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Ranjith Kumar ND

M.V. Sc. Scholar,

Department of Livestock Production
and Management (LPM), Veterinary
College, Hebbal, Bengaluru,
Karnataka, India

Prasanna SB

Professor & Head, Department of
Livestock Production and
Management (LPM), Veterinary
College, Hebbal, Bengaluru,
Karnataka, India

Naveen Kumar BT

Assistant Professor (Agronomy),
Livestock Research and Information
Center (LRIC), Amruthmahal,
Konehally, Karnataka, India

Vivek M Patil

Professor & Head, Department of
LFC Veterinary College, Hebbal,
Bengaluru, Karnataka, India

Umashankar BC

Associate Professor, Department of
LFC Veterinary College, Hebbal,
Bengaluru, Karnataka, India

Yeshavanth SL

Assistant Professor (SS)
Department of Biological Production,
Institute of Animal Health and
Veterinary Biologicals (IAHVB),
Karnataka, India

Mahadevappa D Gouri

Associate Professor, Department of
LFC Veterinary College, Hebbal,
Bengaluru, Karnataka, India

Shankarappa Bhajantri

Associate Professor, Department of
LFC Veterinary College, Hebbal,
Bengaluru, Karnataka, India

Corresponding Author:

Ranjith Kumar ND

M.V. Sc. Scholar, Department of
Livestock Production and
Management (LPM), Veterinary
College, Hebbal, Bengaluru,
Karnataka, India

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

**Ranjith Kumar ND, Prasanna SB, Naveen Kumar BT, Vivek M Patil,
Umashankar BC, Yeshavanth SL, Mahadevappa D Gouri and
Shankarappa Bhajantri**

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Abstract

India's energy production is growing significantly, increasing by 7.06% to reach 1,739.1 billion units in 2023-24. However, a shortfall of 4.1 billion units persists due to population growth and industrialization, sparking interest in renewable energy sources like biogas. This study investigates the impact of *Pseudomonas fluorescens* on biogas production from sheep dung. 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. Results showed that cumulative biogas production increased over time for all treatments, with 6% treatment S6 consistently yielding the highest amounts. Notably, significant differences ($p < 0.05$) emerged between S6 and the control starting from day 21, with S6 producing 8.92% more biogas than S0. In contrast, S3 produced 7.79% more than the control. The 6% inoculum level enhanced hydrolysis of organic compounds and cellulose accessibility, while the 3% level did not achieve sufficient bacterial activity to improve yields. This study illustrates the potential of *Pseudomonas fluorescens* to significantly boost biogas production from sheep dung, especially at optimal inoculum levels.

Keywords: Biogas, *Pseudomonas fluorescens*, Sheep dung, Inoculum, Cumulative production, Renewable energy

1. Introduction

India's energy production is experiencing significant growth, with an annual increase of 7.06%, reaching 1,739.1 billion units in 2023-24 (CEA, 2024). Despite this progress, there remains a shortfall of 4.1 billion units needed to meet the nation's energy demands, primarily due to rising population and industrialization. This situation has led to a heightened interest in exploring new and renewable energy sources such as solar, wind, tidal, and biogas.

In addition, India has the largest livestock population globally, estimated at 535.78 million in 2019. It also ranks third in sheep production, with a total sheep population of 74.26 million (BAHS 2024). The vast amounts of animal manure generated present both challenges and valuable opportunities for energy production through biogas, derived from the anaerobic digestion of organic materials. This eco-friendly technology has gained traction over the last decade for its effectiveness in waste management and clean energy generation (Romero-Güiza *et al.* 2016) ^[9].

By harnessing biogas, India can effectively address its energy shortfall, improve waste management practices, and promote sustainable energy solutions, all contributing to a healthier environment. Therefore, the current study aims to evaluate the impact of biological treatment of sheep dung using *Pseudomonas fluorescens* on biogas production.

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 efficiency and yield of biogas generation

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

Proximate Composition	DM%	OM%	C: N	Lignin%	Hemicellulose%	Cellulose%
	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 (P1)	No culture added
3% inoculum (P2)	0.6gms
6% inoculum (P3)	1.2gms

The treatment groups were designated as S0 (control), S3 (3% inoculum), and S6 (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 monitored over a 50-day period, with each treatment group having three replicates. Every 10 days, six syringes from each group were collected 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 Sheep dung in different biological treatment groups at various time points are summarized in the Table. 3. and the same has been represented 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	S0	S3	S6	P value
0	0	0	0	
10	27.80 $\pm$ 5.862	28.38 $\pm$ 5.988	33.82 $\pm$ 7.340	0.768
20	93.96 $\pm$ 5.044	95.21 $\pm$ 4.913	106.81 $\pm$ 5.098	0.1556
30	141.44 ^b $\pm$ 4.098	143.58 ^b $\pm$ 4.275	156.37 ^a $\pm$ 4.654	0.044
40	180.60 ^b $\pm$ 3.358	182.94 ^b $\pm$ 3.349	198.91 ^a $\pm$ 3.349	0.0011
50	210.01 ^b $\pm$ 2.131	212.31 ^b $\pm$ 2.039	228.87 ^a $\pm$ 2.022	0.001

Means in a row with different superscripts (a, b, c) differ significantly ($p < 0.05$) between treatment groups S0-control, S3-3% inoculum, S6-6% inoculum.

