POTENTIAL OF BIOGAS PRODUCTION FROM SWINE MANURE SUPPLEMENTED WITH GLYCERINE WASTE

ODORICO KONRAD[1], FABIO F. KOCH[2], MARLUCE LUMI[3],
JAQUELINE F. TONETTO[4], ALBERTO BEZAMA[5]

ABSTRACT: In this study, was studied the biogas generation from swine manure, using residual glycerine supplementation. The biogas production by digestion occurred in the anaerobic batch system under mesophilic conditions (35°C), with a hydraulic retention time of 48 days. The experiment was performed with 48 samples divided into four groups, from these, one was kept as a control (without glycerin) and the other three groups were respectively supplemented with residual glycerine in the percentage of 3%, 6% and 9% of the total volume of the samples. The volume of biogas was controlled by an automated system for reading in laboratory scale and the quality of the biogas (CH_4) measured from a specific sensor. The results showed that the residual glycerine has high potential for biogas production, with increases of 124.95%, 156.98% and 197.83% in the groups 3%, 6% and 9%, respectively, relative to the sample control. However, very high organic loads can compromise the process of digestion affecting the quality of the biogas generated in relation to methane.

KEYWORDS: biogas, residual glycerin, swine manure, renewable energy.

POTENCIAL DE PRODUÇÃO DE BIOGÁS ATRAVÉS DE DEJETOS SUÍNOS SUPLEMENTADOS COM GLICERINA RESIDUAL

RESUMO: Neste trabalho, estudou-se a geração de biogás a partir de dejetos suínos utilizando suplementação de glicerina residual. A produção de biogás ocorreu por meio de digestão anaeróbia no sistema em batelada sob condições mesofílicas (35°C), com tempo de retenção hidráulica de 48 dias. O experimento foi realizado com 48 amostras divididas em quatro grupos; destes, um se manteve como controle (sem adição de glicerina), e os outros três grupos foram suplementados, respectivamente, com glicerina residual nos percentuais de 3%, 6% e 9% do volume total das amostras. O volume de biogás gerado foi controlado por um sistema automatizado para leitura em escala laboratorial, e a qualidade do biogás (CH_4), medida a partir de um sensor específico. Os resultados obtidos demonstraram que a glicerina residual tem potencial para suplementar a produção de biogás, com incrementos de 124,95%, 156,98% e 197,83% nos grupos 3%, 6% e 9%, respectivamente, em relação à amostra-controle. Porém, cargas orgânicas muito elevadas podem comprometer o processo de biodigestão, afetando a qualidade do biogás gerado em relação ao metano.

PALAVRAS - CHAVE: biogás, glicerina residual, dejetos suínos, energia renovável.

[1] Dr. mont. Engenharia Ambiental e Sanitária, Prof. Doutor, Centro de Ciências Exatas e Tecnológicas, UNIVATES/Lajeado – RS, Fone: (51) 3714-7000 r.5201, okonrad@univates.br;
[2] Gestor ambiental, Mestre em Ambiente e Desenvolvimento, Programa de Pós Graduação em Ambiente e Desenvolvimento, UNIVATES/Lajeado - RS, fabio.fernades@naturovos.com.br;
[3] Graduanda em Eng. Ambiental, Centro de Ciências Exatas e Tecnológicas, UNIVATES/Lajeado – RS, marlucelumi@gmail.com;
[4] Bióloga, Mestranda em Ambiente e Desenvolvimento, Programa de Pós Graduação em Ambiente e Desenvolvimento, UNIVATES/Lajeado - RS, jaquetonetto@gmail.com;
[5] Dr.mont. Dipl.-Ing., Researcher, Department of Bioenergy, Helmholtz Centre for Environmental Research - UFZ, Leipzig, Germany, alberto.bezama@ufz.de.
Recebido pelo Conselho Editorial em: 25-4-2013
Aprovado pelo Conselho Editorial em: 6-5-2014

INTRODUCTION

In recent years, the residual glycerin was extensively tested in anaerobic co-digestion of various organic substrates for energy production through the biogas production. This practice has become increasingly common, especially in some European countries, like Germany, which has subsidized policy for production and use of renewable energies (BAIRD & CANN, 2011).

