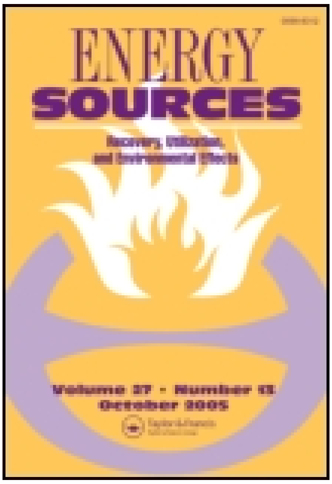

Energy Sources, Part A: Recovery, Utilization, and Environmental Effects
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ueso20

# Effects of Over-liming on Wastewater Detoxification for Enhancement of Biogas Production

H. Seyedy Niasar[a], H. Zilouei[a], V. Rahmani[a] & K. Karimi[a]
[a] Department of Chemical Engineering, Isfahan University of Technology, Isfahan, Iran
Published online: 10 Jul 2015.

*Energy Sources, Part A: Recovery, Utilization, and Environmental Effects*, 37:1560–1567, 2015

ISSN: 1556-7036 print/1556-7230 online
DOI: 10.1080/15567036.2011.617807



# Effects of Over-liming on Wastewater Detoxification for Enhancement of Biogas Production

H. Seyedy Niasar,[1] H. Zilouei,[1] V. Rahmani,[1] and K. Karimi[1]
[1]*Department of Chemical Engineering, Isfahan University of Technology, Isfahan, Iran*

Detoxification by overliming was applied to enhance biogas production from an industrial baker's yeast production wastewater. Various operational parameters affecting the detoxification, i.e., temperature, duration time, and pH, were studied for improvement of biogas production. Overliming was carried out at different pH levels (9 to 12) and temperatures (20 to 60°C) for different periods of time (0 to 12 h). Then, the detoxified wastewaters were subjected to anaerobic digestion. The maximum biogas production of 425 ml $CH_4$/g COD, and the maximum yield of methane production of 3.06 l $CH_4$/l wastewater were achieved under optimum conditions for overliming of wastewater.

*Keywords*: anaerobic digestion, baker's yeast wastewater, biogas, inhibitors, overliming

## 1. INTRODUCTION

In today's energy-demanding lifestyle, exploring and exploiting new sources of renewable energy, which are eco-friendly, is necessary (Yadvika et al., 2004). Biogas is a renewable source of energy that is used as a car fuel and for the production of heat and electricity (Zheng et al., 2010; Kavasic and Topaloglu, 2010). Methane production has been evaluated as one of the most energy efficient and environmentally benign ways of producing vehicle fuel. As an example, the European Union (EU) set a target of increasing the share of biofuels in vehicles to 5.75% by the year 2010 in all member states (European Parliament, 2003).

High concentrations of organic compounds in wastewater resulted in serious problems in the wastewater treatment facilities and in the environment (Kobya and Delipinar, 2008). The high load of organic materials, e.g., sugars, carbohydrates, and fermented products, is one of the main characteristics of the wastewater discharge from the food industry. The effluents of agro-industries, such as baker's yeast, beer, sugar, and starch, are too strong for direct discharge into the environment (Deveci and Çiftçi, 2001). On the other hand, the wastewater from the food industries can be used as a substrate for biogas production by anaerobic digestion (Komemoto et al., 2009). Anaerobic digestion provides numerous significant advantages, such as low sludge production, low energy requirement, and the possibility of high-energy recovery (Chen et al., 2008).

Address correspondence to Dr. H. Zilouei, Department of Chemical Engineering, Isfahan University of Technology, Isfahan 84156-83111, Iran. E-mail: hzilouei@cc.iut.ac.ir

Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/ueso.

