Evaluation of anaerobic biodegradability of wastewater from tebuconazole manufacturing
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ABSTRACT
This study aimed to verify the biological anaerobic treatability of tebuconazole effluent manufacturing. For this purpose, two preliminary batch tests were performed using an ‘anaerobic respirometer’ adjusting the initial pH of the effluent at 7.0 and 8.0, respectively (first phase). In addition, two tests (second phase) were run using sequential batch anaerobic fermenters, the first operated at different hydraulic detention times (10 and 16.7 days) and the second with different initial dilutions of the effluent (5 and 20%). The chemical oxygen demand (COD) removal observed on the anaerobic respirometer tests was in the range of 66–81%. According to the preliminary batch tests an optimal value of initial COD concentration and amount of biomass was identified, which was considered for the fermenters start up. However, it was observed that the optimal relation provided by the respirometer test was not a good parameter of operational control for the fermenters due to the accumulation of inhibitory substances, which affected the microbial activity and took the system to collapse. The initial dilution of the effluent (5 and 20%) was essential for the stability of the anaerobic system, allowing COD removals above 74% during this study.

Key words | anaerobic treatability, batch test, COD removal, methane production, tebuconazole

INTRODUCTION
The use of fungicides in Brazil is related to the significant increase of national wheat production in the last 20 years. In the early 1990s, a fungicide with the active ingredient tebuconazole was recorded for the wheat crop and was widely used by Brazilian producers (Maciel & Chaves 2008). Since that time, the market trend indicates more agricultural use of fungicides, a fact that provides growth opportunity for business leaders in the synthesis of these compounds (Scholze 2006).

Tebuconazole is a triazole fungicide systemic, broad spectrum, used in various crops such as barley, wheat, peanuts, among others. Its fungicidal activity is based on the inhibitory function of the enzyme lanosterol dimethylase, which decreases the biosynthesis of ergosterol, the main sterol of the plasma membrane of most fungi. This sterol is necessary to maintain the fluidity and permeability of the fungi cell (Filipov & Lawrence 2001; Moser et al. 2001).

In Brazil, the use of triazoles peaked in 2003–2004, when the soybean crop across the Cerrado and the south was hit by rust, a disease whose etiologic agent is fungus. The fungicide that showed the best performance in combating soybean rust was tebuconazole resulting in an exceptional demand, increasing the industry production. In addition, a substantial complexity of the production was observed, generating more waste (Scholze 2006).

It is known that this fungicide can cause adverse effects to human health, such as skin and eye irritation, respiratory complications and may be toxic to aquatic organisms, mainly to algae and fish (Milenia 2009). Therefore, the challenge was to find an efficient and cost-effective treatment process for the effluent, as this fungicide has low mobility and is not readily biodegradable. An estimated halftime of degradation in surface water is around 200 days (Directive 98/8/EC 1998). Furthermore, tebuconazole molecule is extremely stable under normal storage and stockpiling. Once isolated in crystal form, it supports temperatures above its melting point without undergoing significant changes in the content of active ingredient (Scholze 2006).

Some authors suggest the use of advanced processes, such as photo-Fenton (Teixeira et al. 2005) and photocatalytic degradation (Prestes et al. 2008, Navarro et al. 2009).
However, these processes are very expensive and may generate byproducts with high toxicity.

This study aimed to identify alternatives for the treatment of tebuconazole effluent, testing its treatability by anaerobic process, more suitable for wastewater with high organic concentration.

**METHODOLOGY**

Initially, a physical and chemical characterization of the industrial effluent was carried out according to procedures described in APHA (1998). The parameters tested were chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), ammonia, total phosphorus, alkalinity, and total volatile and fixed solids.

The experimental work was divided into two phases. The first was based on the measurement of the anaerobic biodegradation based on a single batch anaerobic test. The second phase involved the operation of two sequencing batch anaerobic reactors.

The microbial seed used in the experiments came from a full-scale UASB reactor treating soybean processing industrial effluent.

**First phase**

The tests were performed on an ‘anaerobic respirometer’ consisting of eight reaction flasks, with a total volume of 500 mL (Monteggia 1991). The tests were carried out by using a biomass concentration of 2.000 mg TVS L\(^{-1}\) in all flasks, making it possible to test a range of different initial influent concentrations. In addition, two different values of initial pH were tested, namely pH 7 and 8. The duration of the tests was about 80 hours, identified by the decrease of methane production.

For the first test (pH 7), the following values of initial COD were tested: 500; 1.000; 1.500; 2.000; 2.500; 3.000; 3.500 mg L\(^{-1}\). For the second test (pH 8), the following values of initial COD concentration: 1.500; 2.000; 2.500; 3.000; 3.500; 4.000 and 4.500 mg L\(^{-1}\).

