

Biochemical Methane Potentials and Organic Matter Degradation of Swine Slurry under Mesophilic Anaerobic Digestion

Arumuganainar Suresh^{1,2*}, Hong Lim Choi², Nallakumar Kannan¹ and Kalyanaraman Rajagopal¹

¹Department of Biotechnology, School of Life Sciences, Vels Institute of Science, Technology and Advanced Studies, Chennai - 600117, Tamil Nadu, India; blueyellowsnu@gmail.com, ponkannanbt@gmail.com, hodbiotechnology@velsuniv.org
²Department of Agricultural Biotechnology and Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul, 151-921, Republic of Korea; ulsoo8@snu.ac.kr

Abstract

Background/Objectives: Swine slurry is generally used as raw liquid fertilizer and leads environmental pollution. Therefore to overcome that, anaerobic digestion before its field application would reduce pollution and give bioenergy. **Methods/Statistical Analysis:** The dynamics of biochemical parameters in swine slurry are not fully evaluated in anaerobic system. In this study, basic changes in physico-biochemical character (pH, EC, solids, organic matters, nutrients, heavy metals, pathogens and methanogens) of swine slurry under mesophilic anaerobic digestion using batch system is evaluated. **Findings:** During mesophilic anaerobic digestion of swine slurry was shown the removal rate of organic matters at 85% and 75% in terms of BOD₅ and SCOD_{cr}. The pathogens of *Salmonella* and fecal coliforms removed at 100% and 97%. Interestingly, the nutrients contents were increased at 19% and 12% in terms of NH₃-N and available phosphorous, respectively. The biochemical methane potentials of swine slurry was observed at 236 L/kg COD_{added}' and 307 L/kg VS_{added}' respectively. The methane accounted for 54.3% of the biogas produced with the dominant population of *Methanosarcina* sp. **Conclusion/Improvements:** It is concluded that the mesophilic anaerobic digester is greatly desirable for swine slurry with regards of bioenergy, ecofriendly liquid biofertilizer production and significant biodegradability of organic waste.

Keywords: Anaerobic Digestion, Biogas, Biomethane, Pathogens, Swine Slurry

1. Introduction

Swine waste is one of the single-largest components of the organic waste stream produced at the rate of kg/day/1000-kg animal unit from 1.2 billion pig population in the world¹. The Swine waste includes feces and urine that is diluted with rainfall or cleaning water may contain a small proportion of remains of feeds². The most part of the world, this organic waste is disposed in landfill and sullied the environment. In light of rapidly increasing public health concerns, environmental quality biodegradation, costs associated with energy supply for waste disposal, the conversion of swine wastes to energy is becoming a more economically viable practice. Swine

wastes can be slight variable depending on their sources. Some characteristics of swine wastes that have been reported in the literature^{3,4}, indicating total solids ranges 0.6–12.6%, Volatile Solids to total Solids Ratio (VS/TS) of 57–84%, and carbon to nitrogen and phosphorous ratio (COD:N:P) of 15:1.2:0.7. Due to relatively high moisture content of swine waste, bioconversion technologies, such as anaerobic digestion, are more suitable compared to thermochemical conversion technologies, such as combustion and gasification.

Anaerobic digestion is becoming more and more attractive for the treatment of high strength organic wastes such as swine manure⁵⁻⁹, dairy manure¹⁰, poultry waste¹¹ and paddy straw¹², since it produces renewable

* Author for correspondence

energy, methane, and valuable digested residues, liquid fertilizer and soil conditioner. In spite of these advantages, an anaerobic digester for treating swine manure has not been attractive in the world, due to lack of process dynamics, improper process design and frequent operation failures¹³⁻¹⁵. In addition, a strong demand for renewable energy generation has gradually increased the interest in anaerobic digestion technology¹⁶. Moreover the physical, chemical characteristics and biochemical dynamics of the organic waste are important information for designing and operating anaerobic digesters, because they affect biomethane production and process stability during anaerobic digestion.

