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Bioaugmentation of cattle dung with *Pseudomonas fluorescens*: Effects on lignocellulose degradation for bioenergy production

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Abstract

India's energy production is growing at a rate of 7.06%, reaching 1,739.1 billion units in 2023-24, but the country still faces a deficit of 4.1 billion units. This demand, driven by population growth and industrialization, has depleted conventional energy resources and increased the need for renewable options like solar, wind, and biogas. Biogas, produced through anaerobic digestion (AD) of biomass, is a potential energy source, but breaking down lignocellulosic structures remains a major challenge. This study examines the effect of the bacterium *Pseudomonas fluorescens* on degrading lignocellulose in cattle dung. Fresh cattle dung was mixed with water to achieve a 10% dry matter content and inoculated with 3% and 6% concentrations of *P. fluorescens*. After 50 days, the study measured the degradation of hemicellulose, cellulose, and lignin. The results showed only slight changes among the groups, with no significant differences in fiber breakdown. Some improvements were noted with the higher inoculum (6%) concentration, though they were not statistically significant.

Keywords: Energy production, biogas, anaerobic digestion, lignocellulose, *Pseudomonas fluorescens*, renewable energy

1. Introduction

India's energy production is increasing at a rate of 7.06%, reaching 1,739.1 billion units in 2023-24, yet it still faces a shortfall of 4.1 billion units in meeting annual energy needs. This rising demand, spurred by population growth and industrialization, has led to the depletion of conventional energy sources, highlighting the necessity to explore renewable options such as solar, wind, tidal, and biogas. Biogas, generated through anaerobic digestion (AD) of biomass, represents a viable energy source (CEA, 2024). However, a major challenge in biogas production is the hydrolysis of lignocellulosic structures, which serves as a rate-limiting step (Martin-Ryals *et al.*, 2015) [2]. To address this recalcitrance, new strategies are essential to enhance hydrolysis and improve the economic feasibility of biogas plants. One promising approach involves treatment methods - physical, chemical, or biological-aimed at reducing biomass resistance to enzymatic degradation by increasing surface area and pore size, thus improving cellulose and hemicellulose accessibility for microorganisms during the AD process (Millati *et al.*, 2020) [3]. While physical treatments consume considerable energy and chemical methods may produce harmful by-products and environmental pollution (Carrere *et al.*, 2016) [1], biological treatments offer cost-effective and eco-friendly alternatives to enhance AD performance (Paritosh *et al.*, 2018) [5]. Therefore, the current study aims to evaluate the impact of biological treatment of cattle dung using *Pseudomonas fluorescens* on lignocellulose degradation.

2. Materials and Methods

2.1 Work plan

For this experiment, fresh cattle dung from a uniform age group of animals was utilized as the substrate. It was analysed

for proximate principles as represented in Table 1 and standardized to contain 10% dry matter before its introduction into the digester to enhance the efficiency and lignocellulose degradation.

Table 1: Proximate analysis of different Cattle dung on DM basis

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

A slurry with a dry matter content of 10% was prepared by adding the appropriate amount of water to the substrate. To this mixture, inoculum of *Pseudomonas fluorescens* was introduced at concentrations of 3% and 6% as detailed in Table 2.

Table 2: Plan of work for the experiment

Particulars	Cattle Manure
Moisture%	81.94
Dry matter%	18.06
Fresh Dung (Grams)	11.11
Fresh dung on DMB	2.00
Water added (ml to maintain DM 10%)	8.89
Total volume	20
Culture added to standardized slurry	
Control group (C1)	No culture added
3% inoculum (C2)	0.6gms
6% inoculum (C3)	1.2gms

The treatment groups were designated as C1 (control), C2 (3% inoculum), and C3 (6% inoculum). The prepared syringes containing 20 ml of this slurry were then incubated in a water bath at 30 °C, the optimal temperature for the

growth of *Pseudomonas fluorescens*. Lignocellulose degradation was calculated by collecting samples from each syringe once in 10 days, till 50th day and subjecting it to for fiber analysis techniques as per AOAC (2016).

