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

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Abstract

Pigs significantly contribute to affordable protein sources and livelihoods in developing nations like India, yet their waste management remains a challenge. The global pig population has doubled since the 1970s, generating approximately 10.1 million tons of pig excreta annually in India. Despite its potential as an organic fertilizer and renewable energy source, social taboos and poor practices hinder the effective use of pig manure, leading to pollution. This study investigates the effect of biologically treating pig dung with *Pseudomonas fluorescens* on biogas production. Freshly collected pig dung was analysed and prepared with 10% dry matter for digestion, inoculated with 3% and 6% *P. fluorescens*. Treatment groups included a control (P0), a 3% inoculum (P3), and a 6% inoculum (P6). Over 50 days, biogas production was measured, with P6 consistently outperforming other groups. Initial biogas yields were similar across groups, but from days 11-50, P6 showed significantly higher production compared to controls ($p < 0.05$), resulting in 12.51% more cumulative biogas than P3 and 11.93% more than P0. The present study illustrates the potential of *Pseudomonas fluorescens* to significantly boost biogas production from pig dung, especially at optimal inoculum levels.

Keywords: Renewable energy, biogas, *Pseudomonas fluorescens*, inoculum, pig dung, organic fertilizer

1. Introduction

Pigs play a crucial role as versatile livestock, providing an affordable source of protein and enhancing livelihoods in developing nations such as India. Since the 1970s, the global pig population has doubled, with forecasts suggesting a further increase of 25% by 2030 (Hu *et al.*, 2021) [4]. However, swine farming generates significant waste; in India, the pig population produces approximately 10.1 million tons of excreta annually (Kaur *et al.*, 2017) [5]. Importantly, pig manure is richer in nutrients compared to cow dung (Makara *et al.*, 2019) [7]. Despite its potential as an organic fertilizer and renewable energy source, social taboos and poor management practices hinder the effective utilization of pig waste, leading to environmental pollution and health risks (Ramírez *et al.*, 2020) [12]. Utilizing pig manure for biogas production can alleviate some of these challenges, helping to reduce reliance on non-renewable resources (Hamelinck *et al.*, 2005) [3] while simultaneously addressing issues such as environmental degradation and energy shortages. Therefore, this research aims to investigate the impact of biologically treating pig dung with *Pseudomonas fluorescens* on biogas production.

2. Materials and Methods

2.1 Work plan

In this study, we employed fresh pig dung sourced from uniform age groups of animals as the substrate. To optimize biogas production, the dung was analysed for proximate principles as detailed in Table.1 and standardized to contain 10% dry matter before its introduction into the digester to enhance the efficiency and yield of biogas generation.

Table 1: Proximate analysis of different pig dung on DM basis.

Proximate	DM%	OM%	C: N	Lignin%	Hemicellulose%	Cellulose%
Composition	30.84	77.24	18.67	7.44	14.98	21.90

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	Pig Manure
Moisture%	69.59
Dry matter%	30.41
Fresh Dung (Grams)	6.56
Fresh dung on DMB	2
Water added (ml to maintain DM 10%)	13.44
Total volume	20
Culture added (in gm) to standardized slurry	
Control group (P0)	No culture added
3% inoculum (P3)	0.6gms
6% inoculum (P6)	1.2gms

The treatment groups were designated as P0 (control), P3 (3% inoculum), and P6 (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*. Biogas production was observed over a 50-day period, with three replicates for each treatment group. Every 10 days, six syringes from each group were retrieved for fiber analysis and bacterial 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 as per Snedecor and Cochran (1994). The Duncan Multiple Range Test (DMRT) was used for further comparison of significant differences.

3. Results and Discussion

3.1. Result

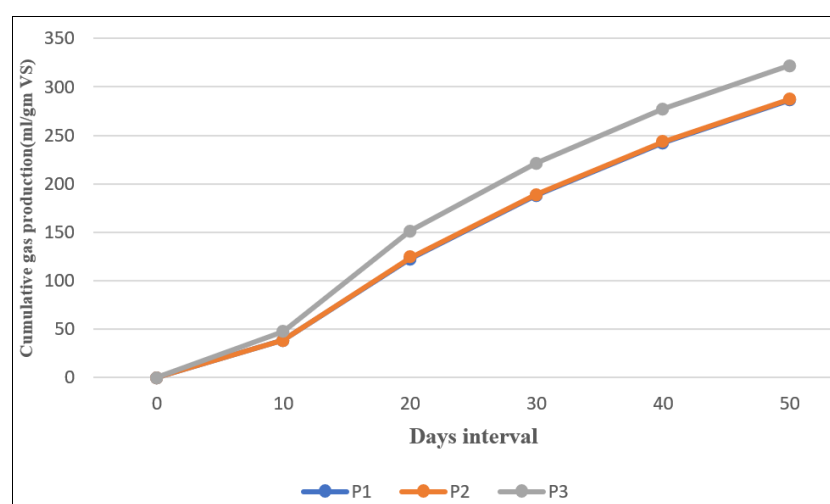
3.1.1 Cumulative gas production

Cumulative biogas production (ml/gm VS) from Pig dung in different biological treatment groups across various time intervals is summarized in the Table. 3 and the trend is depicted in the line diagram in Figure. 1 below.