The quality of biogas was identified by gas chromatography, using the methodology described by Morimoto et al. (2004). At the end of the tests, residual COD and total volatile solids (TVS) were determined and related to biogas production.

**Second phase**

The second phase involved the operation of two anaerobic fermenters, operated on a sequential batch mode and two different feeding criteria. The working volume of each fermenter was 5 L and the mixture was provided by continuous agitation system. Fermenters were inoculated with the same seed microbial sludge described previously. The main operating parameters of each test are described below:

**First test**

- Hydraulic detention time:
  - fermenter 1 = 10 days
  - fermenter 2 = 16.7 days
- Biomass inoculation: TVS = 5.743 mg L\(^{-1}\)
- Feeding criteria:
  - fermenter 1: 300 mL of effluent and 200 mL of dilution water (initial COD of 52.800 mg L\(^{-1}\)).
  - fermenter 2: 300 mL of raw wastewater (initial COD 88.000 mg L\(^{-1}\)).

To maintain the pre-defined hydraulic detention time for both fermenters, the reactor 1 was fed with raw wastewater diluted to 40%. Reactor 2 was fed with raw wastewater without dilution.

**Second test**

- Hydraulic detention time of 10 days for both fermenters;
- Biomass inoculation:
  - fermenter 1: TVS = 5.743 mg L\(^{-1}\)
  - fermenter 2: TVS = 19.144 mg L\(^{-1}\)
- Feeding criteria:
  - fermenter 1: raw wastewater diluted to 5% (COD = 4.400 mg L\(^{-1}\))
  - fermenter 2: raw wastewater diluted to 20% (COD = 17.600 mg L\(^{-1}\)).

The working volume was maintained constant by removing the same volume inserted before daily feeding. The fermenters were monitored by collecting effluent samples twice a week. The biogas was also analyzed by gas chromatography as previously described.

**RESULTS AND DISCUSSION**

The physical and chemical characterization of the raw wastewater from tebuconazole production effluent is presented in Table 1.

The results presented in Table 1 indicate that the effluent in this study has a high concentration of organic
matter, total solids and nutrients. The high COD concentration strongly indicates the convenience of the anaerobic treatment as a first step of treatment.

Due to the high pH value of the raw wastewater, a previous neutralization step was taken in order to adjust the pH at the neutral range. Hence, a required amount of sulfuric acid was added to the raw wastewater to adjust the pH to 7 to provide favorable environmental conditions to the anaerobic microorganisms. The pH value of 8 was also tested in this study in order to reduce the consumption of neutralizing agent, adding a certain economy to the process.

**FIRST PHASE – ANAEROBIC RESPIROMETER**

The respirometric tests were designed in order to evaluate a wide range of initial feed concentrations (COD basis) related to a fixed amount of biomass concentration in each reaction flask, which in this study was adjusted to 2.000 mg TVS L⁻¹.

Figure 1 shows the results of COD removal considering the initial concentration range of 1.500 to 3.500 mg L⁻¹ and initial pH values of 7 and 8.

When the initial pH was adjusted to 7, the COD removal was in the range of 75 to 81% with a maximum value corresponding to the initial COD of 2.500 mg L⁻¹. When initial pH was adjusted to 8, no significant effect was observed upon the COD removal, which ranged from 66 to 80% removal.

Hence, the pH of the raw wastewater was adjusted to 8 for the subsequent tests, in order to reduce the addition of chemicals to the anaerobic reactors.

Figure 2 shows the volume of methane produced as a function of COD feeding. The initial pH 8 caused slightly higher values compared with pH 7, achieving the maximum value at the COD concentration of 3.500 mg L⁻¹ (580.4 and 639.6 mL of CH₄ for pH 7 and 8, respectively).

No inhibitory effects were detected in the wide range of initial COD concentrations tested.

According to Figure 3, the methane yield varied in the range of 0.16 and 0.22 LCH₄.gCODremoved⁻¹ when the initial pH was adjusted to 7. Similar values resulted when the initial pH was adjusted to 8 (0.14 to 0.27 LCH₄.gCODremoved⁻¹). These values are a bit far from the theoretical value (0.35 LCH₄.gCODremoved⁻¹), indicating that...
other groups of microorganisms may have contributed to the COD removal under anaerobic conditions.

These results also indicate that the increase of initial COD concentration did not cause inhibition effects to the methanogenic microorganisms.

These batch tests provided preliminary operational conditions for the operation of the anaerobic bath reactors, such as the range of tebuconazole loading per biomass concentration. These batch tests also provided satisfactory anaerobic biodegradation of tebuconazole effluent, indicating that the microorganisms present in anaerobic microbial seed were able to convert the organic substrate to methane. Also, no toxicity was detected in the initial concentration of COD compared, in a range of 1.500 to 3.500 mg L$^{-1}$, indicating that anaerobic digestion could be considered a feasible technology in this particular case.