The biodegradability of a feedstock is indicated by the biomethane yield and percentage of solids (total solids or volatile solids) that are destroyed in the anaerobic digestion. The methane yield is measured by the amount of methane produced per unit of volatile solids in the feedstock after subjecting it to anaerobic digestion for a sufficient amount of time under a given temperature. Author in¹³ determined the methane yield was 403 mL/gVS swine wastes at 35°C and 20 days of digestion time. Which correspond to 65% of the stoichiometric methane yield, based on elemental composition of raw materials and not studied other biochemical dynamics during anaerobic digestion.

Moreover, extensive literature search showed that little information is available on the biochemical dynamics and biodegradability of swine waste under mesophilic conditions. Some researchers suggested that an increase in the temperature resulted in a reduction of the methane yield, due to the increased inhibition of free ammonia (NH₃) which increases with increasing temperature^{5,8}. Therefore in this study we selected mesophilic temperature to evaluate the biochemical dynamics of swine waste under anaerobic condition to assess its potential as feedstock for anaerobic digester. This study was initiated to examine the feasibility of converting the swine waste into biomethane energy.

2. Materials and Methods

2.1 Experiment Setup

The purpose of this study was to obtain basic trend of biochemical changes of swine waste under mesophilic anaerobic batch system. For this study, 150 mL serum bottles were used at 50 mL of working volume with 50% seed as inoculum. Seed was collected from working

mesophilic anaerobic digester (15m³) at Seoul National University livestock farm, Suwon. Bottles were closed with butyl rubber stoppers and sealed using aluminum seal, then flushed with N₂ gas for 1 min to remove air contamination. Subsequently bottles were incubated at 35°C at 100rpm for 30days.

2.2 Gas Sample Quantification and Analysis

Only two bottles were removed every 5days, but gas samples were quantified in all bottles with 100 mL glass syringe equipped with 23-gauge needles. The glass syringe was lubricated with deionized water before measurement. The syringe was held horizontal for measurement and volume determinations were made by allowing the syringe plunger to move (gently twirling to provide freedom of movement) and equilibrate between the bottle and atmospheric pressures. Readings are verified by drawing the plunger past the equilibrium point and releasing, the plunger should return to the original equilibration volume. The gas samples were collected in Tedlar bag so that other incubation bottles were free from the gas pressure. Some amount of gas samples were checked for flame test using glass syringe and ignited with lighter. The gas samples were analyzed CH₄ and CO₂ by gas chromatography using 60/80 Carboxen-1000 packed column with TCD detector at 250°C. The 0.6mL gas sample was injected, at 50°C inlet temperature, and oven temperature at 35°C (5 min) to 225°C at 20°C/min. The helium was used as carrier gas at 30 mL/min and standard gas mixtures (Supelco Cat. No. 501697) were used for calibration.

2.3 Physico-Biochemical Analysis

The sample pH and Electrical Conductivity (EC) were measured using a pH meter (Inolab, WTW, GmbH, Weilheim, Germany), and an EC meter (EC214, Hanna Instruments, Ltd., Sarmeola di aarubano, Italy). During EC and pH particular attention was paid to the previous homogenization and mixing of the sample. TS, VS, TSS, and BOD₅ were determined as per APHA¹⁷. COD_{cr}, SCOD_{cr}, TN, NH₃-N, and TP were analyzed using HACH (DR 5000) chemicals methods. For heavy metals (As, Cd, Cr, Co, Ni, Pb) and micronutrients (Na, K, Cu, Fe, Mg, Mn, Zn, Al), 10 mL of the sample was first digested with concentrated nitric acid (APHA, 2005), subsequently, the solution was made up to 100 mL in a volumetric flask and quantitatively analyzed by Inductively Coupled Plasma (ICP) Atomic Emission Spectroscopy (AES) (ICPS-7510, Shimadzu Corp., Kyoto, Japan).