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 as per Snedecor and Cochran (1994). The Duncan Multiple Range Test (DMRT) was used for further comparison of significant differences.

3. Result and Discussion

3.1. Result

3.1.1 Mean percentage degradation of different Fiber fractions in cattle dung

Percentage degradation of Fiber fractions like hemicellulose, cellulose and lignin in Cattle dung treated with *Pseudomonas fluorescens* over different time periods is shown in Table 3. and it is represented graphically in Fig. 1 (Hemicellulose), Fig. 2 (Cellulose), Fig. 3 (Lignin).

Table 3: Percentage degradation of different fiber fractions in Cattle dung due to biological treatment

Hemicellulose (%)				
Day	C1	C2	C3	p value
10 th	12.37	12.53	14.30	
20 th	10.23	10.14	11.53	
30 th	8.41	8.40	9.61	
40 th	6.73	6.70	7.68	
50 th	4.21	4.20	4.81	
Mean ± SE	8.39 ± 1.40	8.39 ± 1.43	9.59 ± 1.62	0.597
Total degradation (%)	41.94	41.97	47.94	
Cellulose (%)				
Day	C1	C2	C3	p value
10 th	11.40	11.44	12.06	
20 th	9.12	9.14	9.65	
30 th	7.60	7.63	8.05	
40 th	6.08	6.07	6.43	
50 th	3.80	3.80	4.02	
Mean ± SE	7.60 ± 1.29	7.62 ± 1.30	8.04 ± 1.37	0.826
Total degradation (%)	38.00	38.10	40.21	
Lignin (%)				
Day	C1	C2	C3	p value
10 th	4.07	4.20	4.46	
20 th	3.25	3.90	4.21	
30 th	3.08	3.30	4.08	
40 th	2.20	2.72	3.05	
50 th	1.43	1.66	1.67	
Mean ± SE	2.80 ± 0.454	3.15 ± 0.453	3.49 ± 0.514	0.602
Total degradation (%)	14.03	15.78	17.47	

Means in a row with different superscripts (a, b, c) differ significantly ($p < 0.05$) between treatment groups C1-control, C2 -3% inoculum, C3 - 6% inoculum.

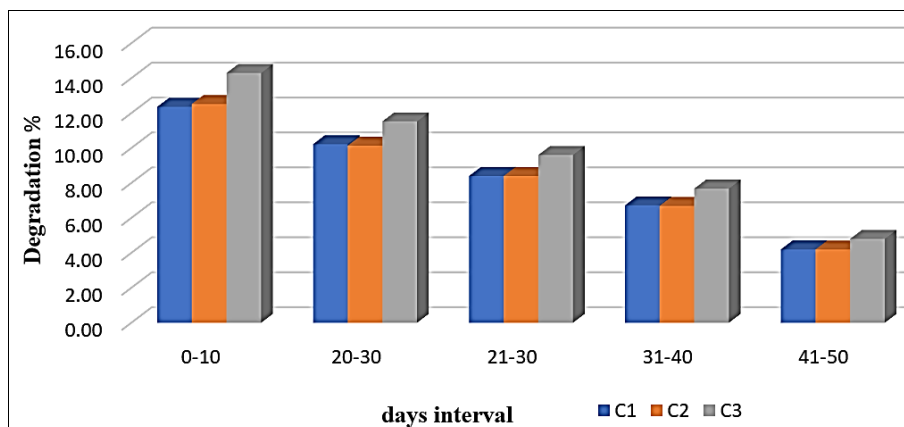


Fig 1: Percentage degradation of hemicellulose in cattle dung due to biological treatment at different time intervals

Hemicellulose degradation was highest in the first 10 days (C1-12.366, C2-12.529, C3-14.303) and gradually decreased over the subsequent intervals. At each time point, the 6% treatment consistently showed the greatest reduction compared to the control (C1) and 3% treatment (C2). Total percentage degradation of hemicellulose for over 50 days in

control (C1), 3% inoculum (C2) and 6% inoculum (C3) was 41.94%, 41.97%, and 47.94% respectively. The mean degradation values were approximately 8.39% for C1 and C2, and 9.59% for C3. The p value of 0.597 indicates no statistically significant difference in hemicellulose degradation among treatments.

