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% reduction of chemical oxygen demand in a digester with a semi-continuous feed and retention time of 10 and 20 days respectively and a productivity of 0.406 g CH₄/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

NOMENCLATURE

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>BOD</td>
<td>Biochemical oxygen demand (mg/L)</td>
</tr>
<tr>
<td>COD</td>
<td>Chemical oxygen demand (mg/L)</td>
</tr>
<tr>
<td>VS</td>
<td>Volatile solids (g/L)</td>
</tr>
<tr>
<td>TS</td>
<td>Total solids (g/L)</td>
</tr>
<tr>
<td>FS</td>
<td>Fixed solids (g/L)</td>
</tr>
<tr>
<td>C/N</td>
<td>Ratio between the content of carbon and nitrogen</td>
</tr>
<tr>
<td>Loading rate</td>
<td>VS amount introduced to the digester per liter and day (g/L.d)</td>
</tr>
<tr>
<td>CH₄</td>
<td>Methane</td>
</tr>
<tr>
<td>Yield</td>
<td>Grams of methane produced per gram of VS consumed.</td>
</tr>
<tr>
<td>Retention time</td>
<td>Ratio between the reactor volume and the influent flow (h)</td>
</tr>
<tr>
<td>VRR</td>
<td>Volumetric COD removal rate</td>
</tr>
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</table>

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 CO₂ contribution to the atmosphere is zero. Most lemon industry solid wastes are carried away and disposed of on land with 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. 200a, 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, and human and industrial raw material (Bampidis & 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 are few 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 (two parts pulp, one part manure).

Moisture, carbon/nitrogen (C/N) ratio, biodegradability and percentage of solids were determined in each.

**Analytical methods**

  Ten grams 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.
- Chemical oxygen demand (COD): measured by reflux method and titration (APHA 1998).
- Biochemical oxygen demand (BOD): 5 days’ determination was according to APHA (1998).
- Total solids (TS), volatile solids (VS) and fixed solids (FS): the methods of APHA (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.

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 to determine whether it should be digested alone or mixed with manure.

**Semi-continuous feed assays**

The same digesters used in batch assays 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 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·d)) 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).

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: $y = 0.0001x + 2.34$ ($R^2 = 0.98$), obtained from the calibration curve.

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

### 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. Lemon pulp and horse manure analyses are shown in Table 2.

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).
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 (Tayganides 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 only manure 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.

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 oxidation reduction potential 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 O₂/L. With pre-digested pulp, acetogenic digestion was completed in 5 days and a substrate with 27% less organic matter, with COD = 8,979 mg O₂/L, was 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 then became stable until the end of the process (initial COD 9,000 mg O₂/L, final COD (63 days) 1,000 mg O₂/L). In digester 4 (retention time = 15 days) final COD values of 1,400 mg O₂/L were obtained. They were similar to

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

<table>
<thead>
<tr>
<th></th>
<th>Pulp</th>
<th>Manure</th>
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</thead>
<tbody>
<tr>
<td>% Nitrogen</td>
<td>1.9 ± 0.12</td>
<td>6.2 ± 1.16</td>
</tr>
<tr>
<td>% Carbon</td>
<td>49.9 ± 1.48</td>
<td>43.7 ± 1.34</td>
</tr>
<tr>
<td>C/N</td>
<td>26.5 ± 0.84</td>
<td>16.4 ± 1.41</td>
</tr>
<tr>
<td>pH</td>
<td>3.6 ± 1.02</td>
<td>8.1 ± 1.55</td>
</tr>
<tr>
<td>% Moisture</td>
<td>90.0 ± 1.97</td>
<td>65.0 ± 1.55</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>COD (mg O₂/L)</th>
<th>BOD (mg O₂/L)</th>
<th>BOD/COD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12,300 ± 0.80</td>
<td>7,975 ± 0.96</td>
<td>0.79 ± 0.19</td>
</tr>
<tr>
<td>Total solids (g/L)</td>
<td>61 ± 1.81</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volatile solids (g/L)</td>
<td>31.46 ± 1.93</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fixed solids (g/L)</td>
<td>29.54 ± 1.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Moisture</td>
<td>65.0 ± 1.55</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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 O₂/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 per cent 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 percentage 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 CH₄/h in reactor 1 and 0.138 g CH₄/h in reactor 6, but the percentage of methane decreased gradually as retention times shortened: from 54% in digester 1 to 37% in digester 6 due to the effect of acid inhibition.

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

<table>
<thead>
<tr>
<th>Digester</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theoretical average retention time (day)</td>
<td>30</td>
<td>25</td>
<td>20</td>
<td>15</td>
<td>11.1</td>
<td>10</td>
</tr>
<tr>
<td>Volatile solids (g/L)</td>
<td>13.8±0.88</td>
<td>16.6±0.63</td>
<td>20.7±0.76</td>
<td>27.6±1.04</td>
<td>36.8±1.44</td>
<td>41.4±1.87</td>
</tr>
<tr>
<td>Loading rate (g VS/(d·L))</td>
<td>0.76±0.15</td>
<td>0.92±0.06</td>
<td>1.15±0.12</td>
<td>1.53±0.13</td>
<td>2.04±0.08</td>
<td>2.30±0.05</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Digester</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>g gas/h</td>
<td>0.176±0.09</td>
<td>0.158±0.003</td>
<td>0.145±0.011</td>
<td>0.142±0.010</td>
<td>0.141±0.010</td>
<td>0.138±0.003</td>
</tr>
<tr>
<td>% CH₄ in gas</td>
<td>54±1.20</td>
<td>46±1.20</td>
<td>43±1.31</td>
<td>40±0.98</td>
<td>39±0.91</td>
<td>37±1.20</td>
</tr>
<tr>
<td>VRR (g COD/(L·d))</td>
<td>6.86</td>
<td>8.06</td>
<td>9.96</td>
<td>13.14</td>
<td>17.53</td>
<td>19.04</td>
</tr>
<tr>
<td>Yield (gCH₄/g VS)</td>
<td>0.306</td>
<td>0.275</td>
<td>0.252</td>
<td>0.247</td>
<td>0.245</td>
<td>0.240</td>
</tr>
<tr>
<td>Redox potential (mV)</td>
<td>−337±19.09</td>
<td>−330±8.48</td>
<td>−320±5.65</td>
<td>−350±10.60</td>
<td>−300±8.48</td>
<td>−390±9.89</td>
</tr>
</tbody>
</table>

Figure 2 | Change in percentage of methane in gas produced (a) and per cent removal of COD in different digesters (b).
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, volumetric COD removal rate (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 not only is feasible and succeeds in 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 VRR 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 VRR.

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