Anaerobic treatment of residual lemon pulp in digesters with semi continuous feed

A. R. Navarro, Z. Lopez, J. Salguero and M. C. Maldonado

ABSTRACT

Lemon growing areas in the north of Argentina have industries that produce concentrated juice, peel and essential oil and generate a significant amount of liquid and solid waste as lemon pulp. In Argentina, despite the potential applications that the pulp has as animal feed and human and industrial raw material, only 10% is used for these purposes and the rest is discarded into the environment causing many ecological and economic problems. There is little information in the literature on biotechnologies for the treatment of this industrial waste. This paper shows that lemon pulp is a suitable substrate to be treated by anaerobic digestion. We obtained 86 and 92% of COD reduction in a digester with a semi-continuous feed and retention time of 10 and 20 days respectively and a productivity of 0.406 g CH_4/g VS h. Comparative tests showed that pre-digesting the pulp improved the process of digestion and increased biogas generation by 20%.

Key words | anaerobic digestion, biogas, digester, lemon pulp, methane

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NOMENCLATURE

BOD	Biochemical oxygen demand (mg/L)
COD	Chemical oxygen demand (mg/L)
VS	Volatile solid (g/L)
TS	Total solid (g/L)
FS	Fixed solid (g/L)
C/N	Ratio between the content of carbon and nitrogen
Loading rate	VS amount introduced to the digester per liter and day (g/L.d)
CH_4:	methane
Yield	grams of methane produced per gram of VS consumed.
Retention time	Ratio between the reactor volume and the influent flow (h)
VRR	volumetric COD removal rate

INTRODUCTION

Anaerobic processes have been used for industrial wastewater treatment for more than a century. They have many advantages and have become more efficient with new improvements (Lettinga 1995).

Anaerobic biodegradability and methanogenic toxicity are strongly dependent on the characteristics of the effluent (Cates *et al.* 1995). Fruit and vegetable solid wastes represent a potential energy source because they can be biologically converted to methane. They are renewable and their net CO_2 contribution to the atmosphere is zero. Most lemon industry solid wastes are carried away and disposed of on land with the subsequent physical and chemical changes, generating odors, changing soil pH and contaminating the groundwater.

Available data on methane production and the kinetics of microbial conversion of lemon solid waste are scarce.

In the lemon industry, pulp is the semi-solid waste from juice centrifugation, mainly consisting of residual endocarp membranes, vesicles and, to some extent, albedo and endocarp. It is rich in sugars, fibers and other residual substances. Its high water and organic matter contents make it difficult to eliminate by conventional solid waste treatment (Crupi *et al.* 2001a, b). The lemon industry in Argentina annually processes about 975,000 tons of lemons and besides producing lemon juice, peel and essential oils it generates 97,500 tons of lemon pulp with 93% moisture in the process. In spite of the potential applications that the pulp has as animal feed, human and industrial raw material (Bampidis &

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Q1 Robinson 2006; Ibrahim *et al.* 2011), only 10% is used for these purposes and the rest is discarded into the environment causing many ecological and economic problems.

Lemon pulp treatment in anaerobic digesters is an interesting alternative that would solve the pollution problem, produce biogas with high methane content and a final liquid effluent whose economic value is high (Kellner 1990).

In the literature there is little data on citrus industry effluent treatment (Navarro *et al.* 2008, 2009).

This is the first study reporting on the technology used in methane production from residual lemon pulp. Our study is aimed at demonstrating that it is a suitable substrate for anaerobic biological treatment to reduce pollution and produce biogas as an energy source.

MATERIALS AND METHODS

Substrates

Substrates used were: lemon pulp, horse manure and a mixture of both (2 parts pulp, 1 part manure).

Moisture, C/N ratio, biodegradability and % solids were determined in each.

Analytical methods

Soil pH: measured using the technique of Rand *et al.* (1985). Ten gram of sample were added to 25 mL of distilled water, stirred for 10 min and allowed to stand for 30 min before measurement.

Moisture: measured by placing the sample in an oven at 105 °C until reaching constant weight. Nitrogen: Microkjeldahl technique (Method AOAC 1990).

Carbon: Walkley-Black method (Walkley & Black 1946).

COD: measured by reflux method and titration (APHA, AWWA, WEF-1998). BOD: 5 days determination was according to APHA, AWWA, WEF-1998.

