The Pharma Innovation Journal 2022; SP-11(6): 271-273

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(6): 271-273

www.thepharmajournal.com
Received: 24-04-2022
Accepted: 29-05-2022

**Seema Yadav**
Ph.D. Scholar, Livestock Production Management, IVRI, Izatnagar, Bareilly, Uttar Pradesh, India

**Chandrahas**
Principal Scientist, ICAR-CARI, Izatnagar, Bareilly, Uttar Pradesh, India

**Rajneesh Thakur**
Ph.D. Scholar, Livestock Production Management, IVRI, Izatnagar, Bareilly, Uttar Pradesh, India

**Raj Narayan**
Principal Scientist, ICAR-CARI, Izatnagar, Bareilly, Uttar Pradesh, India

**Corresponding Author**
**Seema Yadav**
Ph.D. Scholar, Livestock Production Management, IVRI, Izatnagar, Bareilly, Uttar Pradesh, India

# Effect of dilution rates of pig faeces on bacterial load of bio-slurry produced during anaerobic digestion technology

**Seema Yadav, Chandrahas, Rajneesh Thakur and Raj Narayan**

**Abstract**
The present study was conducted to see the effect of dilution rates of pig faeces on bacterial load of bio-slurry produced during anaerobic digestion (AD) technology for its safer use as natural fertilizer. The study period was of 3 months from June to August 2021. Four groups having different dilution rates of 1:8 ($T_0$= control), 1:6 ($T_1$), 1:4 ($T_2$) and 1:2 ($T_3$) were taken. The bacterial load for control as well as all the treatments was analysed using pour plate technique on day 0, 45 and 90. In case of TBC reduction, $T_0$ group performed better, whereas, for *E. coli* reduction $T_2$ group performed better than others. For salmonella and alike microbes reduction, all the groups did not show any significant difference. The results of the present study showed that AD technology have marked effect in the reduction of bacterial load of bio-slurry, making it safe for the use as natural fertilizer.

**Keywords:** Anaerobic digestion, bacterial load, bio-slurry, dilution rate

## Introduction
In India, pig population is 9.06 million (20th livestock census, DADF). Some researchers have quantified potential pig excreta production of India to be 10.1 MT/year, taking average excreta produced from an adult pig is 2.7 Kg/day (9% of body weight, Kaur *et al.*, 2107). According to Bhatia *et al.* (2020) [4] pig manure has more biogas production potential (0.30 $m^3$/ Kg VS) than cow (0.20 $m^3$/Kg VS)**.** The N, P, K (manurial value) of pig faeces is 0.59, 0.46, 0.43 respectively, greater than cow dung (0.30, 0.18, 0.18) (Makara *et al.*, 2019) [8] which may be used as natural fertilizer. Despite of having lots of hidden potential of pig excreta as manure, it is not used wisely and wasted without proper use. Although swine manure can pollute environment and may have many harmful effects but if it is used properly can be a precious resource to overcome the problems of growing population (Xiaoa *et al.* 2019) [13]. Furthermore, improper disposal of pig faecses generates significant negative impacts causing the economic losses, health hazard to human being, contaminating the water table, pathogen spread and eutrophication of water bodies (Ramírez *et al.*, 2020) [10]. There are many technologies which can use swine faeces efficiently for its proper utilization like anaerobic digestion, composting, use of spent slurry as natural fertilizer for enhanced crop yield etc. But the pig faeces as well as spent slurry contain many pathogens which may adversely affect its handlers. So, the present study was done to see the effect of dilution rates of pig faeces on bacterial load of bio-slurry produced during anaerobic digestion (AD) technology for its safer use as natural fertilizer.

## Materials and Methods
### Location of experiment
The present study was conducted at swine production farm (SPF) IVRI, Izatnagar, Bareilly from June to August 2021. The location of farm is having longitude of 79°24' east, latitude of 28°22' northand an altitude of 169.2m. This location comes under Upper Gangetic Plain Region of India (*Shastri, 1995*). The climatic conditions of Bareilly are humid, sub-tropical with very hot summer (44 °C) and dry winter (2 °C). The monthly mean temperatures ranged from 14 °C to 33 °C (58°F to 92°F) with annual mean temperature is 25 °C (77°F).