The industrial production of yeast by fermentation generally uses molasses, a by-product of sugar manufacturing, as a raw material. This process produces a large quantity of high-strength liquid wastewater (Zak, 2005). However, a part of the non-sugar substances in the molasses are not assimilable by the yeast and are released unchanged into the processing wastewater. In addition, the chemicals added during fermentation, e.g., antifoams and nutrients, as well as the yeast metabolites and residual yeast cells exist in the wastewater. These effluents have high biological oxygen demand (BOD) and chemical oxygen demand (COD) concentrations, which need to be treated before discharging (Kobya and Delipinar, 2008). Furthermore, the sugar-beet molasses contains high amounts of betaine (up to 6% dry solids) and soluble nitrogenous compound. The betaine is not usually consuming during the baker's yeast fermentations, thus becoming a significant constituent of wastewater (Zub et al., 2008).

Presence of a variety of compounds, which act as potent inhibitors of microbial metabolism, affects their kinetics activity and limits yield of biogas production. A wide variety of inhibitory substances are the primary cause of anaerobic digester upset or failure since they exist in substantial concentrations in wastes (Chen et al., 2008). Phenolic compounds are the main inhibitors existing in baker's yeast wastewater that affect bacterial metabolism and cause fail in biogas production. Approximately 0.5–10 mg/L phenolic compounds can cause inhibition to acidogenic and methanogenic populations (Chen et al., 2008). These are toxic to many organisms by disrupting the proton gradient across membranes and interfering with energy transduction of cells. Some other inhibitors are also present in baker's yeast wastewater, such as ammonia, which affects the bacterial metabolism in high concentrations. The effects of these inhibitors are much less than phenolic compounds (Chen et al., 2008).

There are several methods for detoxification of industrial wastewater, such as chemical, physical, biological, and combined methods for overcoming the negative effects of inhibitors (Palmqvist and Hägerdal, 2000). Biological methods of treatment involve the use of specific enzymes or microorganisms that act on the toxic and organic compounds present in the wastewaters and change their compositions (Mussatto and Roberto, 2004). Chemical methods include precipitation of toxic compounds and ionization of some inhibitors under certain pH values, the latter being able to change the toxicity of the compounds to some degree. Theoretically, the process may remove inhibitory components by precipitation, chemically convert toxic components to non-toxic forms, and/or add some substance to the wastewater that enhances the biogas production capability of microorganisms (Mussatto and Roberto, 2004).

Overliming has been used for the removal of inhibitors especially for detoxification of acid hydrolyzates of lignocelluloses (Millati et al., 2002; Horváth et al., 2005). Larsson et al. (1999) compared the effects of 12 different methods for detoxification, including alkali treatment with NaOH and $Ca(OH)_2$, on the chemical composition and fermentability of a dilute-acid hydrolysate of spruce. Overliming was one of the most efficient methods and much more efficient than using NaOH under similar conditions (Larsson et al., 1999; Horváth et al., 2005). Millati et al. (2002) used this treatment for eliminating furfural and hydroxymethylfurfural in the hydrolyzate produced from forest residuals. The result was an increase in fermentability. Optimization of detoxification conditions often aims at eliminating inhibitors without decreasing the amount of organic matter considered sacred for biogas production (Hansen et al., 2007).

In this study, baker's yeast wastewater was used as a substrate for anaerobic digestion and biogas production. The aim was using overliming as a chemical method for the detoxification of baker's yeast wastewater and investigating various conditions (pH, temperature, and duration time) of overliming for enhancing biogas production.

## 2. MATERIALS AND METHODS

### 2.1. Wastewater

Baker's yeast wastewater was obtained from Shahrekord Baker's Yeast Factory (Shahrekord, Iran). It was stored at 4°C before use. Characteristics of the wastewater are shown in Table 1.

### 2.2. Inoculum

Inoculum was obtained from an anaerobic digester of Isfahan municipal wastewater treatment plant (Isfahan, Iran), The digesters are two mesophilic reactors with 4,500 $m^3$ working volume operated at 37°C. The inoculum was incubated under anaerobic conditions for 3 days at 37°C and 120 rpm shaking before use.

