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

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Abstract

India's energy production is witnessing a robust growth of 7.06%, reaching 1,739.1 billion units in 2023-24. However, the country still faces a deficit of 4.1 billion units, driven by rising population and industrial demands. This situation has intensified the need for alternative energy sources as conventional resources dwindle, leading to increased interest in renewables like solar, wind, and biogas. Biogas, generated through anaerobic digestion (AD) of organic materials, offers a promising solution but faces challenges in breaking down lignocellulosic structures. This study explores the impact of the bacterium *Pseudomonas fluorescens* on the decomposition of lignocellulose found in sheep dung. To conduct the research, fresh sheep dung was mixed with water to achieve 10% dry matter, and inoculated with concentrations of *P. fluorescens* at 3% and 6%. After 50 days, the study assessed the degradation of hemicellulose, cellulose, and lignin. The findings revealed minimal changes across the groups, with no significant variations in fiber degradation. Although some enhancements were observed with the higher inoculum concentration of 6%, these differences were not statistically significant, indicating that further research may be needed to optimize the process.

Keywords: Biogas, *Pseudomonas fluorescens*, anaerobic digestion, lignocellulose, sheep dung, renewable energy

1. Introduction

India's energy production is on the rise, growing at a rate of 7.06% and reaching 1,739.1 billion units in 2023-24. Despite this growth, the country still faces a shortfall of 4.1 billion units in meeting its annual energy demands. This increasing demand, driven by population growth and industrialization, has resulted in the depletion of conventional energy sources, underscoring the need to explore renewable alternatives like solar, wind, tidal, and biogas. Biogas, produced through anaerobic digestion (AD) of biomass, is a promising energy source (CEA, 2024). However, a significant challenge in biogas production is the hydrolysis of lignocellulosic structures, which acts as a rate-limiting factor (Martin-Ryals *et al.*, 2015) ^[2]. To overcome this barrier, innovative strategies are required to enhance hydrolysis and improve the economic viability of biogas plants. One effective approach involves various treatment methods - physical, chemical, or biological - that aim to reduce the biomass's resistance to enzymatic degradation by increasing surface area and pore size. This improvement allows better accessibility of cellulose and hemicellulose for microorganisms during the AD process (Millati *et al.*, 2020) ^[3]. While physical treatments can be energy-intensive and chemical methods may generate harmful by-products, biological treatments present a cost-effective and environmentally friendly alternative to enhance AD performance (Paritosh *et al.*, 2018) ^[5]. Consequently, this study intends to assess the effects of biological treatment on lignocellulose degradation in sheep dung using *Pseudomonas fluorescens*.

2. Materials and Methods

2.1 Work plan

For this experiment, fresh sheep 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 lignocellulose degradation efficiency.

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

Proximate	DM%	OM%	C: N	Lignin%	Hemicellulose%	Cellulose%
Composition	31.85	72.05	27.10	11.83	19.23	21.29

A slurry was prepared by adding the appropriate amount of water to have dry matter content of 10%. 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	Sheep Manure
Moisture%	68.15
Dry matter%	31.85
Fresh Dung (Grams)	6.29
Fresh dung on DMB	2
Water added (ml to maintain DM 10%)	13.71
Total volume	20
Culture added to standardized slurry	
Control group (S1)	No culture added
3% inoculum (S2)	0.6gms
6% inoculum (S3)	1.2gms

The treatment groups were designated as S1 (control), S2 (3% inoculum), and S3 (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) [10]. The Duncan Multiple Range Test (DMRT) was used for further comparison of significant differences.

3. Results and Discussion

3.1 Results

3.1.1 Mean percentage degradation of different Fiber fractions in sheep dung

Percentage degradation of Fiber fractions like hemicellulose,

cellulose and lignin in Sheep 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 Sheep dung due to biological treatment