### Design of experiment
To perform anaerobic digestion on pig faeces four digester was used with 1:8 dilutions as control ($T_0$) and three treatments with dilution rate of 1:6 ($T_1$), 1:4 ($T_2$) and 1:2 ($T_3$).

Water was taken to dilute the faeces, wheat straw was used to balance C/N ratio as 25:1 and 2% (v/v)rumen liquor was used as methanogen culture. On day zero, all the ingredients were filled inside the digester and sealed properly to make anaerobic condition. After 15 days of digester sealing, daily turning and feeding of biogas digester done for the rest of the periods.

**Temperature and pH recordings of bio-digesters**

The physicochemical properties like pH and Temperature of bio-slurry of each bio-digester was estimated by using digital pH meter (Perfect India digital pH meter) whereas temperature was recorded by portable temperature meter (Konvio®). The recordings were taken as soon as slurry obtained from outlet to overcome error due to environment. Recordings were taken at fortnightly intervals.

**Sample collection**

For estimation of bacterial load the slurry samples from all (4) biogas digester were collected on day 0, 45 and 90 in properly sterilized 50 ml centrifuge tube. After collection the samples were immediately taken to laboratory for further processing.

**Bacterial load of the bio-slurry**

To examine the bacterial load of bio-slurry, spent slurry sample of 20 ml was taken from control and each treatment. Approximately 1.0 ml of spent slurry was suspended in 10 ml of PBS and thoroughly mixed by little shaking. The bacterial load was calculated using Pour Plate Technique (Miles and Misra, 1938). The Hichrome *E. coli* agar media (HiMedia® Mumbai) was used for identification of *E. coli* (Blue colonies) from non-*E. coli* bacteria. Total bacterial count (White colonies) was determined by using Brain Heart Infusion agar media (HiMedia® Mumbai). Salmonella and Salmonella like Bacteria were identified using Hektoen-Enteroagar media (HiMedia® Mumbai). The following equation is used to calculate the number of colony forming units (CFU) per gram from the original aliquot / sample:

CFU per gram = Average number of colonies for a dilution x dilution factor

**Statistical analysis**

After collection and calculation, data were analyzed using Software Package for Social Sciences (SPSS, version 20.0). To examine the difference between various treatment groups, recorded data were subjected to one way ANOVA (Snedecor and Cochran, 1989). The significant difference between different treatments groups were compared by Tukey's b test.

**Results and Discussion**

**pH and temperature of bio-slurry**

The pH and slurry temperature of control and different treatments has been presented in Table 1.The average pH was significantly ($P<0.01$) higher in $T_3$ than $T_0$ and $T_1$ with non-significant differences among them. The pH value of $T_2$ group was in-between $T_3$ and other two groups. The average temperature (°C) of spent slurry was significantly ($P<0.01$) higher in $T_0$ than the rest of groups having slightly lower values. The physicochemical properties of spent slurry (also called bio-slurry) viz, pH and slurry temperature depends on the type of raw material or organic material used for biogas production. It also depends on the microbes and microbial process going inside the digester. The physicochemical properties of freshly collected bio-slurry more or less indicated the conditions inside the biogas digester in which methanogens thrived

**Table 1:** Physicochemical properties of spent slurry in different treatment groups

| Parameters | $T_0$ (n=12) | $T_1$ (n=12) | $T_2$ (n=12) | $T_3$ (n=12) | Sig. Level |
|---|---|---|---|---|---|
| pH | $6.09^{a}\pm0.21$ | $6.21^{a}\pm0.17$ | $6.33^{ab}\pm0.18$ | $6.85^{b}\pm0.09$ | $P<0.05$ |
| Temperature (˚C ) | $31.85^{b}\pm0.16$ | $30.18^{a}\pm0.23$ | $29.96^{a}\pm0.19$ | $29.91^{a}\pm0.28$ | $P<0.01$ |