### 2.3. Overliming

Overliming was carried out in 250-ml Erlenmeyer flasks. $Ca(OH)_2$ was added to 100 ml of baker's yeast wastewater until reaching the desired pH and mixed at a desired temperature for a specific period of time. After the overliming, the sediments were isolated using centrifuge (5000 rpm for 15 min) and the supernatant was stored at 4°C until use. The experiments were performed at different pH levels (9, 10, 11, and 12), different temperatures (20 and 60°C), and for different periods of time (0, 2, and 12 h). The treatment for 0 h means pH adjustment to the desired pH and then immediate decreasing of the pH to 5.5. Then, pH of the treated wastewater was adjusted to 5.5 ± 0.1, using 1 N sulfuric acid. The treated samples were named (*x, y, z*) corresponding to time, temperature, and pH, respectively. All of the detoxified wastewaters were then anaerobically digested in batch cultivation for biogas production.

### 2.4. Anaerobic Digestion

Anaerobic digestions of the treated and untreated wastewater were performed in 118-ml bottles as digester reactors. All reactors were closed with aluminium caps and septum using a crimper. Following the completion of set-up, the reactors were flushed for 2 min with $N_2$ gas to ensure anaerobic conditions in the headspace of the reactors. Then, reactors were placed in a shaker incubator at 37°C and 140 rpm. Two samples containing only water and inoculums were used as blanks to measure the methane production originating from the inoculums. All of the reactors were monitored by analysis of the produced gas from the headspace by gas chromatography (GC).

Equal substrate concentration was used in all experiments. In order to compensate for the loss of COD during overliming, various volumes of detoxified wastewater were used according to the following formula to have equal substrate in each digester:

TABLE 1
Characteristics of Baker's Yeast Wastewater

| *Parameter* | *Value* |
|---|---|
| Total COD (mg/lit) | 9,500–10,000 |
| $BOD_5$ (mg/lit) | 5,000–5,500 |
| Total VS (mg/lit) | 160–200 |
| Phenolic compounds (mg/lit) | 860–880 |
| Ammonium (mg/lit) | 320–350 |
| pH | 4.5–5 |

$$V(ml) = \frac{10000 \times 25\ (\text{ml})}{\text{COD}\ (\text{mg/l})},$$

where 10,000 and 25 ml refer to COD and volume of untreated baker's yeast wastewater, respectively. Moreover, 25 ml of untreated baker's yeast wastewater contained 0.25 g COD, which was used in all digestions. Furthermore, each reactor contained 20 ml of inoculum and 15 ml of distilled water for pH adjustment.

### 2.5. Analytical Methods

COD, BOD, total solid (TS), and volatile solid (VS) were measured based on the procedures given in the standard method for the examination of water and wastewater (APHA-AWWA-WEF, 1998). COD of the overlimed wastewaters was measured prior to digestion. Phenolic compounds and ammonium were measured by the ASTM D1783-91 and ASTM D3590-02 methods, respectively. The volume of the produced biogas was measured by surface moving water system and its composition was analyzed daily using a gas chromatograph (Sp-3420A, Porapack Q column, TCD detector, Beijing Beifen Ruili Analytical Instrument Co., Beijing, China). Helium was used as a carrier at a flow rate of 25 ml/min. Temperatures of column, injector, and detector were 50, 90, and 140°C, respectively.

## 3. RESULTS AND DISCUSSION

The detoxified and undetoxified wastewaters were subjected to anaerobic digestion in batch reactors. The results of accumulated produced methane are shown in Figure 1. The amount of biogas produced from the wastewater detoxified at 20°C reveals that the treatments at pH levels of 9 and 10 were not enough to make the wastewater suitable for biogas production (Figure 1a). However, with treatments at pH levels of 11 and 12, anaerobic digestion resulted in 426 and 360 ml $CH_4$ per g COD, respectively. Detoxification under the same period of time at 60°C (Figure 1b) resulted in successful biogas production. The wastewater detoxified for 2 h at 20°C and pH level 9 (Figure 1c) did not produce biogas. However, with treatments at pH levels of 10, 11, and 12, the biogas was successfully produced. All of the samples overlimed for 2 h at 60°C produced biogas, but in a lower amount compared to the wastewater treated at the same pH level at 20°C (Figure 1d). Detoxification at 20°C and pH = 10 resulted in the highest amount of biogas production, 410 ml $CH_4$/g COD, among the wastewaters detoxified for 2 h. All of the wastewaters experiencing the overliming conditions for 12 h resulted in successful production of biogas. Generally, the amount of biogas produced from these treated wastewaters detoxified at 60°C (Figure 1f) was lower than the amount of biogas produced from the one detoxified at 20°C (Figure 1e).