Cumulative biogas production increased over time for all treatment groups, with S6 consistently yielding the highest amounts. During the first two intervals (days 0-10 and 11-20), the biogas production values ranged from 27.80 to 28.38 ml/gm VS and 93.96 to 106.81 ml/gm VS, respectively, with no significant differences observed among the treatments.

However, from day 21 onwards, S6 demonstrated significantly higher biogas production, with values of 156.37, 198.91, and 228.87 ml/gm VS for subsequent intervals. In contrast, S0 and S3 exhibited similar production levels during these periods. The differences between the treatment groups became statistically significant ($p < 0.05$) starting on day 21 and continued through till day 50.

Overall, cumulative biogas production steadily increased for all treatments, with S6 (6% inoculum) generating 8.98% more biogas compared to the control group (S0), while S3 (3% inoculum) produced 7.77% more than the control.

3.2 Discussion

During the first 10 days, there were no significant differences ($p > 0.05$) in cumulative gas yields among the control group (S0) and the 3% (S3) and 6% (S6) culture-treated groups. This likely reflects a short lag phase for the methanogenic bacteria, resulting in a relatively slow start of the hydrolytic processes. However, from days 21 to 30 onward, the 6% culture group (S6) showed significantly higher cumulative gas production ($p < 0.05$) compared to the controls. This trend continued through day 50, with the 6% treatment consistently outperforming both the control and the 3% treatments.

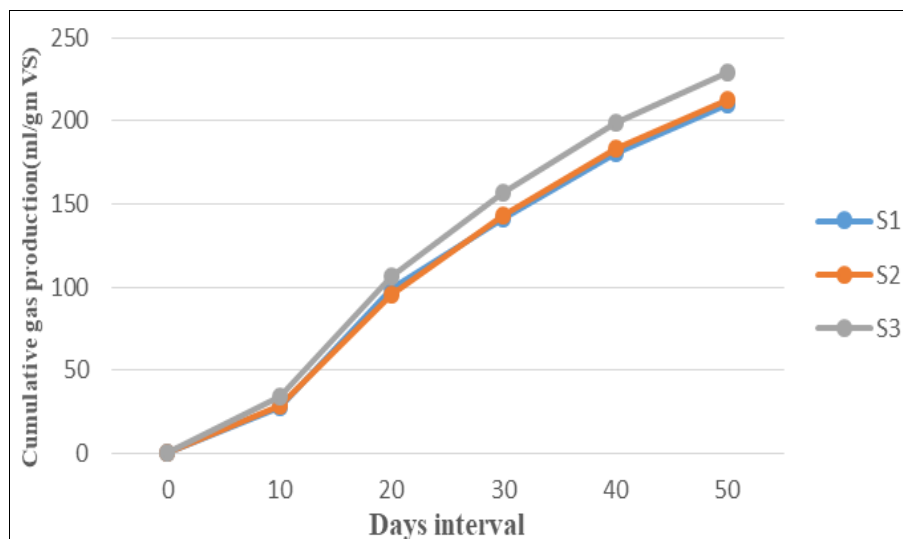


Fig 1: Cumulative biogas production (ml/gm VS) for 50 days in Sheep dung from different biologically treated groups at 10 days interval.

The findings of this research indicate that a 6% inoculum of *Pseudomonas fluorescens* can enhance biogas yield in sheep manure in a reproducible and dose-dependent manner. In contrast, the 3% inoculum did not reach the necessary threshold for significant improvement. Specifically, the sheep dung treated with the 6% inoculum yielded 8.92% more biogas, while the 3% treatment produced 7.79% more compared to the control.

This improved production at the 6% level can be attributed to enhanced hydrolysis of readily degradable organic compounds and partial degradation of lignocellulose. *Pseudomonas fluorescens* produces beneficial enzymes, including dyp-type peroxidases (Rahmanpour and Bugg, 2014) and endo- β -1,4-glucanase and exo- β -1,4-glucanase (Pathak *et al.*, 2016) [7], which facilitate the breakdown of lignin and increase 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.

The delayed response observed in sheep dung where significant improvements were noted only from days 21 to 30 can be explained by the lower availability of soluble, readily degradable nutrients in sheep manure. This is due to the higher digestibility of sheep diets, which leaves less soluble substrate for immediate microbial action. Research by Li and Triolo *et al.* (2011) [10] emphasized that differences in manure composition and prior digestibility influence biogas yield, resulting in lower or delayed responses to biological treatment.

Similar findings have been reported by Mustafa *et al.* (2016) [6], who observed a 120% increase in yield compared to unprocessed rice straw, and Fugol *et al.* (2023) [4], who noted a 62% increase in biogas production from maize silage treated with *Pseudomonas* spp. compared to the control

to 3% and control.

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4. Conclusion

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

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