Means with different superscript in a row differ significantly

**Bacterial load of spent slurry (bio-slurry)**

The microbial load like TBC, *E. coli*, *Salmonella* and Salmonella like microbes for different groups has been enlisted in Table 2. TBC count (in log10) on day 0 was significantly ($P<0.05$) higher in $T_3$ followed by $T_2$, $T_0$ and $T_1$ with non-significant differences between last two groups. At day 45, TBC was significantly ($P<0.05$) higher in $T_3$ than $T_0$ and $T_1$ groups with non-significant differences among them, however, it was in-between $T_3$, $T_0$ and $T_1$ for $T_2$ group. Similarly at 90 days, the trend of TBC was significantly higher ($P<0.05$) in $T_3$ followed by $T_2$, $T_1$ and $T_0$ with non-significant difference among last two groups. The *E Coli* count at day 0 was significantly ($P<0.05$) lower in $T_0$ than rest of the groups. At 45 days, it was significantly ($P<0.05$) higher in $T_3$and $T_2$ than $T_1$ and $T_0$ groups. At 90 days also, the *E Coli* count was significantly ($P<0.05$) higher in $T_3$ followed by $T_1$, $T_0$ and $T_2$ with non -significant difference among last two groups. *Salmonella* and *Salmonella* like microbes did not show any significant differences at 0, 45 as well as at 90 days.

The substrate used for anaerobic digestion has numerous microbes, few of them may be pathogens, whose quantity depends on the source of waste (Jenkins *et al.*, 2007) [6] may be due to which TBC and *E. coli* has higher in $T_3$ as well as $T_2$ as these groups were subjected to lower dilution and higher OLR (higher concentration of substrate) under present study. The anaerobic digestion technology greatly helps to reduce the bacterial load of organic waste and safe discharge of digested slurry which may be used as fertilizer (Avery *et al.*, 2014) [1]. Similar trend has been observed for TBC and *E. coli* under present study. The key factor involved in the reduction of pathogens during AD is unfavorable environments inside the digester which may restrict growth and development of pathogens. Among these factors temperature and pH is most crucial. In the same way the essential factors required for growth and multiplication of pathogens depends on the operational factors of digester (Organic loading rate, Hydraulic retention time, temperature and pH), type of feedstock, competitive inhibition and anaerobic conditions (Chen *et al.*, 2016).

The reduction in the log values of TBC in slurry from day 0 to day 90 was17.76% in $T_0$, 17.25% in $T_1$, 12.96 in $T_2$ and 9.22% in $T_3$ which may be attributed to the hostile environment other than temperature and pH. As in this experiment the temperature of slurry was in the range of 29-

32ºC and pH 6.0-7.0 for all groups (Table 1), it may not be a good explanation of reduction of log value. The other factors like competitive inhibition, HRT, moisture content etc. may be attributed to explanation of pathogen reduction from start to end of the experiment (Smith, 2005) [12]. The little reduction in log values from 0 to 90 days may be due to continuous type of biogas digester (Sahlstrom, 2008).Bagge, (2005) [2] also advocated that the batch reactor was better than continuous type in case of pathogen reduction. The significantly ($P$<0.05) lesser log value of TBC in control and $T_1$ than $T_2$ and $T_3$ may be because of the difference in the OLR and HRT values along with other less studied factors. Similarly in case of *E. coli*, reduction from 0 to 90 days was 4.1% in $T_0$, 3.84% in $T_1$, 7.06% in $T_2$ and 4.21% in $T_3$ and may be explained by the abovementioned factors. Paudel (2009) in a study found that the AD technology reduces the pathogen load in bio-slurry than bio-waste (loaded organic substrate). In case of *Salmonella spp.* and *salmonella* like bacteria, the non-significant difference among the groups may be because of their lower rate of growth and favourable condition for the survivability, as multiplication of bacteria is temperature sensitive process.