On the other hand, the alkali treatment resulted in a dramatic decrease in the COD value in some treated wastewaters. Considering economical reasons, the productivity is not the only parameter that should be considered for choosing the optimal conditions of detoxification.

Phenolic compounds are more easily oxidized when present in their corresponding ionized forms. Generally, phenol groups are ionized around a pH of 10 (25°C) (Horvath et al., 2005). The concentration of phenols decreased in most of the conditions used. Concentrations of phenolic compounds, ammonium, and COD for all of the detoxified wastewaters are shown in Table 2. The amount of ammonium and phenolic compounds are reported as the percentages relative to their amount in the untreated wastewater.

There is a considerable variation in the inhibition levels reported for most substances in the literature for anaerobic digestion. As ammonia concentrations were increased in the range of

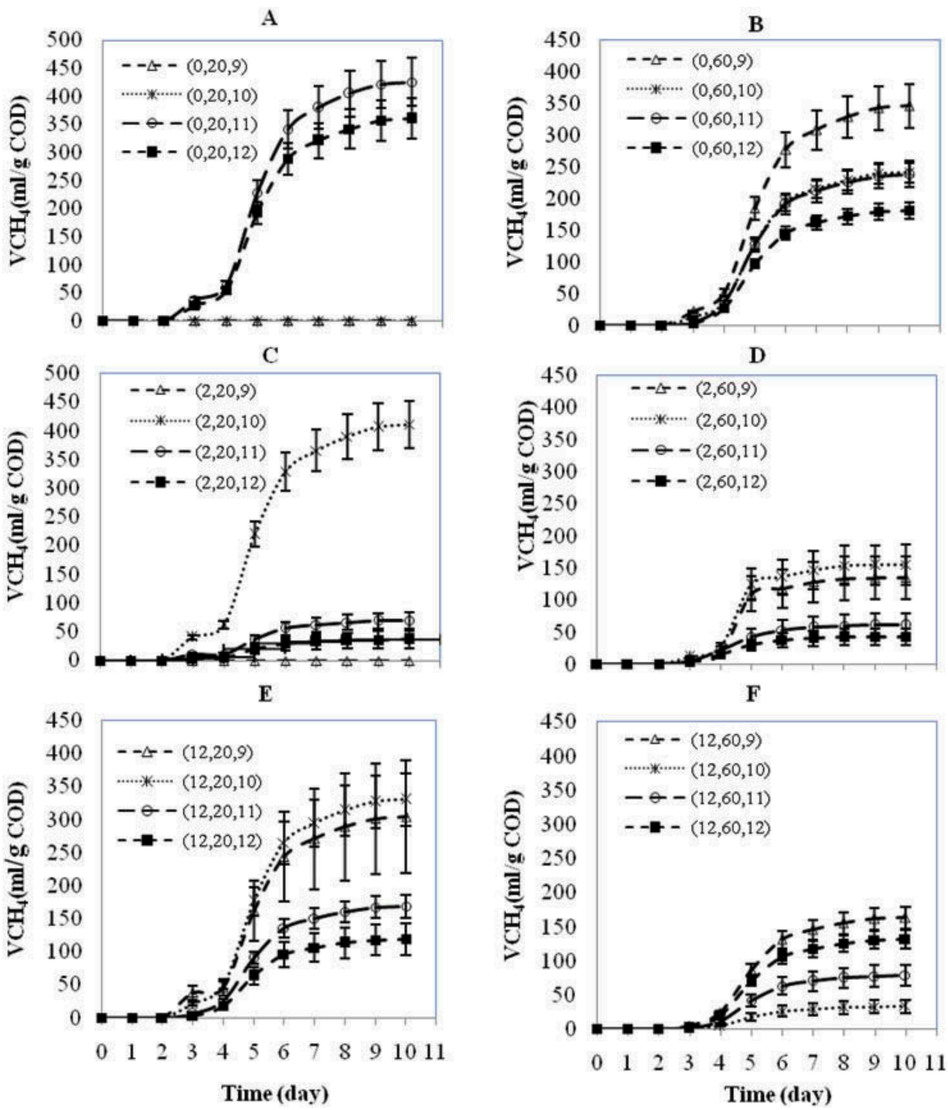


FIGURE 1 Accumulated methane production from undetoxified and detoxified wastewater in different conditions: (a) at 20°C for 0 h, (b) 60°C for 0 h, (c) 20°C for 2 h, (d) 60°C for 2 h, (e) 20°C for 12 h, and (f) 60°C for 12 h. The numbers in parentheses in the figures indicate time, temperature, and pH in overliming, respectively.

4,051–5,734 mg /L, the acidogenic populations in the granular sludge were hardly affected while the methanogenic population lost 56.5% of its activity. This parameter is approximately 0.5–10 mg/L for phenolic compounds (Chen et al., 2008). Measurement of phenolic compounds and free ammonia of untreated baker's yeast wastewater showed that the concentration of ammonia was not in the inhibitory range, but the concentration of phenolic compounds was in the inhibitory range and could affect the metabolism of anaerobic bacteria. These two parameters were measured for all the detoxified samples, results of which are shown in Table 2. These results indicate that over-liming is a promising method for elimination of phenolic compounds from baker's yeast