Table 3: Mean $\pm$ SE Cumulative biogas production (ml/gm VS) for 50 days in Sheep dung from different biologically treated groups at 10 days interval

Days	P0	P3	P6	p value
0	0	0	0	
10	38.68 $\pm$ 7.737	38.70 $\pm$ 7.893	47.81 $\pm$ 9.811	0.6867
20	122.29 ^b $\pm$ 6.952	123.94 ^b $\pm$ 6.937	151.54 ^a $\pm$ 8.174	0.0146
30	187.94 ^b $\pm$ 5.801	192.30 ^b $\pm$ 5.717	221.39 ^a $\pm$ 6.015	0.00042
40	242.42 ^b $\pm$ 4.501	243.79 ^b $\pm$ 4.558	277.40 ^a $\pm$ 4.706	0.001
50	286.23 ^b $\pm$ 3.754	287.71 ^b $\pm$ 3.739	322.06 ^a $\pm$ 3.804	0.001

Means in a row with different superscripts (a, b, c) differ significantly ($p < 0.05$) between treatment groups P0-control, P3 -3% inoculum, P6 - 6% inoculum.

**Fig 1:** Cumulative biogas production (ml/gm VS) in Pig dung from different biologically treated groups 10 days interval.

Biogas output increased over time for all groups, with P6 consistently producing the highest amounts. In the 0-10 days interval, the values ranged from 38.68 to 47.81 ml/gm VS, with no significant difference among treatments ($P = 0.6867$). From day 11 onwards, P6 showed significantly higher production (151.54, 221.39, 277.40, 322.06) compared to P0 and P3, which had similar values within each interval. The

differences among treatments were 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 6% inoculum (P6) generating 12.51% and 3% inoculum (P3) generating 11.93% more compared to control.

3.2 Discussion

The impact of biological treatment with *Pseudomonas fluorescens* on cumulative biogas production from pig dung was evident throughout the 50-day experimental period. During the initial phase (days 0-10), cumulative gas yields did not show significant differences ($p > 0.05$) among the control, 3%, and 6% culture-treated groups, which likely reflects a short adaptation (lag) phase for the methanogenic bacteria, resulting in a slow start to hydrolytic processes.

However, starting from days 11-20, the 6% treatment group (P6) demonstrated significantly higher cumulative gas production compared to the control ($p < 0.05$), and this trend continued through day 50, with the 6% treatment consistently outperforming both the control and the 3% treatment groups. These results suggest that a 6% inoculum level of *P. fluorescens* provides a reproducible and dose-dependent increase in biogas yield, whereas the 3% level failed to achieve a substantial improvement. The enhancement observed at the 6% level may be attributed to improved hydrolysis of readily degradable organic compounds, such as simple sugars, soluble carbohydrates, proteins, and lipids, alongside partial lignocellulose degradation.

Pseudomonas fluorescens is known to produce DyP-type peroxidases (Rahmanpour and Bugg, 2014) [11], and some strains of *Pseudomonas* exhibit endo- β -1,4-glucanase and exo- β -1,4-glucanase activities (Pathak *et al.*, 2016) [9], which are extracellular enzymes with potential lignin-degrading and cellulose-acting capacities. Thus, the enzyme secretion by *P. fluorescens* likely contributes to the degradation of the lignin-carbohydrate complex, enhancing the accessibility of cellulose and hemicellulose.

The lack of significant enhancement at the 3% inoculum level suggests that a critical threshold of bacterial biomass or enzyme activity was not reached, limiting substrate modification and biogas yield. Similar findings were noted by Bruni *et al.* (2010) [1], who reported that suboptimal dosing of microbial cultures had negligible effects on methane production.

Similar findings have been reported by Mustafa *et al.* (2016) [8], who observed a 120% increase in biogas yield from unprocessed rice straw, and Fugol *et al.* (2023) [2], who noted a 62% increase in biogas production from maize silage treated with *Pseudomonas* spp. In other studies, Phutela *et al.* (2011) [10] found a 53.8% increase in biogas yield compared to controls, while Tuesorn *et al.* (2013) [13] reported a 55% increase. Kaur *et al.* (2014) [6] also demonstrated a 30.7% rise in biogas yield compared to untreated paddy straw in their biological treatment experiments with various substrates.

4. Conclusion

The present study suggests that inoculation of sheep dung slurry with *Pseudomonas fluorescens* showed significant enhancement in biogas yield at 6% inoculum compared to 3% and control.

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