2.4 Microbiological Analysis

Samples were analyzed for fecal coliforms and *Salmonella* to check pathogens reduction during mesophilic anaerobic digestion. 10 mL aliquot of a well mixed slurry sample was first mixed with 90 mL of sterile Ringer solution (NaCl 2.25 g/L, KCl 0.105 g/L, CaCl₂ 0.045 g/L, NaHCO₃ 0.05 g/L, and citric acid 0.034 g/L) and subsequently serially diluted up to 10⁻⁹. For determination of fecal coliforms, membrane filtration method using M FC agar (Merck, USA) plates and incubated at 44.5 ± 0.2°C for 24 h. For determination of *Salmonella* count, 3-tube MPN method was followed (APHA, 2005). Selenite cystine broth was used for enrichment, while *Salmonella shigella* (SS) agar and Triple Sugar Iron (TSI) agar (Difco) were used for confirmation. For Methanogens, the samples were placed under a fluorescence photomicroscope (Axiophot, Zeiss, Germany), and their fluorescence and morphology were observed.

3. Results and Discussion

3.1 Dynamics of Physico-Biochemical Parameters

pH and EC of the swine waste was increased from 6.82 to 7.49 and 10.6 to 13 mS cm⁻¹ during mesophilic anaerobic digestion for 30 days, respectively (Table 1. and Figure 1). Ammonium is released during the anaerobic hydrolysis of organic nitrogen compounds (proteins), causing an increase of the pH value¹⁸. In our experiment the ammonia nitrogen was increased from 1480 to 1760 mg l⁻¹. In anaerobic digestion for biomethane production, acid-forming bacteria needs pH around 5.0 for better degradation, but methane forming bacteria does not grow below 6.2¹⁹. However anaerobic digester performs well within a pH range of 6.8-7.2. In case of EC, the increase due to the ionic nutrients (NH₃-N) rise in anaerobic digestion (Table 1). These cations are dependable for EC elevation in the effluents. Thus effluent could be used as a quality bio-liquid fertilizer.

The average TS content was 2% and its liquid form could be pumped easily into the digester. The SCOD_{Cr} proportion was observed to be almost 59% of TCOD_{Cr} (3.1%), which is higher than that found in other animal waste. Moreover, the average VS/TS ratio was observed

at 73%, hence swine slurry is an excellent feedstock for an anaerobic digester for biomethane production. The kinetics of solids contents were observed (Figure 2.), and calculated the removal percentage of 22%, 32% and 49% on TS, VS and TSS, respectively. Interestingly, the solids contents were decrease from 0 day to 20 days, and then it slightly increased on 25 day, which might be the anaerobic bacterial growth.

Similar to the solids parameters, drastic changes were also observed in COD_{Cr} and BOD₅ (Figure 3.), between the influent (3.1% and 1.04%, respectively) and effluent (1.5% and 0.16%), respectively. Based on the mesophilic batch system, the biodegradability of organic matter was evaluated for the swine waste as 22%, 32%, 52%, 73% and 85% of TS, VS, COD_{Cr}, SCOD_{Cr} and BOD₅, respectively (Figure 4).

Table 1. The swine slurry composition of before and after anaerobic digestion in a batch system at mesophilic condition

Parameters	Units	0 day (Influent)	30 days (Effluent)
pH		6.82	7.49
EC	mS/cm	10.6	13
TS	mg/L	19730	15480
VS	mg/L	14405	9810
TSS	mg/L	10700	5500
BOD	mg/L	10440	1588
COD _{Cr}	mg/L	31125	14900
SCOD	mg/L	12800	3450
Total N	mg/L	1750	1770
NH ₃ -N	mg/L	1480	1760
Total P	mg/L	2740	2700
Avi.P	mg/L	900	1010
TK	mg/L	115	116
Ca	mg/L	58	53
Na	mg/L	26	26
Mg	mg/L	29	25
Fe	mg/L	9.2	7.9
Zn	mg/L	2.32	2.06
Al	mg/L	2.74	2.59
Mn	mg/L	1.27	1.07
Cu	mg/L	1.27	1.26
Fecal coliforms	CFU/mL	290	<10
<i>Salmonella</i>	MPN/mL	960	<0.03