The anaerobic digestion is an example of the process of transformation of biomass in energy that can be applied as a treatment of organic wastes of animal or vegetable origin, resulting in the biogas, and significant reduction in emissions of greenhouse gases (DEUBLEIN & STEINHAUSER, 2008; XAVIER JR & LUCAS, 2010).

Biogas units can contribute significantly to sustainable development in rural areas, as well provide to farmers new income opportunities (Diretiva 2009/28 / EC). The biogas generation plants are economically viable according to CERVI et al. (2010), but this viability depends on some factors such as the technical dimension of energy to be used and the energy supply proposal.

Pretreatments (chemical, thermal, ultrasonic and enzymatic) can be applied to increase biogas production and / or waste can be co-digested with other wastes for synergistic effects and make the anaerobic digestion process profitable (SILES et al., 2009). The treatment of supplemented waste with crude glycerin is a very attractive option for rural areas (CASTRILLÓN, et al., 2013).

Anaerobic reactors can be used for biodigestion of different biodegradable substrates with glycerin supplementation targeting power generation through methane, the main biogas constituent. The methane generated from organic waste, is a renewable source of energy and reduced pollution potential, if compared to fossil fuels, primarily responsible for the emission of greenhouse gases. (BAIRD & CANN, 2011).

The industrial biodiesel generates millions of tons of crude glycerin residue each year (SILES et al. 2009), and therefore, biogas production can be optimized through the use of inoculum and glycerin as a carbon source, mainly nitrogen-rich substrates. The inoculum consists of using part of the material that has already passed through the biodigestion process, through the reincorporation of microorganisms adapted to the environment, which favors the efficiency of the process (XAVIER JR & LUCAS, 2010;. ROBRA et al, 2010).

Thus and to consort the use of different substrates for biogas production, tests on mesophilic conditions were made in order to determine the proper dosage of residual glycerin to be added to swine manure in different percentages (3%, 6% and 9%), evaluating the biogas quality in terms of methane composition as well the volume of biogas generated per assay.

MATERIALS AND METHODS

The swine manure was collected in breeding farm of a rural property in Vale do Taquari region in Rio Grande do Sul, and the glycerin used in the study is from the biodiesel production in the same state. The study was conducted at the Laboratory of Bioreactor UNIVATES in Lajeado/ RS.

To conduct the study, were prepared 48 samples of 600 mL each, composed of 30% inoculum and 70% of swine manure, divided into four representative groups, as follows: control group (no glycerin addition), 3% glycerin, 6% glycerin and 9% glycerin and the glycerin dosages were respectively of 18 mL, 36 mL and 54 mL of total volume used of sample on each reactor, as the glycerin application were divided into five distinct stages over the experiment, always respecting the time which noted the decrease in biogas production. The reactors remained packed in a bacteriological incubator, set at a constant temperature of 35°C for 48 days. According to a study by SOUZA et al. (2005), the anaerobic digestion of swine manure occurs satisfactorily to the temperature of 35°C.

The biogas quantification was performed by using the gas measurement system based on the fluid displacement (Figure 1) developed in the Laboratory of Bioreactors UNIVATES, which was based on the methodology described by VEIGA et al. (1990). The biogas volume generated was determined by the combined equation of the ideal gas, which describes that the relationship between temperature, pressure and volume of a gas is constant according to the equation below (HALLIDAY, 2009).

$$(P1.V1)/T1=(P2.V2)/T2 \qquad (1)$$

where,

P = pressure of ideal gas, hPa;

V = volume of ideal gas, L, and

T = temperature, ºC.

FIGURE 1. Specific sensor for measuring the methane concentration.

The determination of methane content existent in biogas was performed daily injecting biogas on a specific sensor to measure the methane concentration, in percentage, called Advanced Gasmitter, manufactured by PRONOVA Analysentechnik GmbH & Co (Figure 2), which had being calibrated through a standard gas.

FIGURE 2. Automated system measurement of biogás.

The determination of total nitrogen and total organic carbon were performed according to the methodology of Standard Methods for Water and Wastewater Examination (1995). The nitrogen analysis is based on the oxidation of nitrogenous organic matter, according to the Kjeldahl method, which leads to the formation of ammonium sulfate, which remains in solution. Later, proceed alkali treatment, which releases ammonia, which is conducted, under drag, from the boiling aqueous solution, into a vial suitable collector seeking subsequent titration (VOGEL, 1960; STRICKLAND & PARSONS, 1972).