TS, VS and FS: the methods of APHA, AWWA, WEF-1998 were used.

Conductivity and redox potential: with a U22 HORIBA multi-parameter electrode.

Digesters: Six 20 L (18 L working volume) airtight plastic digesters were used. A rubber hose connector for gas discharge was placed on top.

Batch assays

Digesters were fed as follows:

Digester A: 3 kg pulp (90% moisture) + 6 L water +1 L inoculum. Digester B: 2 kg pulp (90% humidity) + 1 kg manure (65% humidity) + 6 L water + 1 L inoculum.

Digester C: 3 kg manure (65% moisture) + 6 L water + 1 L inoculum. Inoculum: the effluent from an anaerobic digester fed with 70% pulp and 30% manure, was used as inoculum.

All substrates were brought to pH 8.5 with a saturated solution of lime (lime water). To achieve anaerobic conditions nitrogen was injected into the digesters to dislodge air. The digesters were placed in a temperature-controlled room at 30 °C.

The manure assays were carried out to compare results with those obtained from lemon pulp and determine whether it should be digested alone or mixed with manure.

Semi continuous feed assays

The same digesters used in a batch were modified to be semi continuously fed (Figure 1).

The anaerobic digester used can be classified as horizontal and intermittent feed.

Each digester was loaded with 16 L of lemon pulp (90% moisture) at pH 7 and 2 L of inoculum. The digesters were placed in a temperature-controlled room at 30 °C and gas production began 20 days later. From this time digesters were fed daily with the same substrate at the same time according to the desired retention time. Preliminary tests revealed that it was better to feed the digesters with a substrate at pH 7 to obtain an effluent with a pH close to neutrality.

Originally, the pulp for the digesters was stored in a container at room temperature, but when it started to decompose we decided to put the pulp in sealed containers (with a gas trap) to encourage a pre-digestion that would enhance efficiency and productivity of the anaerobic digestion process. Pulp was neutralized with lime water at pH 8–8.5 and kept in a closed 20 L container for 5 days at 30 °C. This reservoir was fed daily and served as a buffer tank to feed the digesters.

In order to optimize system performance, digester N° 5 was fed with 1,620 mL pulp + 180 mL of effluent of the same digester.

Substrate amounts and theoretical average retention times for each digester are shown in Table 1.

Loading rate (gVS/L day): was determined from the feed conditions of each digester and the pulp characterization values.

Biogas volume: was determined by liquid displacement (Rodriguez *et al.* 1997).

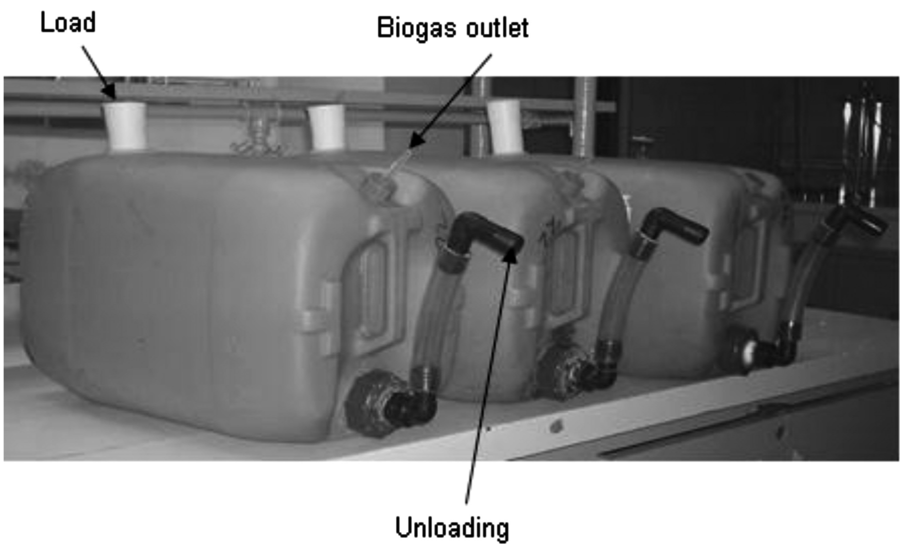

Figure 1 | Diagram of the semi-continuous feed digester.