CFU, colony forming unit; MPN, most probable number

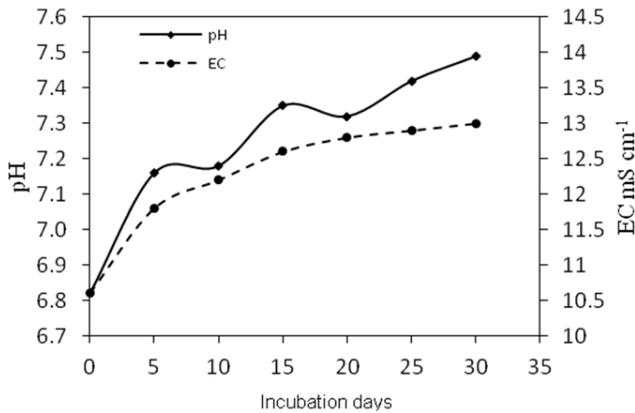


Figure 1. Kinetics of pH and EC in swine slurry during mesophilic anaerobic digestion.

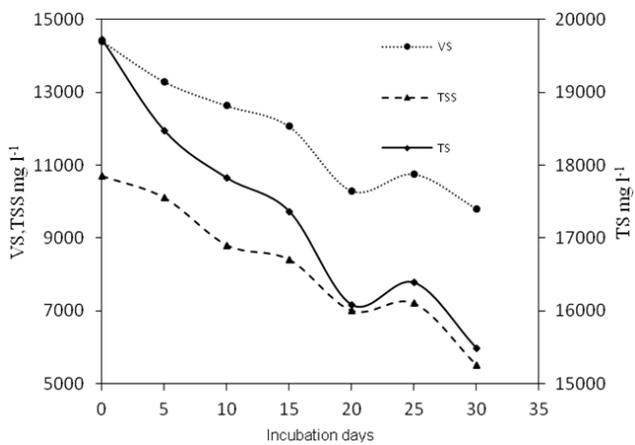


Figure 2. Kinetics of solids in swine slurry during mesophilic anaerobic digestion.

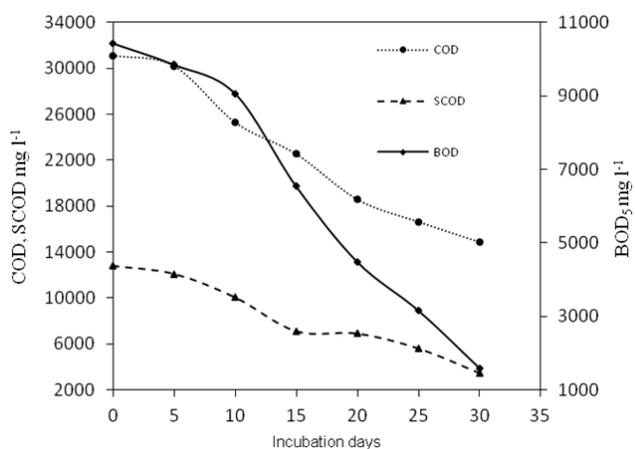


Figure 3. Kinetics of chemical and biochemical oxygen demand in swine slurry during mesophilic anaerobic digestion.

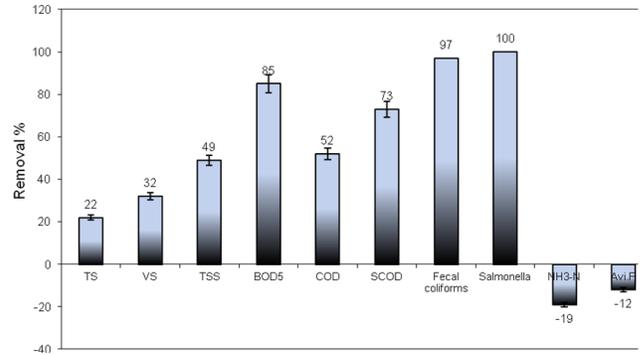


Figure 4. Removal percentage of various physico-biochemical parameters and pathogens during mesophilic anaerobic digestion of swine slurry.