The organic carbon was obtained by titrimetric procedure of oxidation-reduction, which consists of the oxidation of organic carbon contained in a given volume of sample, with 0.1 N solution of potassium dichromate in an acidic medium, followed by titration of the excess of dichromate with 0.05 N ammonium ferrous sulphate solution, using ferroin solution as indicator (STRICKLAND & PARSONS, 1972).

The determination of total and volatile solids was obtained by gravimetric method using analytical balance, heater for 24 hours at 105°C (total solids) and muffle for 8 hours at 550°C (volatile solids) according to the methodology of the Association of Official Analythical Chesmitry, Official Methods of analysis (1995). For the determination of pH, it was used a pH meter Digimed, model DM-20, whereas for the determination of biological oxygen demand, it was used the equipment Oxitop Wtw model IS6 by dilution (sample volume: 22.7 ml and dilution factor: 100) and incubated at 20°C for 5 days.

RESULTS AND DISCUSSION

SANTOS et al. (2007) evaluated the digestion of swine manure for 120 days and concluded that most organic matter removal occurs between the 30th and the 60th day of storage. In this regard, the experiment proved to be able to reduce the BOD on 81.8% for the period of 48 days, as shown in Table 1, in accordance with the results identified by RODRIGUES et al. (2010) during the evaluation of the performance of UASB reactor for the treatment of wastewater of suine culture.

It stands out that the glycerine supplementation increased values of BOD in the essays of 3%, 6% and 9%, due to the residual carbon not consumed during biodigestion, with emphasis to the group of 6% and 9% of glycerin which has presented a final BOD higher than observed in the initial sample.

A particularly important aspect for the performance of anaerobic digestion is the carbon/ nitrogen (C/N) relation. Waste with high nitrogen content must be digested preferably with residues that have a low content of nitrogen and at the same time have an adequate presence of organic carbon, allowing to the biological metabolism to proper C/N ratio of 30/1 (ALVAREZ & LIDÉN, 2007). It is observed that the initial sample showed C/N ratio of 8.75/1, something quite unbalanced, and potentially could have compromised the biological metabolism and consequently, the consumption of carbon available, but also can observe from Table 1 that the C/N ratio has decreased in the control group, 3% and 6%, while in the experiment with an increase of 9% of glycerin, the final ratio was greater than the original, this is explained by the supplementation of organic charge by adding of glycerin.

The pH remained stable, in agreement with the ideal range recommended by PEREZ (2007) between 6.0 to 8.0. However, the groups that received the treatment with glycerin showed an increase of acidity, possibly due to the incorporation of fatty acids.

The total solids (TS) are characterized as the remaining material after remove the water, while the volatile solids (VS) refer to organic matter present in the liable substrate of transformation of biogas, since the greater the concentration of SV the greater the biogas yield (OLIVEIRA, 2010). According to Table 1 is visualized that in all treatments the levels of total and volatile solids decreased, and the largest reduction occurred in the SV group 3% and the lowest in the Control group. With respect to total solids, the Control sample obtained the highest reduction (16.85%), followed by groups 3% (12.3%), 9% (6.59%) and 6% (4.81%). It was observed that the low reduction of ST indicates that there was a high concentration of them in all groups after the end of the experiment. This may be related to the low C/N ratio at the beginning of the process, not happening efficient degradation of organic load present. The difference shown between the control and the supplemented groups with glycerin showed that, the same one caused an increase in finals ST.

TABLE 1. Values of C/N, BOD (mg/L O_2), pH, totals and volatile solids (%) pre-treatment and post-treatment control group, 3%, 6% and 9% glycerin by anaerobic digestion.