TABLE 2
Concentration of Phenolic Compound, Ammonium, COD, Lag Phase, and Maximum Speed of Biogas Production for Undetoxified and All Detoxified Wastewater

| *Overliming Conditions* | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Time, h* | *Temperature, °C* | *pH* | *Phenolic Compounds, %* | *Ammonium, %* | *COD, mg/lit* | *Lag phase, day* | *Max. $CH_4$ Prod. Speed, ml/g COD/day* |
| 0 | 20 | 9 | 54.6 | 80.9 | 8,465 | — | — |
| 0 | 20 | 10 | 43 | 48.4 | 7,395 | — | — |
| 0 | 20 | 11 | 59.3 | 67.5 | 6,357 | 2 | 165 |
| 0 | 20 | 12 | 72.1 | 82.2 | 5,287 | 2 | 140 |
| 0 | 60 | 9 | 43 | 61.9 | 7,027 | 2 | 125 |
| 0 | 60 | 10 | 38.4 | 48.1 | 6,056 | 2 | 70 |
| 0 | 60 | 11 | 90.2 | 96.6 | 5,086 | 2 | 70 |
| 0 | 60 | 12 | 104.8 | 95.6 | 4,149 | 3 | 50 |
| 2 | 20 | 9 | 72.9 | 58.4 | 8,633 | — | — |
| 2 | 20 | 10 | 38.8 | 57.3 | 7,462 | 2 | 155 |
| 2 | 20 | 11 | — | — | 6,290 | 2 | 20 |
| 2 | 20 | 12 | 89.1 | 87.5 | 5,119 | 2 | 10 |
| 2 | 60 | 9 | 91.2 | 77.5 | 6,792 | 3 | 90 |
| 2 | 60 | 10 | — | — | 5,789 | 3 | 100 |
| 2 | 60 | 11 | — | — | 4,785 | 3 | 20 |
| 2 | 60 | 12 | — | — | 3,814 | 3 | 10 |
| 12 | 20 | 9 | — | — | 6,290 | 2 | 100 |
| 12 | 20 | 10 | — | — | 5,856 | 2 | 100 |
| 12 | 20 | 11 | — | — | 5,421 | 3 | 60 |
| 12 | 20 | 12 | — | — | 4,986 | 3 | 45 |
| 12 | 60 | 9 | — | — | 6,324 | 3 | 55 |
| 12 | 60 | 10 | — | — | 5,421 | 4 | 10 |
| 12 | 60 | 11 | — | — | 4,517 | 3 | 30 |
| 12 | 60 | 12 | — | | 3,614 | 3 | 45 |
| Untreated wastewater | | | 100 | 100 | 9,369 | — | — |

wastewater. The maximum value of this elimination was 61.2%, resulting in maximum biogas production (2, 20, and 10).