Table 1 | Substrate amount (90% moisture) and retention time for each digester

	Digester					
	1	2	3	4	5	6
Theoretical average retention time (days)	30	25	20	15	11.1	10
ml substrate/day	600	720	900	1,200	1,620 + 180 recycle	1,800

Methane concentration: was determined by gas chromatography. The chromatograph operating conditions were: carrier gas: nitrogen; carrier gas rate: 6 mL/min; injector temperature: 150 °C; column temperature: 215 °C; detector temperature: 260 °C; capillary column with a diameter of 320 μm; length column: 15 m; methane retention time: 0.4 min. Methane concentration was determined with the equation: $y = 0.0001x + 2.34$ (R2 = 0.98), obtained from the calibration curve.

Determinations of loading rate, biogas volume and methane concentration were carried out in triplicate.

Q2 **Table 2** | Lemon pulp and horse manure analyses (wet basis)

	Pulp	Manure
% Nitrogen	1.9 ± 0.12	6.2 ± 1.16
% Carbon	49.9 ± 1.48	43.7 ± 1.34
C/N	26.5 ± 0.84	16.4 ± 1.41
pH	3.6 ± 1.02	8.1 ± 1.55
% Moisture	90.0 ± 1.97	65.0 ± 1.55

RESULTS AND DISCUSSION

Lemon pulp composition

Lemon pulp moisture content was within the range considered suitable for biological treatment and methane production (Cajigas Ceron *et al.* 2005). Similar compositions were found for other substrates that had been found to be suitable for anaerobic digestion by Hupping Stoner (1978). Sosa *et al.* (1999) indicated that the ideal ratio of C/N for methanogenesis values is between 20:1 and 30:1.

The manure C/N ratio was 16.4 but it can also be considered appropriate for anaerobic biological treatment.

The results obtained by other authors in the determination of BOD_5/COD (Martín García *et al.* 2006) and total solid values for lemon pulp (Loher 1974), and those reported for manure (Kaltwasser 1980) indicate that they are suitable substrates for biological treatment and are consistent with the results obtained in this work (Table 3).

Table 3 | Biodegradability index and pulp solid contents with 90% moisture content

BOD (mgO_2)/L: 9 735 ± 0.96	Total solids (g/L): 61 ± 1.81
COD (mgO_2)/L:12 300 ± 0.80	Volatile solids (g/L): 31.46 ± 1.93
BOD/COD: 0.79 ± 0.19	Fixed solids (g/L): 29.54 ± 1.64

Batch phase results

The pH of the three substrates reached values between 7.00 and 7.23 in the batch anaerobic treatment for 60 days at 30 °C. They were considered appropriate for digestion (Taiganides 1980; Youngfu *et al.* 1989).

During the first 28 days of treatment, the pulp alone produced a lower biogas volume than the pulp-manure mixture. This could be attributed to the difficulty bacteria have in digesting the pulp, rich in cellulose, hemicellulose and lignin (Bampidis & Robinson 2006; Ibrahim *et al.* 2011).

The manure-pulp mixture had a higher biogas production than pulp alone. This synergistic effect takes place because manure provides a significant amount of microorganisms to the digester and the pulp contributes nutrients. Starting on day 24 both substrates began to increase their gas output exponentially. These results are similar to those obtained in a study with *Eichhornia crassipes* (bora) and cattle manure (Rodriguez *et al.* 1997).

Biogas production in the digester fed with manure only was similar to that obtained with the manure-pulp mixture in the first 24 days. From then on production was lower and continued to decline gradually, possibly for depletion of carbon sources as C/N ratio became lower.

Q3 López *et al.* (2000) note that the start of any anaerobic reactor is slow due to low biomass production in relation to the substrate consumed. Hence, inoculation with microorganisms adapted to the system is advisable.

The starting time depends on biological, chemical and physical parameters. The beginning of the digestion is influenced by wastewater concentration and composition, volume, activity and adaptation of the inoculum, environmental conditions, operating parameters and reactor configuration. All of these are closely related (Noyola 1994).

The three substrates tested were inoculated with effluent from a 20 L anaerobic digester that worked with pulp and manure as a substrate so that we could be sure we had used an inoculum adapted to the system.

Manure produced methane until day 32. Pulp instead, began producing methane at day 24, but did so until the end of the experiment (60 days) because different materials have different chemical compositions and produce biogas at different rates.