3.2 Dynamics of Nutrients and Heavy Metals

The contents of various nutrient elements in the swine waste are shown in Table 1. The macro and micronutrients are very essential for bacterial growth in the digester, therefore the nutrients concentration are very important to observe. Swine slurry was observed as COD/NH³-N ratio of 21, which is most favorable for anaerobic biogas production. Interestingly, the ammoniacal nitrogen (NH³-N) and available phosphorous content was increased at 19% and 12% after anaerobic digestion, respectively. This ionic nutrient source could be used as liquid fertilizer in soil. In addition metals and other microelements were also analyzed, since the total concentration of each of these nutrients will not change significantly during the digestion hence the digester effluents would provide the essential elements for plant growth if they are used as organic fertilizers²⁰.

3.3 Biogas Production and Composition

The biogas and methane yield during mesophilic digestion of swine waste were shown in Figures 5 and 6. The total biogas and methane amount was quantified at 666 and 307 ml g⁻¹ VS added. The biogas production severely decreased from 25 to 30 days of incubation due to exhaustion of organic matter. The maximum biogas and methane was observed at 165 ml and 82 ml g⁻¹ VS added on 15th day of incubation, respectively. The biogas composition during mesophilic digestion of swine slurry was shown in Figure 6. Almost constant methane content was obtained at 54.3% on 15 to 25 days of incubation while CO₂ revealed at 20.3%. Thus an average energy content of 20.3 MJ/m³ could be estimated for the biogas produced

from swine waste based on 54.3% methane content and 37.3 MJ/m³ energy content of methane. However, the biogas produced from the swine slurry was lower compare with other studies¹³. They observed 403 ml methane g⁻¹ VS added with 65.3%. The flame test was performed with 100 ml syringe, which showed blue stable flame (picture not shown). The steady state of biogas production was observed on 15 to 25 days at the average of 139 ml g⁻¹ VS added with 54.3% methane and estimated production rate of 179 ml CH₄/L/day. This may suggest that the swine waste used in the experiments had an optimal digestion rate on mesophilic condition.

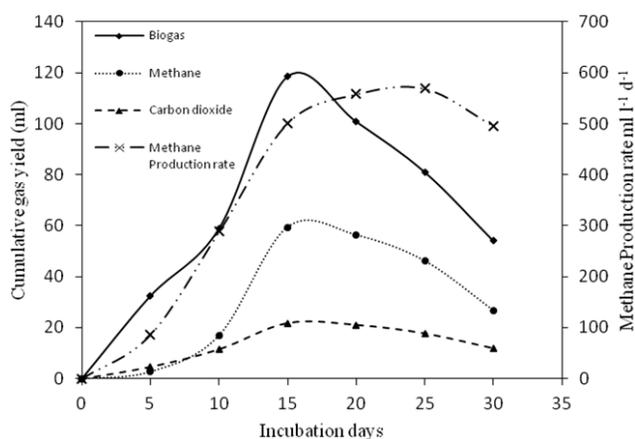


Figure 5. Biogas yield of swine slurry during mesophilic anaerobic digestion.

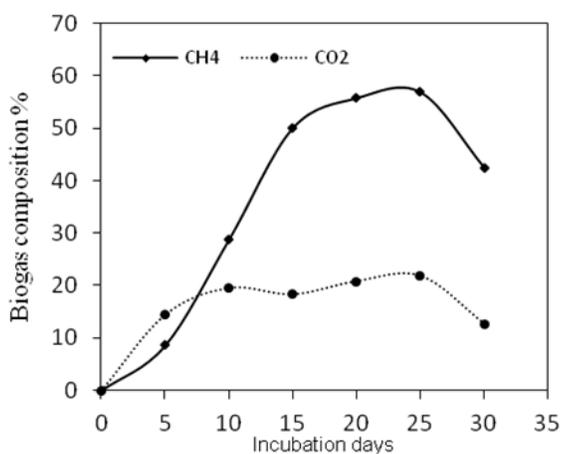


Figure 6. Biogas composition of swine slurry during mesophilic anaerobic digestion.