**Table 2:** Bacterial load (log10) of spent slurry variants under different treatments

| Parameters | $T_0$ (n=6) | $T_1$ (n=6) | $T_2$ (n=6) | $T_3$ (n=6) |
|---|---|---|---|---|
| **Total Bacterial Count (TBC)** | | | | |
| Day 0 | $7.60^{a}$±0.02 | $7.71^{a}$±0.05 | $8.41^{ab}$±0.15 | $8.78^{b}$ ±0.08 |
| Day 45 | $7.46^{a}$ ±0.03 | $7.32^{a}$±0.10 | $8.30^{ab}$ ±0.00 | $8.68^{c}$±0.04 |
| Day 90 | $6.25^{a}$±0.05 | $6.38^{a}$±0.00 | $7.32^{ab}$±0.01 | $7.97^{b}$±0.23 |
| ***E. coli*** | | | | |
| Day 0 | $5.26^{a}$±0.00 | $5.41^{ab}$±0.01 | $5.52^{b}$±0.00 | $5.69^{b}$±0.02 |
| Day 45 | $5.17^{a}$±0.10 | $5.14^{a}$±0.05 | $5.37^{b}$±0.03 | $5.46^{b}$±0.00 |
| Day 90 | $5.04^{a}$±0.01 | $5.21^{ab}$±0.014 | $5.13^{a}$±0.16 | $5.45^{b}$±0.06 |
| ***Salmonella* and *Salmonella* like microbes** | | | | |
| Day 0 | 1.91±0.09 | 1.97±0.15 | 1.97±0.15 | 1.91±0.048 |
| Day 45 | 1.97±0.15 | 1.91±0.09 | 1.91±0.09 | 2.06±0.06 |
| Day 90 | 1.91±0.09 | 2.00±0.00 | 1.91±0.08 | 1.91±0.09 |

Means with different superscript in a row differ significantly ($P$<0.05)

## Conclusion

It is evident that microbial load inside the bio-digester depends on crucial factors of growth like temperature, pH, amount of substrate as well as competitive inhibition of among methanogens and other microbes. From the present study, it can be concluded that AD technology assists in the pathogen reduction present in the bio-waste and make safe bio-fertilizer as a by-product of this technology

## References


1. Avery LM, Anchang KY, Tumwesige V, Strachan N, Goude PJ. Potential for pathogen reduction in anaerobic digestion and biogas generation in Sub-Saharan Africa. biomass and bioenergy. 2014;70:112-124.
2. Bagge E, Sahlström L, Albihn A. The effect of hygienic treatment on the microbial flora of biowaste at biogas plants. Water Research. 2005;39(20):4879-4886.
3. Basic Animal Husbandry and Fisheries Statistics. Department of Animal Husbandry, Dairying and Fisheries. Ministry of Agriculture, Govt of India, New Delhi, 2020.
4. Bhatia RK, Ramadoss G, Jain AK, Dhiman RK, Bhatia SK, Bhatt AK. Conversion of waste biomass into gaseous fuel: present status and challenges in India. Bio Energy Research, 2020;13(4):1046-1068.
5. Chen C, Guo W, Ngo HH, Lee DJ, Tung KL, Jin P, *et al*. Challenges in biogas production from anaerobic membrane bioreactors. Renew. Energy. 2016;98:120-134.
6. Jenkins SR, Armstrong CW, Monti MM. Health effects of biosolids applied to land: Available scientific evidence. Virginia Department of Health, 2007.
7. Kaur G, Brar YS, Kothari DP. Potential of livestock generated biomass: Untapped energy source in India. Energ. 2017;10(7):847.
8. Makara A, Kowalski Z, Lelek Ł, Kulczycka J. Comparative analyses of pig farming management systems using the Life Cycle Assessment method. J. Clean. Prod. 2019;241:118305
9. Poudel RC, Joshi DR, Dhakal NR, Karki AB. Evaluation of hygienic treatment of biowastes by anaerobic digestion in biogas plants. Nepal Journal of Science and Technology. 2009;10:183-188.
10. Ramírez-Islas ME, Güereca LP, Sosa-Rodriguez FS, Cobos-Peralta MA. Environmental assessment of energy production from anaerobic digestion of pig manure at medium-scale using life cycle assessment. Waste Manage. 2020;102:85-96.
11. Sahlström L, Bagge E, Emmoth E, Holmqvist A, Danielsson-Tham ML, Albihn A. A laboratory study of survival of selected microorganisms after heat treatment of biowaste used in biogas plants. Bioresource Technology. 2008;99(16):859-7865.
12. Smith SR, Lang NL, Cheung KHM, Spanoudaki K. Factors controlling pathogen destruction during anaerobic digestion of biowastes. Waste Management. 2005;25(4):417-425.
13. Xiao Y, Yang H, Yang H, Wang H, Zheng D, Liu Y, *et al.* Improved biogas production of dry anaerobic digestion of swine manure. Bioresource Technology. 2019;294:122188.