These results are consistent with those published by Youngfu *et al.* (1989) who worked with different types of substrates, confirming that materials with high nitrogen content, such as animal manure, metabolize readily and produce biogas in a short fermentation period while materials with high carbon content have slower decomposition and longer fermentation.

A closed container containing the pulp to feed the reactors initially displays aerobic microbial growth but after about 21 h the ORP drops to 160 mV and development of anaerobic microorganisms begins.

Comparative tests showed that pre-digestion improved the process of digestion and that biogas generation increased by 20%. This could be attributed to the presence of nutrients and reduction/dilution of inhibitory substances due to mixing of waste, according to Braun & Wellinger (2003). After 60 days of experimentation, accumulated gas volumes were different and depended on the substrate used, being 22.9 L with manure, 42.99 L with pulp and 65 L with pulp-manure.

Treatment with intermittent feeding digesters

Based on the pulp volatile solid content (Table 3), and the volumes fed (Table 1) loading rates were determined for each digester used as indicated in Table 4.

The COD of the untreated pulp was 12,300 mg O_2/L. With pre-digested pulp, acetogenic digestion was completed in 5 days and a substrate with 27% less organic matter, with COD = 8,979 mgO_2/L were obtained.

The COD in digesters 1, 2 and 3, where the retention times were 30, 25 and 20 days respectively, decreased rapidly until day 7 (when digesters began to be fed) and

Table 4 | Loading rate and volatile solids in the digesters with semi continuous feeding

	Digesters					
	1	2	3	4	5	6
Theoretical average retention time(day)	30	25	20	15	11.1	10
Volatile solids (g/L)	13.8 ± 0.88	16.6 ± 0.63	20.7 ± 0.76	27.6 ± 1.04	36.8 ± 1.44	41.4 ± 1.87
Loading rate (gVS/d.L)	0.76 ± 0.13	0.92 ± 0.06	1.15 ± 0.12	1.53 ± 0.13	2.04 ± 0.08	2.30 ± 0.05

then became stable until the end of the process (initial COD 9,000 mg O_2/L, final COD (63 days)) 1,000 mg O_2/L. In digester 4 (retention time = 15 days) final COD values of 1,400 mg O_2/L were obtained. They were similar to those of reactor 5 (10 days retention time). This behavior occurred because reactor 5 was fed with 10% recycle. Digester 6 (retention time 10 days) had higher COD (1,700 mg O_2/L) final values due to a shorter residence time. Similar results were reported by Hashimoto (1982) and Sing *et al.* (1998) with different raw materials.

The optimal pH for the anaerobic digestion process is 6.6 to 7.6 (Youngfu *et al.* 1989). Reactors 1, 2, 3, 4 and 5 maintained this pH, unlike digester 6 where the pH was 6.2 because the shorter retention time prevented methanogenic bacteria from converting the short chain organic acids formed by acetogenic bacteria into methane.

As retention times decreased, so did % COD removal. In digesters 1, 2 and 3 it was 92% compared with untreated pulp, 88% in digesters 4 and 5 and 86% in digester 6. The % of methane in the biogas decreased with shorter retention times. Pulp degradation was high (86%) with a shorter retention time, but gas composition was poor in methane (Figure 2).

Biogas production was similar in all reactors, 0.176 g CH_4/h in reactor 1 and 0.138 g CH_4/h in reactor 6, but the percentage of methane decreased gradually as retention times shortened: 54% in digester 1 and 37% in digester 6 due to the effect of acid inhibition.

As retention time decreased, conductivity of the treated effluent increased, probably due to the dissolution of organic acids and some inorganic constituents, especially in reactor 6 where pH was lower.

While the residence time increases, VRR values increase and the yield decreases.

The values of the oxide reduction potential were normal for methanogenic bacteria in all digesters (Table 5).

