cellulose, hemicellulose and lignin) was determined by Van Soest method. Volatile Solids were determined by combustion of the sample at 550°C in muffle furnace for 1 hour.
2.3 Acid hydrolysis biomass and detoxification

Acid hydrolysis was done by single step hydrolysis. 1 kg biomass was mixed with 2 L of 10% H₂SO₄. Flasks containing this composition were autoclaved for 2 hours at 121°C and 15 psi. Hydrolysates were then cooled at room temperature and neutralized by NaOH. The residual biomass was separated from the hydrolysates by centrifugation at 8000 rpm for 15 minutes. The produced supernatant was further used for sugar and other analysis. The filtrate was then checked for reducing sugar by colorimetric and chromatographic methods. Colorimetric method was performed according to Miller. Sugar concentrations in the samples were quantified by high pressure liquid chromatography (HPLC). During the acid hydrolysis of biomass, various types of chemicals were generated that act as fermentation inhibitor. 5 mL of 0.5% sodium sulfite solution was then added to 100 mL of hydrolysate solution for detoxification of fermentation inhibitors.

2.4 Scanning electron microscope and FTIR

The morphology of A. conyzoides before and after acid hydrolysis was studied using scanning electron microscope (SEM) (FE-SEM Quanta 200 FEG). For this, powdered biomass and residues were coated in pure gold after pretreatment. The functional groups present in biomass were identified by Fourier transform infrared spectroscopy (FT-IR 6700, NICOLET).

2.5 Fermentation

A yeast strain, S. cerevisiae, was obtained from food science dept. of Uttaranchal University Dehradun, India. The yeast cells were cultivated on liquid YPD medium (yeast extract, peptone, and dextrose). For the fermentation, 1.5 L hydrolysate solution was fermented with 2% (w/v) of yeast S. cerevisiae. Fermentation was carried out at 32°C and pH-5 for 7 days with agitation at 150 rpm. Distillate obtained from rotary evaporator was used to determine bioethanol concentration colorimetrically using potassium dichromate method. The maximum theoretical ethanol yield was calculated as follows:

\[ Y_{\text{max}}(\%) = \frac{\text{Ethanol produced in reactor (g)}}{\text{Initial sugar in the reactor (g)} \times 0.511} \times 100 \]

2.6 Biogas reactor and anaerobic digestion (residues of acid hydrolysis and fermentation waste water)

A stainless-steel digester was used during the study with a working volume of 3 L (14 cm ID, 38 cm height, 5 mm thickness) (Figure 2). One port was used for substrate feeding, while the second port was connected to a gas outlet. Gas sample was taken via a silicone tube by a pressure-tight gas syringe. The third port acted as outlet. Cow dung was used as an inoculum for this reactor. Residues of acid hydrolysis and fermentation waste water of current study were thermally pretreated in autoclave at 120°C and 15 psi for 30 minutes.
The initial substrate concentration was 1:50 (1 residues of acid hydrolysis and 100 mL fermentation waste water of this study). Substrate to inoculum (S/I) ratio was kept at one third. The initial pH values of experimental sets were in the range of 6.50 to 7.50. After inoculation, the reactor was put in open field. Volume of the gas produced was estimated at intervals of 24 hours using water displacement method.

2.7 Analytical methods

The yield of the biogas was determined by using displacement method. Biogas content was determined by using Gas Chromatography (GC Agilent Technologies, Santa Clara, California).

2.8 Statistical analyses

The data is being presented in the study as the mean ± SE of triplicate experiments. The variation sugar contents was investigated by Graph Pad Prism software (version 6.0f) with $P < .05$.

3 RESULTS AND DISCUSSION

3.1 Characteristics of A. conyzoides

Moisture content, total solid, volatile solids and ash content based on dry matter have been presented in Table 1. Moisture content was found to be 4.03 ± 0.12. Moisture is an important factor as it affects the storage condition, handling, fungus contamination and conversion processes of biomass. Similar proximity content was reported by Bhattacharjee et al in the A. conyzoides. Most of the agricultural biomass is comprised of about 10% to 25% lignin, 20% to 30% hemicellulose and 40% to 50% cellulose.

3.2 ATR-FTIR

The FTIR spectra of A. conyzoides biomass showed varying differences in peak intensity.