3.4 Dynamics of Microbial Parameters

In the case of pathogens, *Salmonella* were removed

completely (100%) in the effluent and 97% for fecal coliforms (Table 1, Figures 4. and 7.), and the result was comparable to previous studies^{21,22}. However, In²³ reported that only thermophilic digesters can execute pathogens removals like fecal coliforms, not mesophilic digesters, which is contradictory to our study. It is believed that a longer HRT (>25 days) may kill all pathogens, even when operating at mesophilic temperatures. This suggests that the anaerobic digestion of swine slurry could reduce pathogens before its application as liquid fertilizers. The 15th day digested samples was observed for methanogens populations and it showed high intensity fluorescence (blue to green), and mostly packed cocci (Figure 8), indicating that the swine waste anaerobic system contains and support *Methanosarcina spp.*, which were dominant in the system.

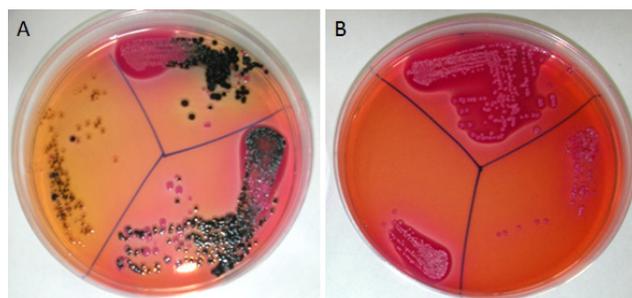


Figure 7. *Salmonella* count in the swine slurry before (A, influent) and after (B, Effluent) mesophilic anaerobic digestion. The black colour colonies are *Salmonella*.

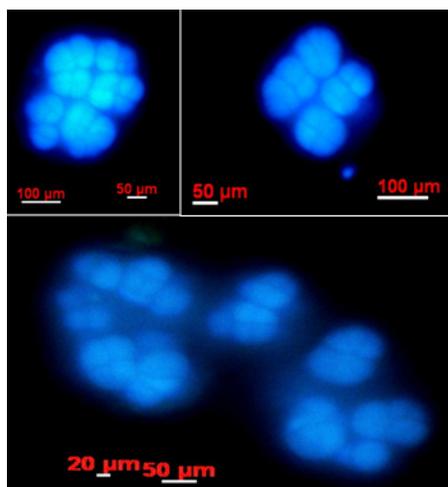


Figure 8. Epifluorescence micrograph of methanogens (*Methanosarcina spp*) population in mesophilic anaerobic digestion of swine slurry.

4. Conclusion

The mesophilic anaerobic digestion of swine slurry significantly produced biomethane, increased available N and P, and reduced organic waste and pathogens. The mesophilic anaerobic system showed that the swine slurry had biochemical methane potential of 236 L/kg COD_{added} and 307 L/kg VS_{added}, respectively. The methane accounted for 54.3% of the biogas produced, and the methane production rate was 179 ml CH₄/L/day. Furthermore, the reduction of the organic waste and pathogens were outstanding; therefore, the effluent can be used as an eco-friendly liquid biofertilizer. In conclusion, the basic design and process criteria of a mesophilic anaerobic system would be highly desirable for the anaerobic digestion of swine waste.

5. Acknowledgement

This research was supported by a grant from Korea Environmental Industry and Technology Institute (KEITI), Republic of Korea.