Parameters	Pre Treatment	After Treatment			
		Control	3%	6%	9%
C/N	8.75/1	4.60/1	4.65/1	6.62/1	10.0/1
BO_5D (mg/L O_2)	22,000	4,000	7,000	26,000	49,000
pH	6.93	8.00	7.92	6.06	6.15
Volatile solids (%)	77.25	72.55	69.44	71.85	72.50
Total solids (%)	7.89	6.56	6.92	7.51	7.37

On figure 3 visualizes the production of biogas from the control triplicate, showing at the beginning of the treatment four days of peaks of biogas generation, a reduction in the volume generated on the 5th day and the resumption of biogas generation until the 9th day. From the 11th day, the system reduced the biogas generation and maintained an average of 150 ml day^{-1}. In the experiment, the methane production in Control triplicate represented 61.4% of the total biogas generated.

Notice that in the Control group, there was a considerable fall in biogas generation from the referred date (Figure 3). It is noteworthy that the concentration of methane gas present in the biogas reached values close to 60% after the eighth day.

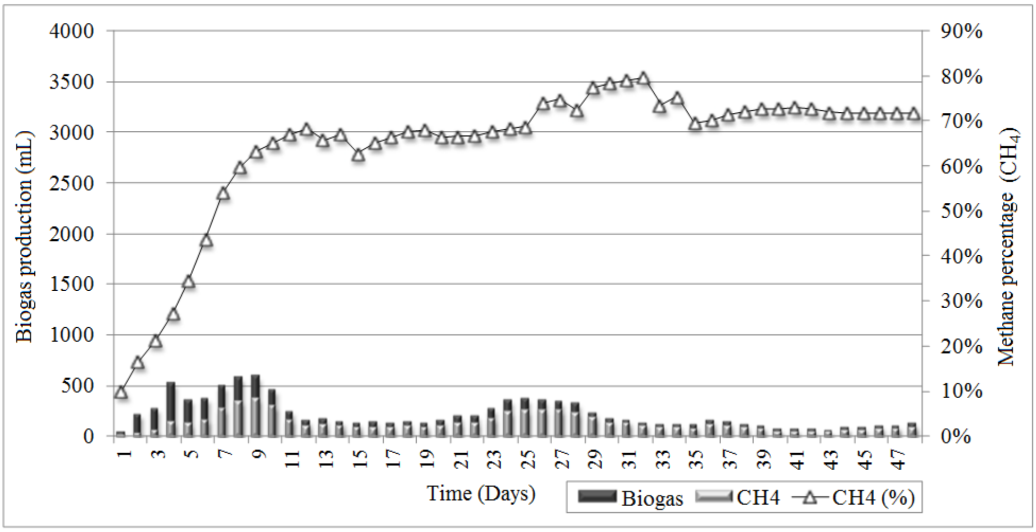

FIGURE 3. Daily biogás production from control triplicate.

The glycerin applications were made at five different times, using as reference the reduction of biogas volume, i.e., as it was observed a significant decrease in biogas production, the glycerin was supplemented to the selected experiments for this purpose (Figures 4, 5 and 6). Note that the glycerin provided an increase in the biogas production a day or two after supplementation in all groups, but also it was observed a reduction at the methane percentage after the dosage of glycerin, which returned to stabilize in subsequent days. This situation is in agreement with KOLESÁROVÁ et al. (2011), who emphasizes that the reduction of methane generation would be related to the presence of high concentrations of propionic and butyric acid, derived from a concentration of free fatty acids and a stressful situation between producers and consumers microorganisms and because according to PÉREZ (2007) the methanogens microorganisms are highly sensitive to disturbances of the process, as such as organic and hydraulic overload, and the presence of toxic or inhibitory substances.

In a study by ROBRA (2006) and BACKES (2011) also showed that the addition of glycerine increases the organic charge in the medium, causing an immediate increase in the biogas production, but then, a sudden fall may occur, as you raise the organic charge content, impairs the production and consumption of intermediate products (acetic acid, propionic, butyric, valeric and others) accumulating carbon dioxide, hydrogen and acetate.

The substrate supplementation with 9% of glycerin showed to be able to provide the best productivity in biogas volume, with 30,554 mL generated during the experiment period, whereas the samples 3% and 6% of glycerin generated 23,077.85 mL and 26,363.44 mL of biogas, respectively. However, the group that received the treatment with 6% of glycerin, presented the volume of methane higher than others treatments, with 15,117 ml, whereas for the samples supplemented with 3% and 9% of glycerine, the methane volume were respectively 14,434.63 mL and 14,631.31 mL.