For an explicit comparison between results, "absolute yield" was used to represent the amount of produced biogas from "1 liter" of detoxified baker's yeast wastewater. This parameter was introduced to make a combined assessment of the effect of the detoxification treatment on both COD losing and removing of inhibitors. Absolute yields of all the detoxified samples are shown in Figure 2.

Results showed that anaerobic digestion of detoxified baker's yeast wastewater in moderate conditions of overliming resulted in a higher yield of biogas compared to soft and harsh conditions. Similar results have been observed from detoxification of lignocellulosic materials' hydrolyzate where medium conditions of overliming were reported as the optimum conditions (Millati et al., 2002). The results also proved that different overliming conditions are effective in the lag phase of biogas production and its rate. These two parameters are shown in Table 2. In general, the rate of biogas production is related to biogas yield and the maximum biogas production rate is relevant to the samples that also have the maximum biogas yield.

Overliming at 20°C and pH level of 11 for 0 h resulted in the maximum biogas production (425 ml $CH_4$/g COD). Furthermore, the amount of biogas produced from the sample

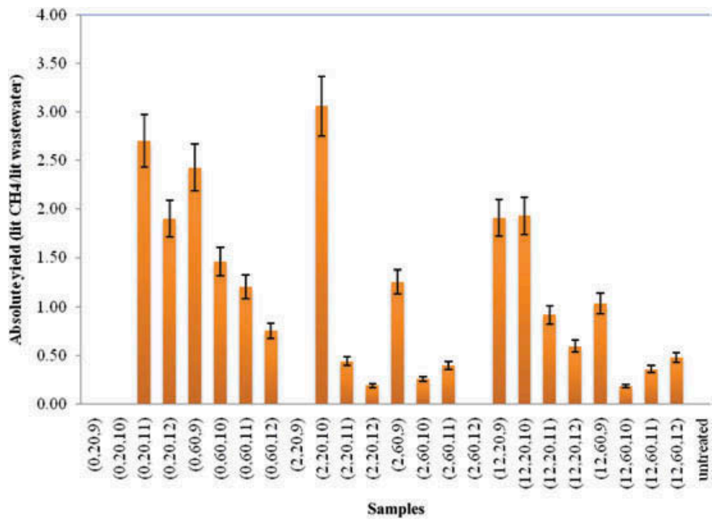


FIGURE 2 Absolute yield (the amount of produced biogas from each liter of detoxified baker's yeast wastewater). The numbers in parentheses in the figures indicate time, temperature, and pH in overliming, respectively.



overlimed at 20°C and a pH level of 10 for 2 h was 410.4 ml $CH_4$/g COD, whose maximum gas production speed of 165 ml $CH_4$/g COD/day was the highest rate among all the treated wastewaters. Also, this substrate ranked second in biogas production yield. The maximum absolute yields of methane production were 3.06 l $CH_4$/l wastewater and 2.71 l $CH_4$/l wastewater for the overliming conditions at 20°C, pH 10, for 2 h and 20°C, pH 11, for 0 h, respectively. In these optimum conditions, the amount of inhibitors is lower than their minimum toxic concentration, and the percentage of eliminated COD is minimal, too, resulting in maximum biogas yield. In this research, alongside producing biogas as a renewable source of energy and also reducing the COD, the detoxification process was dependent on pH and temperature. The effect of the detoxification process depends on pH and temperature. Addition of $Ca(OH)_2$ affected the pH level as did the supply of cation. The more the cation was supplied, the higher and faster the conversion was. At low temperature, the reaction between cation and reactant was instantaneous to produce a complex ion.

Results show that overliming is an effective method for the detoxification of baker's yeast wastewater and increasing biogas yield. The most significant effect of overliming was a sharp decrease in the concentration of anaerobic digestion inhibitors, such as phenolic compounds from the wastewater.

## 4. CONCLUSION

It can be concluded that treatment of baker's yeast wastewater can be significantly improved by overliming. However, the effectiveness of the process highly depends on the operational parameters, i.e., time, pH, and temperature. The mechanism of improvement is detoxification of inhibitors available in the wastewater. Phenolic compounds are the main inhibitors existing in the wastewater whose inhibition can be overcome by overliming. The best overliming conditions were treatment at room temperature and pH = 11.