Hemicellulose (%)				
Day	S1	S2	S3	P value
10 th	11.09	11.11	12.61	
20 th	8.88	8.91	10.09	
30 th	7.39	7.37	8.41	
40 th	3.70	3.70	6.72	
50 th	5.93	5.93	4.20	
Mean ± SE	7.40 ± 1.26	7.42 ± 1.26	8.400 ± 1.43	0.618
Total degradation (%)	37.00	37.01	42.03	
Cellulose (%)				
Day	S1	S2	S3	P value
10 th	9.86	9.87	10.77	
20 th	7.89	7.90	8.62	
30 th	6.57	6.58	7.18	
40 th	5.26	5.25	4.64	
50 th	3.29	3.29	2.52	
Mean ± SE	6.58 ± 1.12	6.58 ± 1.12	6.75 ± 1.45	0.928
Total degradation (%)	32.86	32.88	33.73	
Lignin (%)				
Day	S1	S2	S3	P value
10 th	2.67	2.740	2.79	
20 th	2.23	2.24	2.24	
30 th	1.86	1.92	2.12	
40 th	1.45	1.59	1.83	
50 th	0.93	1.08	1.21	
Mean ± SE	1.82 ± 0.301	1.91 ± 0.281	2.04 ± 0.259	0.870
Total degradation (%)	9.14	9.57	10.19	

Means in a row with different superscripts (a, b, c) differ significantly ($P < 0.05$) between treatment groups S1-control, S2 -3% inoculum, S3 – 6% inoculum.

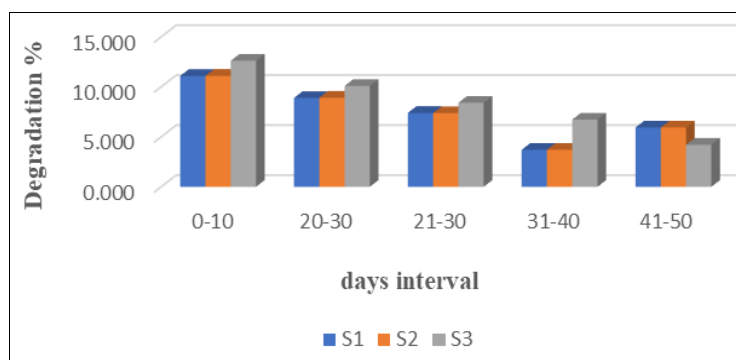


Fig 1: Percentage degradation of hemicellulose in Sheep dung due to biological treatment at different time intervals

The highest hemicellulose degradation occurs within the first 10 days (S1-11.089%, S2-11.11%, S3-12.61%) and decreases in all groups over the subsequent intervals. At each time point, S3 generally showed higher degradation than S1 and S2. Total percentage degradation of hemicellulose for 50 days in control(S1), 3% inoculum(S2) and 6% inoculum(S3) was 37.00%, 37.01%, and 42.03% respectively. The mean degradation values are approximately 7.40% for S1, 7.42% for S2, and 8.40% for S3. The P value of 0.618 indicated no statistically significant difference in hemicellulose degradation among the treatments.

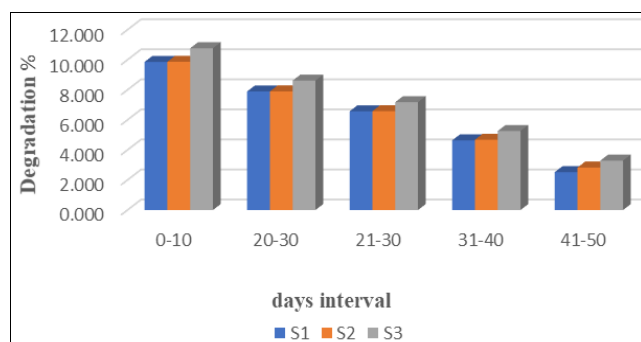


Fig 2: Percentage degradation of different cellulose in Sheep dung due to biological treatment at different time intervals

The highest cellulose degradation occurs within the first 10 days (S1-9.861%, S2-9.868%, S3-10.770%) and declines over time in all groups. At each time point, S3 generally showed higher degradation than S1 and S2. Total percentage degradation of cellulose for 50 days in control(S1), 3% inoculum(S2) and 6% inoculum(S3) was 32.86%, 32.88%, and 33.73% respectively. The mean degradation values were approximately 6.58% for S1 and S2, and 6.75% for S3. The P value of 0.928 indicated no significant difference among the treatments.