Second phase: anaerobic fermenters operated on a sequential batch mode

First test

The efficiency evaluation of the first test was based on COD removal and biogas quality according to the initial COD and biomass concentration, determined by previous respirometry tests.

Figure 4 shows the values of COD removal in the first test with fermenters, during the period of the test.

The decrease of COD removal efficiency shown in Figure 4 clearly indicates that the anaerobic fermenters were operating under unfavorable conditions, probably due to some sort of toxicity caused by the industrial wastewater.

Figure 4 also shows that the downward trend in the efficiency in the fermenter 1 occurred more smoothly than in the fermenter 2. It was probably due to the initial dilution of 40% performed for feeding, in contrast to fermenter 2, which was fed in concentrated form.

The results of biogas quality are shown in Figure 5. After 1 week of operation, it was clearly observed a decrease of methanogenic activity, reaching values near to zero after 2 weeks of operation. According to Speece (1996), the inhibition of methane production by toxic compounds is normally related to their concentration.

At start up of the batch reactors, there was a large dilution of the raw wastewater (about 94%), which decreased with the daily feeding (50% in the fermenter 1 and 18% in the fermenter 2 after 2 weeks of operation). Thus, the feeding system adopted in this case resulted in a progressive accumulation of toxic compounds within the reactor and, as a consequence, a fast decrease in the efficiency of the fermenter, measured by the COD removal and methane production.

Thus, these results demonstrated that the initial concentration of COD related to a certain amount of biomass is not an appropriate procedure to conduct treatability studies when toxicity agents are present in the raw wastewater.

Kortekaas (1998) pointed out that the upfront dilution of the raw wastewater may be a strategy for detoxification of the effluent. According to Paraskeva & Diamadopoulos (2006), the dilution is very often used prior to biological treatment to reduce the toxicity for the microorganisms responsible for organic matter decomposition. Therefore, the influent dilution was adopted as additional operating criteria for the subsequent tests to minimize the toxicity effect of tebuconazole effluent.

Second test

The second test was carried out taking into consideration the necessity of upfront dilution of the raw wastewater to
avoid adverse effects to the anaerobic microorganisms. Hence, two concentrations of the tebuconazole influent were adopted: Fermenters 1 and 2 were fed respectively with 5% and 20% raw wastewater, during a 30-day period. The parameters analyzed were COD removal, mixed liquor volatile solids and methane percentage in the biogas.

According to Figure 6, the efficiency of COD removal was similar for both fermenters, with average value of 78% at fermenter 1 and 74.6% at fermenter 2. However, the organic load removed at fermenter 2 was significantly higher than the one obtained at fermenter 1, due to the higher influent COD concentration.

Figure 6 shows that the biomass was able to adjust and to maintain a reasonable stability in terms of COD removal at both fermenters (5 and 20% feed concentration) without any adverse effect caused by the effluent from tebuconazole manufacturing.

The results of volatile mixed liquor in the fermenters are shown in Figure 7, indicating a decrease in the concentration during the monitoring period (5 weeks).

However, the tendency of biomass decrease concentration was attributed to the adjustment of the amount of active biomass to the influent organic load. Amaral et al. (2008) reported that during the acclimation period, the sludge can be naturally selected favoring only the presence of the groups able to degrade or adapt to the substances present in the wastewater, causing a reduction of the total number of microorganisms.

Figure 8 illustrates the methane content in the biogas produced at the two fermenters.

The methane content averaged 25.5% at fermenter 1 (5% dilution) and reached 39.7% at fermenter 2 (20% dilution) which are values well below the range obtained for non toxic industrial effluents (Metcalf and Eddy Inc. 2003). However, the higher values of methane content in the biogas form fermenter fed with 20% raw wastewater is a further indication of the capacity of the anaerobic process to biodegrade a complex industrial wastewater as the one from the tebuconazole manufacturing.

CONCLUSIONS

The results obtained in this study clearly indicate the potential of anaerobic biodegradation as a first step for the treatment from tebuconazole effluent. Short-term biodegradability tests using a conventional anaerobic respirometer showed satisfactory reduction of COD in the range of 66–80%, establishing an optimum range of initial value of COD per biomass concentration for planning further tests.

However, it was verified that additional parameters and different levels of experimental procedures have to be taken in consideration when laboratory studies are carried out to investigate the treatability of industrial effluents containing toxic residues.

In this case, upfront dilution of the raw effluent from tebuconazole manufacturing was required to avoid adverse effects upon the anaerobic organisms.
REFERENCES


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