Regarding the quality of biogas in terms of concentration of methane gas, the substrate undergoing treatment with 3% of glycerin showed an average grade of 62.86% with a maximum peak of 74.6%, demonstrating superior results to other groups. Treatments with 6% and 9% of glycerin showed methane content of 45.80% and 35.87%, respectively, with a maximum peak of 74.19% and 70.23%. It is noteworthy that the quality of biogas in the Control group had an average concentration of 65.45% of methane and maximum percentage of 79.5%.

It is observed that the addition of glycerin affected the quality of the biogas generated, but at the same time showed an increase in biogas generation, and the higher the percentage of glycerin added, the higher the biogas yield. Furthermore, it is noticed that the largest biogas generation does not indicate greater methane volume, as observed between samples supplemented with 9% and 6% of glycerin.

Regarding the control sample, the 3% group obtained increments in the biogas generation of 124.95% and the 6% and 9% groups, showed increments of 156.98% and 197.83%, respectively. Results obtained by AMON et al. (2006) shows that the amount of glycerin should not exceed 6% for a stable digestion process. It is possible to agree with the results of AMON et al. (2006) when comparing the amount of methane generated, however, when assessing the amount of biogas this statement does not match with the experiment presented here.

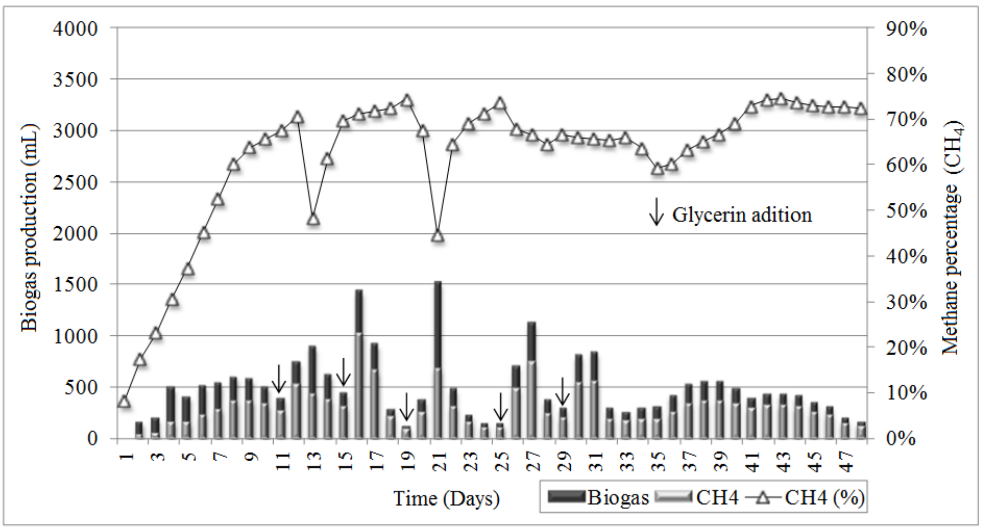

FIGURE 4. Daily biogás production from triplicate supplemented with 3% glycerin.