The peak at 3336.50 cm$^{-1}$ correspond to O-H Stretching shows the presence of acid. The peak at 2521 cm$^{-1}$ represents the C-H$_n$ stretching and shows the presence of aromatic compounds. 2077 cm$^{-1}$ corresponds to N=C=S stretching. 1634 cm$^{-1}$ represent the C=O Stretching, Ketone, Ester, Amide. The peaks at 1432, 1199, 992, 870, 619 cm$^{-1}$ correspond to CH bending, C=O/C=O-C, C=C bending (alkenes), C-N/R-O-C/R-O-CH3 Stretching aromatic C-H and C-C stretching respectively (Figure S1).
### TABLE 1 Proximate analysis and compositions of biomass of *Ageratum conyzoides*

| Parameters          | *A. conyzoides* (%) |
|---------------------|---------------------|
| Ash                 | 10.82 ± 0.01        |
| Moisture content    | 4.03 ± 0.12         |
| Volatile solids     | 68.03 ± 0.2         |
| Fixed Carbon $^a$   | 17.12               |
| Cellulose           | 23.02 ± 0.2         |
| Hemicellulose       | 28.1 ± 0.01         |
| Lignin              | 12.02 ± 0.04        |

* $^a$100 – (ash + moisture + volatile).

### FIGURE 3 Ethanol concentration during fermentation

![Ethanol concentration graph](image)

### 3.3 Release of fermentable sugars and ethanol production

By the single-step acid hydrolysis process, 146.01 ± 02 mg/g of fermentable sugar was released from *A. conyzoides* biomass. H$_2$SO$_4$ and HCl were mainly used for cellulose hydrolysis. Idrees et al have reported that high reducing sugar is obtained when plant material is pretreated at 121°C. The quality and quantity of sugars after acid hydrolysis was analyzed by High Performance Liquid Chromatography. It was observed that the main components present in liquid phase were hexose sugars which included fructose (14.1 mg/g). The others components were inulin, cellulbiose, xylose, and arabinose.

Hydrolysates were further assayed for the presence of fermentation inhibitors by High Performance Liquid Chromatography. 1.2 g/L of Acetic acid was also found. The production of acetic acid was due to hemicellulose degradation taking place during the acid hydrolysis. The fermentation inhibitors produced by carbohydrates hydrolysis are generally furan derivatives, weak acids and phenolic compounds. Acetic acid negatively disturbs the growth of yeast *S. cerevisiae*. After the hydrolysis, the whole slurry containing sugar was further used for fermentation using *S. cerevisiae*. Ethanol concentration was observed over a period of few days. Ethanol concentrations were reported after three, five and 7 days. The ethanol concentration after 3, 5 and 7 days were found to be 7.89 g/L, 8.42 g/L and 11.89 g/L, respectively (Figure 3). Mohapatra et al reported that growth of yeast is extremely influenced when concentration remains more than 2.0 g/L.

### 3.4 Scanning electron microscope

Physical changes of biomass before and after acid hydrolysis was studied using high magnified SEM images (Figure 4). SEM is a powerful tool for the structural analysis of lignocellulosic biomass. Before acid hydrolysis the surface of *A. conyzoides* biomass was found to be smooth, but after acid hydrolysis biomass it showed a rough surface. Sun et al reported that after the acid pretreatment roughness of the wood surface increased. Similar type of rough surface was observed in rice straw biomass after popping pretreatment.
3.5 | Biogas production

Anaerobic digestion of waste biomass, weed and waste water is a sustainable alternative to fossil fuels. Anaerobic digestion of residues obtained from acid hydrolysis and fermented waste water of *A. conyzoides* produced 2% nitrogen, 54.31% methane, 43.17% carbon dioxide and 0.01% hydrogen sulphide (Figure 5) (Table 2). Mogle and Jadhav reported 65% of biogas production from five weeds.40 The final obtained biogas yield was 204 L kg⁻¹VS. After 40 days, biogas production from substrate was almost negligible. The experiment was considered complete after 40 days and biogas obtained was
considered as the final production yields. Caporgno et al reported negligible biogas production from sewage sludge and microalgae after 20 days.\textsuperscript{41}

4 | CONCLUSIONS

The conversion of \textit{A. conyzoides} to bioethanol and gaseous fuel production from it was investigated. 146.01 ± 0.2 mg/g of fermentable sugar was obtained from \textit{A. conyzoides}. The fermentable sugar was further converted into ethanol \textit{S. cerevisiae}. 11.89 g/L ethanol was obtained on seventh day of fermentation. Solid residues of acid hydrolysis and fermented waste water were further used for the biogas production. Obtained yield of biogas was 204 L kg\textsuperscript{-1} VS. Finally, we conclude that \textit{A. conyzoides} has potential to be used for bioethanol and gaseous fuel production.

PEER REVIEW INFORMATION

\textit{Engineering Reports} thanks the anonymous reviewers for their contribution to the peer review of this work.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS

\textbf{Shivam Pandey}: Investigation; methodology. \textbf{Vinod Kumar}: Investigation; methodology; supervision; writing-original draft; writing-review and editing. \textbf{Mikhail Vlaskin}: writing-review and editing. \textbf{Manisha Nanda}: Visualization; writing-original draft.

ORCID

Vinod Kumar\textsuperscript{\textregistered} https://orcid.org/0000-0003-1808-1980

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