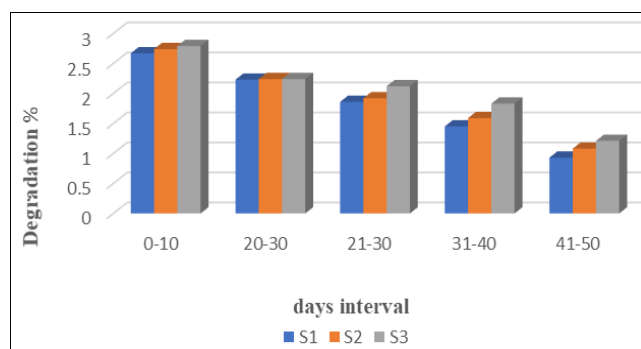


Fig 3: Percentage degradation of different lignin in Sheep dung due to biological treatment at different time intervals

Lignin degradation was highest in the first 10 days, with values of 2.67% for S1, 2.74% for S2, and 2.79% for S3, and it decreases over subsequent intervals. The S3 treatment consistently showed slightly higher degradation percentages compared to S1 and S2 across all time points. Total percentage degradation of lignin for 50 days in control(S1), 3% inoculum(S2) and 6% inoculum(S3) was 9.14%, 9.57%, and 10.20% respectively. The mean degradation values were approximately 1.82% for S1, 1.91% for S2, and 2.04% for S3. The P value of 0.870 indicates no statistically significant difference among the treatments.

3.2 Discussion

The study observed that the fiber degradation patterns in sheep dung showed only minimal changes across different treatment groups over a period of 50 days. The total percentage degradation of hemicellulose after 50 days was 37.00% for the control (S1), 37.01% for the 3% inoculum (S2), and 42.03% for the 6% inoculum (S3). Similarly, the degradation percentages for cellulose were 32.86% (S1), 32.88% (S2), and 33.73% (S3). For lignin, the percentages were 9.14% (S1), 9.57% (S2), and 10.19% (S3). Although the 6% inoculum showed slight enhancements in hemicellulose and cellulose degradation, these changes were not statistically significant ($P > 0.05$), suggesting limited fiber degradation.

P. fluorescens is known to generate enzymes such as dyp-type peroxidases and xylanases (Rahmanpour and Bugg, 2014) [8], in addition to endo- and exo- β -1,4-glucanases (Pathak and Navneet, 2016) [6] capable of degrading lignin and cellulose. However, unless lignin is effectively modified, polysaccharides like cellulose and hemicellulose remain largely unreachable for hydrolytic enzymes. Our study found minimal changes in lignin fractions even after 50 days of treatment, which limited cellulose and hemicellulose degradation. This observation is consistent with other studies; for example, Zhou *et al.* (2012) [12] reported only a 5.7% reduction in lignin following 20 days of treatment on corn stover, with similarly modest decreases in hemicellulose and cellulose. These results support our findings, indicating that structural fiber fractions were largely resistant to bacterial treatment.

In contrast, research on specialized fungal treatments has achieved significantly higher fiber degradation levels. Zulkifli *et al.* (2018) [13] documented up to 60% lignin removal in sheep dung using *Trichoderma* sp., while Rastogi *et al.* (2016) [9] reported 63% lignin degradation in rice straw with *Pyrenophora phaeocomes*. Additionally, Zhang *et al.* (2012) [11] noted nearly 50% lignin loss after treating steam-exploded rice straw with *Phanerochaete chrysosporium*. Trends in cellulose and hemicellulose degradation further highlight these limitations. Other studies found that *Pseudomonas* and *Bacillus* strains exhibited some cellulolytic activity, but their degradation rates were significantly lower compared to fungal treatments, ranging from 3% to 33% (Pathak *et al.*, 2016) [6]. This aligns with our findings, where cellulose degradation was low to moderate and did not display 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) [4].

In summary, the findings indicate that bacterial systems exhibit relatively modest effects on fiber degradation when compared to more effective fungal or combined approaches. The limited degradation observed in this study reflects both the biochemical constraints of *P. fluorescens* in anaerobic conditions and the structural resistance of the lignocellulosic complex in sheep dung.

4. Conclusion

The present suggest that Inoculation of sheep 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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Conflict of Interest

Not available

Financial Support

Not available

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