6. References

1. USDA. Foreign Agricultural Service, Swine Summary Selected Countries. Available from: <http://www.fas.usda.gov/psdonline/psdReport.aspx?hidReportRetrievalName=Swine+Summary+Selected+Countries&hidReportRetrievalID=1649&hidReportRetrievalTemplateID=7>
2. Pain B, Menzi H. Recycling agricultural, municipal, and industrial residues in agriculture network. In Glossary of Terms on Livestock Manure Management 2003. Zollikofen, Switzerland: Swiss College of Agriculture; 2003.
3. Barker JC, Overcash MR. Swine waste characterization: A review. Transactions of the ASABE. 2007; 50(2):651–7.
4. Suresh A, Choi HL, Lee JH, Zhu K, Yao HQ, Choi HJ, Moon OK, Park CK, Kim JJ. Swine slurry characterization and prediction equations for nutrients on South Korean farms. Transactions of the ASABE. 2009; 52(1):267–73.
5. Angelidaki I, Ahring BK. Anaerobic thermophilic digestion of manure at different ammonia loads: Effect of temperature. Water Res. 1994; 28(3):727–31.
6. Angelidaki I, Ahring BK. Methods for increasing the biogas potential from the recalcitrant organic matter contained in manure. Water Science and Technology. 2000; 41(3):189–94.
7. Bonmati A, Flotats X, Mateu L, Campos E. Study of thermal hydrolysis as a pretreatment to mesophilic anaerobic digestion of pig slurry. Water Science and Technology. 2001; 44(4):109–16.
8. Hansen HH, Angelidaki I, Ahring BK. Improving thermophilic anaerobic digestion of swine manure. Water Research. 1999; 33(8):1805–10.
9. Anriansyah R, Choi HL, Suresh A. Underground anaerobic digester to solve the energy balance problem in temperate regions: A pilot study. Applied Engineering in Agriculture. 2015; 31(4):643–51.
10. Vivekanandan S, Kamaraj G. The study of biogas production from rice chaff (karukka) as co-substrate with cow dung. Indian Journal of Science and Technology. 2011; 4(6):657–9.
11. Iyovo GD, Du G, Chen J. Sustainable biomethane, biofertilizer and biodiesel system from poultry waste. Indian Journal of Science and Technology. 2010; 3(10):1062–9.
12. Phutela UG, Sahni N, Sooch SS. Fungal degradation of paddy straw for enhancing biogas production. Indian Journal of Science and Technology. 2011; 4(6):660–5.
13. Chae KJ, Jang A, Yim SK, Kim IS. The effects of digestion temperature and temperature shock on the biogas yields from the mesophilic anaerobic digestion of swine manure. Bioresource Technology. 2008; 99(1):1–6.
14. Hawkes DL. Factors affecting net energy production from mesophilic anaerobic digestion. In: Anaerobic Digestion. Stavord DA, Wheatley BI Hughes DE, editors. London, UK: Applied Science Publishers Ltd; 1980.
15. Fischer JR, Iannotti EL, Durand J. Anaerobic animal manure. In: Agriculture and Energy, Alternative Energy in Agriculture. Goswami I, Yogi D, editor. Florida, USA: CRC Press, Inc; 1986.
16. Van Lier JB, Tilche A, Ahring BK, Macarie H, Moletta R, Dohanyos M, Pol LWH, Lens P, Werstraete W. New perspectives in anaerobic digestion. Water Science and Technology. 2001; 43(1):1–18.
17. Eaton AD, Ann HM. Franson. APHA. Standard Methods for the Examination of Water and Wastewater. 21st ed. Washington DC, USA: American Public Health Association/American Water Works Association/Water Environment Federation; 2005.
18. Seyfried ATV. Technologische Beurteilungskriterien zur anaeroben Abwasserbehandlung (Technological appraisal factors for anaerobic wastewater treatment), 2. Arbeitsbericht des Fachausschusses 7.5, Korrespondenz Abwasser. 1993; 40(2):217–23.
19. Gerardi MH. The microbiology of anaerobic digester. Hoboken, New Jersey: John Wiley and Sons; 2003.
20. Lusk P. Methane recovery from animal manures the current opportunities casebook. Golden, CO: National Renewable Energy Laboratory. 1998; Available at: <http://www.nrel.gov/docs/fy99osti/25145.pdf>
21. Cheunbarn T, Pagilla KR. Aerobic thermophilic and anaerobic mesophilic treatment of sludge. Journal of Environmental Engineering. 2000; 126(9):790–95.
22. Pagilla KR, Kim H, Cheunbarn T. Aerobic thermophilic and anaerobic mesophilic treatment of swine waste. Water Research. 2000; 34(10):747–53.
23. Zabranska J, Dohanyos M, Jenicek P, Zaplatilkova P, Kutil J. The contribution of thermophilic anaerobic digestion to the stable operation of waste water sludge treatment. Water Science and Technology. 2002; 46(4-5):447–53.