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Effect of biological treatment of Cattle dung using *Pseudomonas fluorescens* on biogas production

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Abstract

Biogas, produced through the anaerobic digestion of organic materials, has emerged as an effective solution for waste management and clean energy production. This study assessed the impact of biological treatment of cattle dung using *Pseudomonas fluorescens* on biogas production. Fresh cattle dung was analysed for its proximate composition. A slurry with 10% dry matter content was prepared, inoculated with *Pseudomonas fluorescens* at 3% and 6%, and incubated at 30°C for 50 days. Result showed cumulative biogas production significantly increased over time for all treatment groups, with the 6% inoculum (C6) producing the highest yields. By day 50, C6 generated 268.94 ml/gm VS, showing a 19.81% increase compared to the control group (C0). The initial lag phase of gas production was observed in all groups, but the 6% inoculum demonstrated better hydrolysis of organic compounds, enhancing biogas yields. This study suggests that higher doses of *Pseudomonas fluorescens* significantly improve biogas production from cattle dung, providing a potential pathway for sustainable energy solutions in India

Keywords: Biogas, Anaerobic digestion, *Pseudomonas fluorescens*, Inoculum

1. Introduction

India's energy production is growing at 7.06%, reaching 1,739.1 billion units in 2023-24 (CEA, 2024). However, there is still a deficit of 4.1 billion units compared to the annual energy requirements, attributed to population growth and industrialization. This scenario has prompted the exploration of new and renewable energy options, including solar, wind, tidal, and biogas. India has world's largest livestock population, estimated at 192.49 million cattle in 2019, the substantial quantities of animal manure generated pose both challenges and opportunities. If not managed properly, this manure can lead to environmental pollution and health risks.

Biogas, is one such energy source generated through the anaerobic digestion of organic materials. This environmentally friendly technology has gained popularity for its role in waste management and clean energy production over the past decade (Romero-Güiza *et al.* 2016) [1]. By leveraging biogas, India can address energy deficits, enhance waste management, and promote sustainable energy solutions, contributing to a cleaner environment. Hence, the present experiment was conducted to assess the effect of biological treatment of cattle dung using *Pseudomonas fluorescens* on biogas production (BAHS 2024).

2. Material and Methods

For this experiment, fresh Cattle dung from animals of uniform age group was used as substrate. Before adding substrate to the digester, it was analysed for proximate principles for standardization to get optimized biogas production.

Table1: Proximate analysis of cattle dung on DM basis.

Composition	DM %	OM %	C: N	Lignin %	Hemicellulose %	Cellulose %
	18.05	79.15	29.06	10.56	19.61	27.88

2.1 Experimental design

The cow dung slurry with dry matter content of 10% was prepared, to which *Pseudomonas fluorescens* inoculum (10^8 CFU/g) was added at concentrations of 3% and 6% of the Slurry (10% Dry matter). The treatment groups were labelled as C0 (control), C3 (3% inoculum), and C6 (6% inoculum). The loaded syringes containing the slurry (20 ml) were then placed in a water bath to incubate at 30 °C, which is the optimal temperature for the growth of *Pseudomonas fluorescens*.

Biogas production was monitored over a period of 50 days, with each treatment group consisting of three replicates. Every 10 days, six syringes from each treatment group were removed for Fiber analysis and bacterial growth quantification using qPCR techniques.

2.2 Statistical analysis

Data analysis was conducted using SPSS software, version 16.0. A one-way ANOVA was performed to evaluate differences between treatment groups, with significance determined at the 5% level of significance as per Snedecor and Cochran (1994) [5]. The Duncan Multiple Range Test (DMRT) was used for further comparison of significant differences.

3. Result and Discussion

3.1. Result

Cumulative gas production

Cumulative biogas production (ml/gm VS) from Cattle dung in different biological treatment groups at various time points are summarized in the Table.2. and the same has been represented in the line diagram in Figure.1 below.

Table 2: Mean $\pm$ SE Cumulative biogas production (ml/gm VS) for 50 days in Cattle dung from different biologically treated groups at 10 days interval.

Days	C0	C3	C6	P value
0	0	0	0	
10	31.02 $\pm$ 6.499	32.70 $\pm$ 6.526	43.15 $\pm$ 8.290	0.441
20	100.11 ^b $\pm$ 5.580	103.52 ^b $\pm$ 6.014	123.48 ^a $\pm$ 6.139	0.019
30	152.94 ^b $\pm$ 4.767	159.80 ^b $\pm$ 4.742	185.37 ^a $\pm$ 5.892	0.0002
40	197.58 ^b $\pm$ 3.712	201.85 ^b $\pm$ 3.716	236.37 ^a $\pm$ 3.723	0.001
50	224.40 ^b $\pm$ 2.741	235.52 ^b $\pm$ 3.024	268.94 ^a $\pm$ 2.652	0.001

Means in a row with different superscripts (a, b, c) differ significantly ($P < 0.05$) between treatment groups C0-control, C3 -3% inoculum, C6 - 6% inoculum.

Biogas production increased over time for all treatments, with C6 consistently producing the highest amounts. In the 0-10 days interval, the values ranged from 31.02 to 43.15 ml/gm VS, with no significant difference among treatments ($P = 0.4412$). From days 11-50, C6 showed significantly higher production (123.48, 185.37, 236.37, 268.94) compared to C0 and C3, which had similar values within each interval. The differences among treatments became statistically significant ($P < 0.05$) from day 11 onwards and persisted till day 50. Over all Cumulative Biogas production increased gradually over time for all treatments, with C6(6%) generating 19.81 % and 14.15 % more compared to control C0 and C3 respectively. Similar findings were seen in the experiments of Fugol *et al.* (2023) in maize silage, when treated with microbial

consortium containing *Pseudomonas spp.* Which resulted in 62% increase in biogas production. Yadav, M., and Vivekanand, V. (2021) performed fungal pretreatment of wheat straw, with *Chaetomium globosporum*, which yielded 568 ml/g VS, which represents a 31% increase.

4. Discussion

During the first 10 days, cumulative gas yields were not significantly different ($p > 0.05$) among the control (C0), 3% (C3), and 6% (C6) culture-treated groups across all three manure types, suggesting a short adaptation (lag) phase for the methanogenic bacteria. However, from days 11 to 20, the 6% culture group (C6) showed significantly higher cumulative gas production compared to the controls ($p < 0.05$), a trend that continued through day 50.

The 6% inoculum of *P. fluorescens* yielded 19.81% more biogas than the control, while the 3% treatment provided only a 14.15% increase. This improvement at the 6% level can be attributed to enhanced hydrolysis of readily degradable organic compounds and partial lignocellulose degradation. *P. fluorescens* produces beneficial enzymes, such as dyp-type peroxidases (Rahmanpour and Bugg, 2014) [9] and endo β -1, 4 glucanase activity, exo- β -1, 4 glucanase (Pathak *et al.* 2016) [8], facilitating the degradation of lignin and improving cellulose and hemicellulose accessibility.

The lack of significant enhancement at the 3% level indicates that a critical threshold of bacterial biomass or enzyme activity was not reached, limiting substrate modification and gas yield. This aligns with findings by Bruni *et al.* (2010) [2], who noted that suboptimal microbial dosing had minimal effects on methane output.

5. Conclusion

The present study suggest that Inoculation of cattle dung slurry with *Pseudomonas fluorescens* culture showed significant enhancement in biogas yield at 6% inoculum than compared to 3% and control.

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