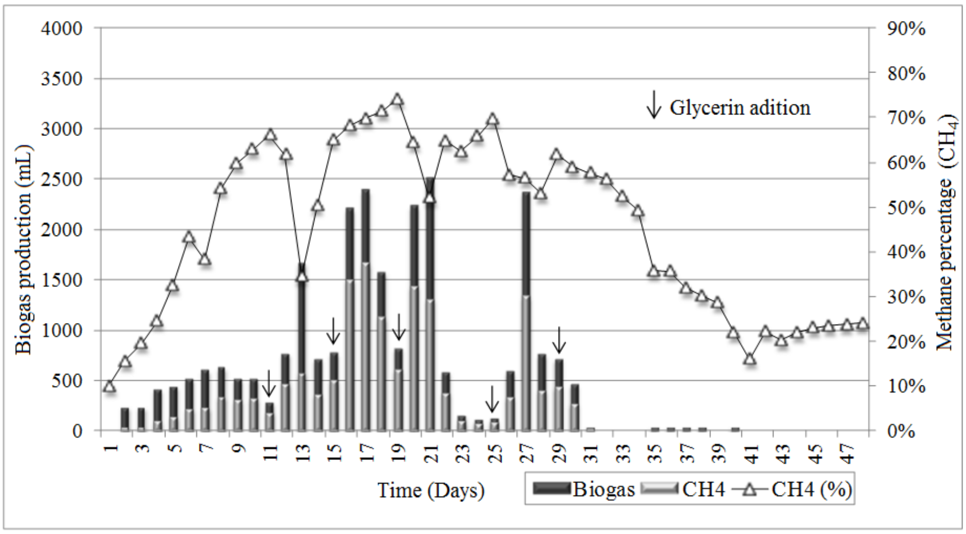

FIGURE 5. Daily biogás production from triplicate supplemented with 6% glycerin.

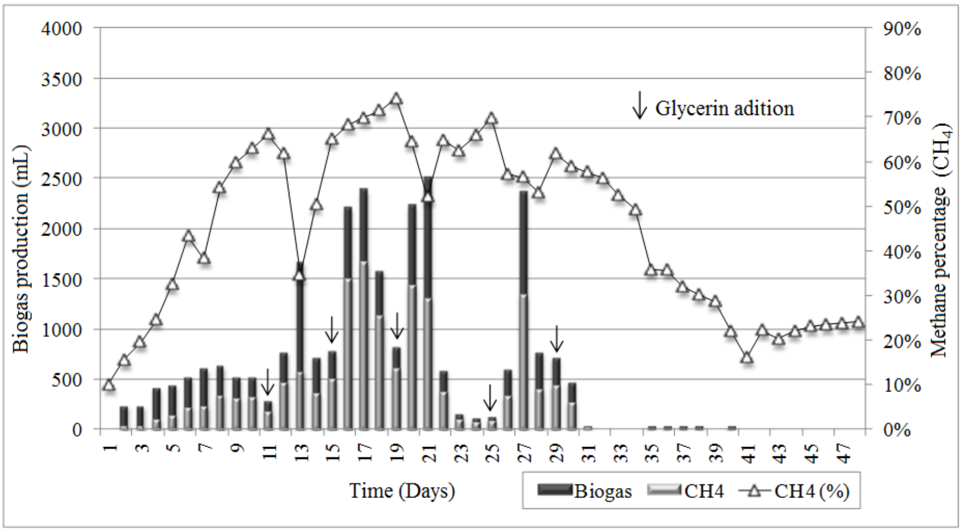

FIGURE 6. Daily biogás production from triplicate supplemented with 9% glycerin.

It was conducted a statistical analysis of the results obtained thought analysis of variance (ANOVA), for comparison test of averages of Scott-Knott ($P < 0.05$). We considered four treatments and three replicates for each of them, being made, therefore, 12 observations. It is noted, Table 2 that the samples supplemented with glycerin differs from the control sample, but statistically does not presents significantly different from each other.

TABLE 2. Variance Analysis (ANOVA) of biogas production from triplicate treatments: control, 3%, 6% and 9% of glycerin, through anaerobic digestion of pig slurry in batch system under mesophilic (35°C), and hydraulic retention time of 48 days.

Treatments	Averages	Results
Control	10013513333	a1
3% Glycerin	21526950000	a2
6% Glycerin	25778540000	a2
9% Glycerin	25862973333	a2

On Results column, letters followed by the same number does not differ.
Harmonic average of the number of repetitions (r): 3
Standard error: 2308.55377518428 C.V.(%) = 19.23 n=12

In Table 3 are shown the values of the Pearson correlation between the biogas volume and the methane percentage of treatments: Control, 3%, 6% and 9% of glycerin. There is a significant positive correlation between the biogas volume and the methane percentage on Control groups (R^2 = 82.11%, p = 0.0001) and 3% of glycerin (R^2 = 64.64%, p = 0.0001), in other words, biogas volume was directly proportional to the percentage of methane, wherein 3% of glycerin has decreased this correlation.

In treatment 6% of glycerin there was a negative correlation, although not significant (R^2 = -7.79%, p = 0.5988), this being, the highest treatment with methane production (15.117 mL). With 9% of glycerin there was a significant negative correlation (R^2 = -47.44%, p = 0.0007), in other words, variation in the percentage of methane and biogas volume are inversely proportional.