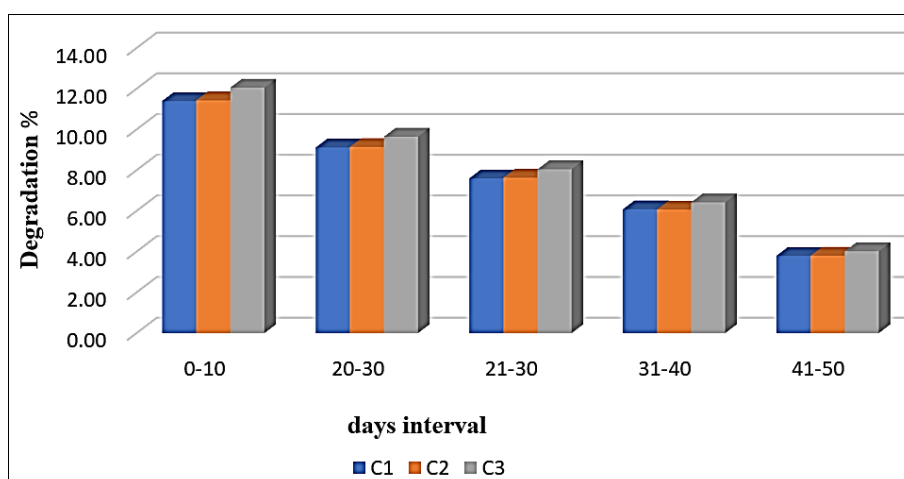


Fig 2: Percentage degradation of cellulose in Cattle dung due to biological treatment at different time intervals

Cellulose degradation was highest in the first 10 days (C1-11.400%, C2-11.436%, C3-12.060%) and steadily decreased over subsequent intervals. The C3 treatment generally showed slightly higher degradation percentages compared to C1 and C2 across all time points. Total percentage degradation of cellulose for over 50 days in control (C1), 3% inoculum (C2)

and 6% inoculum (C3) was 38.00%, 38.10%, and 40.21% respectively. The mean degradation values were approximately 7.60% for C1, 7.62% for C2, and 8.04% for C3. The P value of 0.826 indicates no statistically significant difference in cellulose degradation among the treatments.

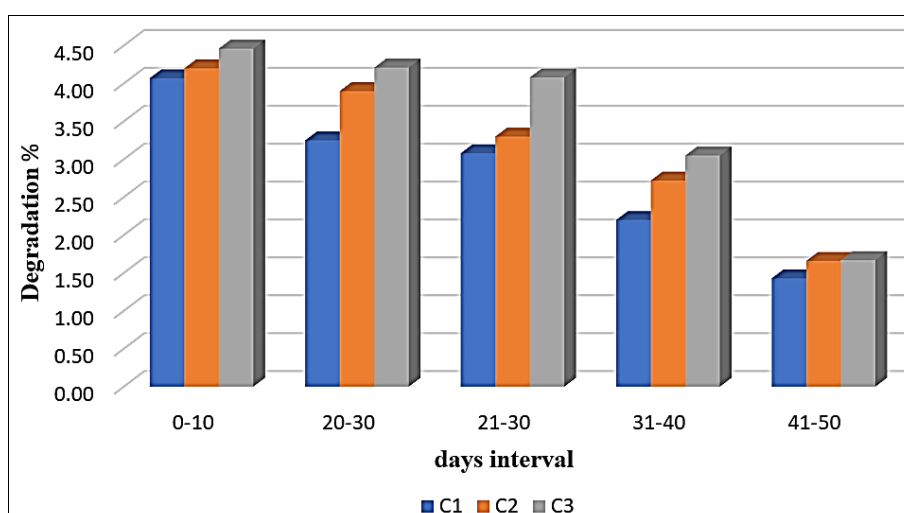


Fig 3: Percentage degradation of lignin in Cattle dung due to biological treatment at different time intervals

Lignin degradation was highest in the first 10 days, with values of C1- 4.07%, C2- 4.20%, and C3-4.46% and decreases over time in all groups. The C3 treatment consistently showed slightly higher degradation percentages compared to C1 and C2 across all intervals. Total percentage degradation of lignin in control(C1), 3% inoculum(C2) and 6% inoculum(C3) was 14.03%, 15.78%, and 17.47% respectively. The mean degradation values were approximately 2.80% for C1, 3.15% for C2, and 3.49% for C3. The *p* value of 0.602 indicates no statistically significant difference among the treatments.