CONCLUSIONS

The results obtained show that the anaerobic digestion of residual lemon pulp is not only feasible and succeeds in

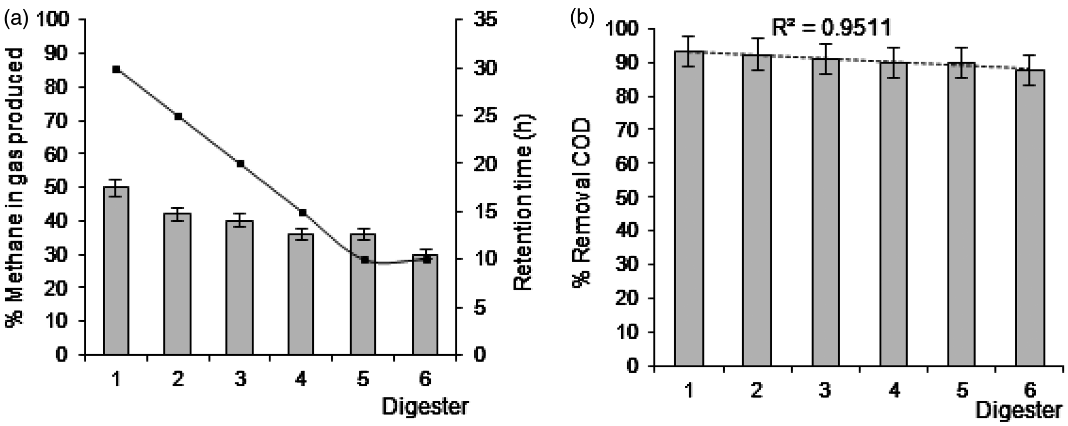

Figure 2 | Change in % of methane in gas produced (a) and % removal of COD in different digesters (b). Q5

Table 5 | Values of the most important parameters obtained from each of the digesters working with intermittent feeding

Digestor N°

	1	2	3	4	5	6
g gas/ h	0.176 ± 0.09	0.158 ± 0.003	0.145 ± 0.011	0.142 ± 0.010	0.141 ± 0.010	0.138 ± 0.003
% $CH_{4\ in\ gas}$	54 ± 1.20	46 ± 1.20	43 ± 1.31	40 ± 0.98	39 ± 0.91	37 ± 1.20
VRR (g COD/L.d)	6.86	8.06	9.96	13.14	17.53	19.04
Yield (gCH_4/ g VS)	0.306	0.275	0.252	0.247	0.245	0.240
Redox potential (mV)	−337 ± 19.09	−330 ± 8.48	−320 ± 5.65	−350 ± 10.60	−300 ± 8.48	−390 ± 9.89

reducing a high percentage of the pollution but also produces methane that can be used as fuel for the same process.

The horizontal digester intermittent feeding was a good choice for the process and the results showed that with decreasing residence time, methane production decreased while the volumetric COD removal rate increased.

Lemon pulp pre-digestion improved the performance of the process and solved the problem of uncontrolled decomposition of the pulp that took place in the feeding tanks.

Recycling of 10% of the effluent from a reactor to feed the same reactor caused no significant improvement in methane productivity or volumetric COD removal rate.

REFERENCES

AOAC 1990 *Assocciation of Oficial Agricultural Chemistry. Oficial Methods of Analysis of the AOAC 12th*. Washington, DC, USA.

APHA, AWWA & WEF 1998 *Standard Methods for the*
Q6 *Examination of Wastewater*, 20th Edition. United States of America.

Bampidis, V. & Robinson, P. 2006 Citrus by product as rumiant feeds. A review. *Animal Feed Science and Technology* **128**, 175–217.

Braun, R. & Wellinger, A. 2003 Potential of Co-digestion. IEA Bioenergy.

Cajigas Ceron, A. A., Pérez Vidal, A. & Torres Lozada, P. 2005 Importancia del pH y la alcalinidad en el tratamiento anaerobio de las aguas residuales del proceso de extracción de almidón de yuca. Scientia et Technica Año XI, No 27, Abril. UTP. ISSN 0122–1701 243.

Cates, D., Eggert, C., Yang, J. & Eriksson, K. 1995 Comparison of effluents from TCF and ECF bleaching of kraft pulps. *Tappi Journal* **78** (12), 93–98.

Crupi, F., Dugo, P., Di Giacomo, G., Rispoli, G. & Di Giacomo, A. 2001a Sulla produzione del succo di aranda in Italia. Parte I. *Essenze Derivati Agrumari* **71**, 121–132.

Crupi, F., Dugo, P., Di Giacomo, G., Rispoli, G. & Di Giacomo, A. 2001b Sulla produzione del succo di aranda in Italia. Parte I. *Essenze Derivati Agrumari* **71**, 207–229.