The percentage correlation of glycerin and methane observed in this study corroborates the data of KONRAD et. al (2010) and AMON et al. (2006) who describe that, the glycerin supplementation always resulted in an increased on methane production. However, is adverse to WOHLGEMUTH (2009), which states that the methanogenic activity is inhibited by adding 2% or more of glycerin.

TABLE 3. Indexes of Pearson Correlation (R^2%) and difference (p) between the volume of biogas and methane percentage of treatments: control, 3%, 6% and 9% of glycerin, through anaerobic digestion of pig slurry in batch system under mesophilic conditions (35°C), and hydraulic retention time of 48 days.

Parameters	Control (R^2 %; p)	3% Glycerin (R^2 %; p)	6% Glycerin (R^2 %; p)	9% Glycerin (R^2 % ;p)
Biogas/Methane	82.11; 0.0001	64.64%; 0.0001	-7.79; 0.5988	-47.44%; 0.0007

Pearson Correlation, significant difference ($p<0.05$).

CONCLUSION

The addition of different percentages of glycerin associated with pig manure provided a significant increase as the biogas and methane generation, being that the percentage of methane per biogas volume was inversely proportional to the amount of glycerin added to the system.

The experiment was able to demonstrate the possible use of anaerobic biodigestion technology for organic substrates of animal and vegetable origin, with a view to energy use. However, the supplementation of high glycerin charges added to the substrate may alter biological and physical-chemical phenomenon which limit the metabolic processes involved in the anaerobic digestion, affecting the biodigestion process.

In the research situation where it was evaluated the biodigestion of pig manure supplemented with residual glycerin indicates the inclusion of 6% (v/v) of residual glycerin in order to achieve better energy efficiency (percentage of methane presents in the biogas).

ACKNOWLEDGMENT

UNIVATES, CNPQ (Conselho Nacional de Desenvolvimento Científico e Tecnológico), FAPERGS (Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul), ECOCITRUS, NATUROVOS and BSBIOS.

REFERENCES

ALVAREZ, R.; LIDÉN, G. Semi-continuous co-digestion of solid slaughterhouse waste, manure, and fruit and vegetable waste. *Renewable Energy*, Oxford, v.33, n.2, p.726-734, 2007.

AMON, T.; AMON, B.; KRYVORUCHKO, V.; BODIROZA, V.; PÖTSCH, E.; ZOLLITSCH, W. Optimising methane yield from anaerobic digestion of manure: effects of dairy systems and of glycerine supplementation. International Congress Series, Amsterdam, v.1293, p. 217-220, 2006.

BACKES, M. G. *Avaliação do processo de digestão anaeróbia na geração de energia a partir de dejetos suínos e bovinos de leite com suplementação de glicerina residual bruta oriunda da produção de biodiesel*. 2011. Dissertação. (Mestrado em Ambiente e Desenvolvimento) - Centro Universitário Univates, Lajeado, 2011.

BAIRD, C; CANN, M. *Química ambiental*. 4.ed. Porto Alegre: Editora Bookman, 2011. 844 p.

CASTRILLÓN, L., FERNÁNDEZ-NAVA, Y., ORMAECHEA, P., MARAÑÓN, E., Methane production from cattle manure supplemented with crude glycerin from the biodiesel industry in CSTR and IBR. *Bioresource Technology*, Essex, v.127, p.312–317, 2013.

CERVI, R.G.; ESPERANCINI, M.ST.; BUENO, O.DE C. Viabilidade econômica da utilização do biogás produzido em granja suinícola para geração de energia elétrica. Engenharia Agrícola, Jaboticabal, v.30, n.5, p.831-844, set./out.2010

DEUBLEIN, D.; STEINHAUSER, A. *Biogas from waste and renewable resources*. Weinheim: Ed. Wiley-VCH, 2008. 443p.

HALLIDAY, D.; RESNICK, R.; WALKER, J. Fundamentos de física 2. 8. ed. Rio de Janeiro: LTC, 2009. 310p.

KOLESÁROVÁ, N.; HUTŇAN, M.; BODIK, I.; SPALKOVÁ, V. Utilization of biodiesel by-products for biogas production. *Journal of Biomedicine and Biotechnology*, Slovakia, 2011.