3.2 Discussion

The degradation patterns of fiber in cattle dung showed only slight changes among different treatment groups, with no significant differences in the breakdown of hemicellulose, cellulose, or lignin between the control group and those treated with *Pseudomonas fluorescens*. Over 50 days, the degradation percentages were as follows: hemicellulose - control (41.94%), 3% inoculum (41.97%), 6% inoculum (47.94%); cellulose - control (38.00%), 3% inoculum (38.10%), 6% inoculum (40.21%); and lignin - control (14.03%), 3% inoculum (15.78%), 6% inoculum (17.47%). Although the 6% inoculum showed some improvements in hemicellulose and cellulose breakdown, these changes were not statistically significant (*p* > 0.05), indicating limited contributions of fiber degradation.

P. fluorescens is capable of producing enzymes such as dyp-type peroxidases and xylanases, (Rahmanpour and Bugg, 2014) [8], and endo- and exo- β -1,4-glucanase (Pathak and Navneet., 2016) [6] capable of degrading lignin and cellulose respectively. Unless lignin is effectively modified, polysaccharides (cellulose and hemicellulose) remain largely inaccessible to hydrolytic enzymes. In our study, lignin fractions showed little change even after 50 days of treatment, limiting the degradation of fiber. Similar observations were noted in other studies; Zhou *et al.* (2012) [12] reported only 5.7% lignin reduction after 20 days of treatment on corn stover, while hemicellulose and cellulose removal were also modest. These findings agree with the present results, where structural Fiber fractions were largely resistant to bacterial treatment.

In contrast, other studies with specialized fungal treatments achieved significantly higher fiber degradation. Zulkifli *et al.* (2018) [13] noted up to 60% lignin removal with *Trichoderma* sp., and Rastogi *et al.* (2016) found 63% lignin degradation in rice straw using *Pyrenophora phaeocomes*. Similarly, Zhang *et al.* (2012) [11] reported nearly 50% lignin loss after treating steam-exploded rice straw with *Phanerochaete chrysosporium*.

Cellulose and hemicellulose degradation trends also reflect this limitation. Although *Pseudomonas* and *Bacillus* strains from waste sites showed some cellulolytic activity (ability to break down cellulose), the degradation was much lower compared to fungal systems, ranging from 3% to 33% (Pathak *et al.*, 2016) [6]. This aligns with our findings, where cellulose degradation was low to moderate and showed no significant differences among treatments.

The broader literature suggests that substantial fiber degradation generally occurs under fungal treatments. For instance, Phutela *et al.* (2011) [7] documented reductions of 34.25%, 39.19%, and 34.12% in cellulose, hemicellulose, and lignin, respectively, using *Thermoascus aurantiacus* MTCC. Furthermore, combined treatment methods, such as milling followed by biological treatments with *Pleurotus ostreatus*,

have demonstrated greater fiber fraction degradation, including a reported 30.4% lignin removal by Mustafa *et al.* (2017).

Overall, the literature suggests that significant fiber degradation typically occurs under optimized fungal treatments or combined methods, like milling followed by biological treatments. The absence of significant Fiber degradation in the present study reflects both the biochemical constraints of *P. fluorescens* in anaerobic settings and the structural resistance of lignocellulosic complex in cattle dung.

4. Conclusion

The present study suggests that inoculation of cattle dung slurry with *Pseudomonas fluorescens* at 3% and 6% showed only moderate changes across treatment groups, with no significant differences (*p* > 0.05) in hemicellulose, cellulose, or lignin degradation between control and treated groups.

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6. Conflict of interest

Not available.

7. Financial support

Not available.

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