Hashimoto, A. G. 1982 Methane from cattle waste: effect of temperature, hydraulic retention time, and influent substrate concentration on kinetic parameter (k). *Biotechnology and Bioengineering* **24** (9), 2039–2052.

Hupping Stoner, C. 1978 *Como Usar Las Fuentes de Energía Natural*, 1ª Edición. Editorial Diana, Mexico, pp. 179–228.

Ibrahim, M., El-Banna, H., Omara, I. & Suliman, M. 2011 Evaluation of nutritive value of some citrus pulp as feedstuf in rabbit diets. *Pakistan Journal of Nutrition* **10**, 667–674.

Kaltwasser, B. 1980 *Biogas, Regenerative Energieerzengung Durch Anaerobe Fermentationorganischer Abfalle in Biogasanlagen*. Ed. Bauverlag, Wiesbaden, FRG, pp. 35–36.

Kellner, C. 1990 Slurry, the difficult advantage. *Biogas Forum* **40**, 4–7.

Lettinga, G. 1995 Anaerobic digestion and wastewater treatment systems. *Antonie Van Leeuwenhoek* **67**, 3–28.

Loher, R. C. 1974 *Agriculture Waste Management*. Academic Press Inc., London and, NY.

López, J., Morgan, J. & Loyola, A. 2000 Arranque de reactores anaerobios industriales: dos casos de estudio.'Memorias de XII Congreso de la Federación Mexicana de Ingenieria Sanitaria y Ciencias Ambientales' (FEMISCA).

Martín García, I., Betancort Rodríguez, J. R., Salas Rodríguez, J. J., Peñate Suárez, B., Pidre Bocardo, J. R. & Sardón Martín, N. 2006 Guía sobre tratamientos de aguas residuales urbanas para pequeños núcleos de población. Mejora de la calidad de los efluentes, 25–26. Daute Diseño, S.L. ISBN: 84–689–7604–0.

Navarro, A. R., Dorado, L. & Maldonado, M. C. 2009 An eco-biotechnological process for the treatment of residual lemon pulp. *Clean Technologies and Environmental Policy* **11**, 323–327.

Navarro, A. R., López, Z. O. & Maldonado, M. C. 2008 A pilot plant for the treatment of lemon industry wastewater. *Clean Technologies and Environmental Policy* **10**, 371–375.

Noyola, A. 1994 Diseño, inoculación y arranque de reactores UASB. 'Memorias del III Taller y seminario Latinoamericano de Tratamiento anaerobio de aguas residuales'. Universidad de la República (Editor). Montevideo Uruguay. Punta Brava La Habana. Cuba Vol.6 N°2. Available from: http://www.sian.info.ve/porcinos/publicaciones/rccpn/rcpp.htm

Rand, M. C., Greenberg, A. R. & Taras, M. J. 1985 *Standard Methods for the Examination of Water and Wastewater*, 16th edn. APHA, Washington, DC, USA.

Rodriguez, R., Julio, C., El Atrach, K., Rumbos, E. & Delepiani, A. G. 1997 Resultados experimentales sobre la producción de biogas a través de la bora y el estiércol de ganado. *Agronomía Tropical* **47** (4), 441–455.

Sing, P. P., Ghuman, B. S. & Grewal, N. S. 1998 Computer model for performance prediction and optimization of unheated biogas plant. *Energy Conversion and Management* **39** (1/2), 51–63.

Sosa, R., Chao, R. & del Río, J. 1999 Aspectos bioquímicos y tecnológicos del tratamiento de residuales agrícolas con producción de biogas. *Revista Computarizada de Producción Porcina* **6** (2). Q4

Taiganides, E. P. 1980 Biogas: recuperación de energía de los excrementos animales. *Zootecnia* **35**, 2–12.

Walkley, A. & Black, L. 1946 An examination of the Degjtjareff method for determining soil organic matter and proposed modification of the cromic acid titration method. *Soil Science* **37**, 29–38.

Youngfu, Y., Yibo, Q., Yunxuan, G., Hui, Z., Yuansheng, X., Chenyong, X., Guoyuan, F., Jienquan, X., Taiming, Z. & Gan, L. 1989 *The Biogas Technology in China*. Agricultural Publishing House. Beijing, pp. 20–54.

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