KONRAD, O.; HEBERLE, A.N.A.; CASARIL, C.E.; KAUFMANN, G.V.; LUMI, M.; DALL'OGLIO , M.; SCHMITZ, M. Avaliação da produção de bio**g**ás e geração de metano a partir de lodo de estação de tratamento de efluentes e glicerina residual. *Revista Destaques Acadêmicos*, Lajeado, n.4, 2010.

OLIVEIRA, P.R. Avaliação de desempenho de reator UASB no tratamento de águas residuárias na suinocultura. *Engenharia Agrícola e Ambiental*, Campina Grande, v. 14, n.1, p.94-100, 2010.

PERÉZ, F.J.M. *Comportamiento dinámico de digestores anaeróbios*. 2007. 250 f. Tese (Doutorado em Engenharia Química) – Universidade de Santiago de Compostela, Santiago de Compostela, 2007.

ROBRA, S. U*so da glicerina bruta em biodigestão anaeróbica: Aspectos tecnológicos, ambientais e ecológicos*. 2006. Dissertação (Mestrado em Desenvolvimento Regional e Meio Ambiente)-- Universidade Estadual de Santa Cruz, Ilhéus, 2006.

ROBRA, S., SERPADA, R. C., OLIVEIRA A. M., ALMEIDA NETO, J. A., SANTOS, J. V. Generation of biogás using crude glycerin from biodiesel production as a supplement to cattle slurry. *Biomass and Bioenergy*, Oxford, v.34, n.9, p.1330-1335, 2010.

RODRIGUES, L.S.; SILVA, I.J.; ZOCRATO, M.C.O.; PAPA, D.N.; SPERLING, M.V.; OLIVEIRA, P.R. Avaliação de desempenho de reator UASB no tratamento de águas residuárias de suinocultura. *Revista Brasileira de Engenharia Agrícola e Ambiental*, Campina Grande, v.14, n.1, p. 94-100, 2010.

SANTOS, M.A.A.; SCHMIDT, V.; BITENCOURT, V.C.; MAROSO, M.T.D. Esterqueiras: avaliação físico-química e microbiológica do dejeto suíno armazenado. *Engenharia Agrícola*, Jaboticabal, v.27, n.2, p.537-543, 2007.

SILES, J.A., MARTÍN, M.A., CHICA, A.F., MARTÍN, A. Anaerobic digestion of glycerol derived from biodiesel manufacturing. *Bioresource Technology*, Essex, v.100, p.5609– 5615, 2009.

SOUZA, C.F.; JÚNIOR, J.L.; FERREIRA, W.P.M. Biodigestão anaeróbia de dejetos de suínos sob efeito de três temperaturas e dois níveis de agitação do substrato – considerações sobre a partida. *Engenharia Agrícola*, Jaboticabal, v.25, n.2, p.530-539, 2005.

STRICKLAND, J. D. H.; PARSONS, T. R. *A practical handbook of seawater analysis*. Ottawa: Fisheries Research Board of Canada, 1972. (Bull.,167)

THE EUROPEAN PARLIAMENT AND THE COUNCIL OF THE EUROPEAN UNION. *Directive 2009/28/EC*. Disponível em: <http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=Oj:L:2009:140:0016:0062:en:PDF> Acesso em: 20 jan. 2014.

VEIGA, M.C.; SOTO, M.; MÉNDEZ, R.; LEMA, J.M.; A new device for measurement and control of gas production by bench scale anaerobic digesters. *Water Research*, New York, v.24 n.12, p.1551-1554, 1990.

VOGEL, A. I. *Química analítica cuantitativa*. Buenos Aires: Editora Kapelusz, 1960. v.1, 812 p.

WOHLGEMUTH, O. *Co-digestion of hog manure with glycerol to boost biogás and methane production*. 2009. 90f. Dissertação (Mestrado) - Faculty of Graduate Studies, University of Manitoba, Winnipeg, 2009.

XAVIER, C. A. N.; LUCAS JÚNIOR, J. Parâmetros de dimensionamento para biodigestores batelada operados com dejetos de vacas leiteiras com e sem uso de inóculo**.** *Engenharia Agrícola*, Jaboticabal, v.30, n.2, p.212-223, 2010.