## ACKNOWLEDGMENTS

The authors gratefully acknowledge Ilona Sarvari Horvath at the University of Borås for the scientific support, Mr. Harati and Mr. Gholami at Isfahan Municipal Wastewater Treatment for providing the inoculum, and Mr. Safi at Shahrekord Baker's Yeast factory for providing the baker's yeast wastewater.


## REFERENCES


APHA-AWWA-WEF. 1998. *Standard Methods for the Examination of Water and Wastewater*, 20th ed. Washington, DC: American Public Health Association.

Chen, Y., Cheng, J. J., and Creamer, K. S. 2008. Inhibition of anaerobic digestion process: A review. *Bioresour. Technol*. 99:4044–4064.

Deveci, N., and Ciftçi, G. 2001. A mathematical model for the anaerobic treatment of Baker's yeast effluents. *Waste Manage*. 21:99–103.

European Parliament. 2003. *Directive 2003/30/EC of the European Parliament and of the Council of* 8 May 2003 *on the promotion of the use of biofuels or other renewable fuels for transport*. Brussels, Belgium: European Parliament.

Hansen, T. L., Jansen, J. C., Davidsson, A., and Christensen, T. H. 2007. Effects of pre-treatment technologies on quantity and quality of source-sorted municipal organic waste for biogas recovery. *Waste Manage*. 27:398–405.

Horváth, I. S., Sjöde, A., Alriksson, B., Jönsson, L. J., and Nilvebrant, N. O. 2005. Critical conditions for improved fermentability during overliming of acid hydrolysates from spruce. *Appl. Biochem. Biotechnol*. 124:1031–1044.

Kavasic, B., and Topaloglu, B. 2010. Biogas production from co-digestion of a mixture of cheese whey and dairy manure. *Biomass Bioenergy* 34:1321–1329.

Kobya, M., and Delipinar, S. 2008. Treatment of the baker's yeast wastewater by electrocoagulation. *J. Hazard. Mater*. 154:1133–1140.

Komemoto, K., Lim, Y. G., Nagao, N., Onoue, Y., Niwa, C., and Toda, T. 2009. Effect of temperature on VFA's and biogas production in anaerobic solubilization of food waste. *Waste Manage*. 29:2950–2955.

Larsson, S., Reimann, A., Nilvebrant, N. O., and Jönsson, L. J. 1999. Comparison of different methods for the detoxification of lignocellulose hydrolyzates of spruce. *Appl. Biochem. Biotechnol*. 77–79:91–103.

Millati, R., Niklasson, C., and Taherzadeh, M. J. 2002. Effect of pH, time and temperature of overliming on detoxification of dilute-acid hydrolyzates for fermentation by *Saccharomyces cerevisiae. Process. Biochem*. 38:515–522.

Mussatto, S. I., and Roberto, I. C. 2004. Alternatives for detoxification of diluted-acid lignocellulosic hydrolyzates for use in fermentative processes: A review. *Bioresour. Technol*. 93:1–10.

Palmqvist, E., and Hägerdal, H. B. 2000. Fermentation of lignocellulosic hydrolysates. I: Inhibition and detoxification. *Bioresour. Technol*. 74:17–24.

Yadvika, S., Sreekrishnan, T. R., Kohli, S., and Rana, V. 2004. Enhancement of biogas production from solid substrates using different techniques—A review. *Bioresour. Technol*. 95:1–10.

Zak, Z. 2005. The use of Fenton's system in the yeast industry wastewater treatment. *Environ. Technol*. 26:11–19.

Zheng, M., Li, L., Li, X., Xiong, J., Mei, T., and Chen, G. 2010. The effects of alkaline pretreatment parameters on anaerobic biogasification of corn stover. *Energy Sources, Part A* 32:1918–1925.

Zub, S., Kurissoo, T., Menert, A., and Blonskaja, V. 2008. Combined biological treatment of high-sulphate wastewater from yeast production. *Water Environ. J*. 22:274–286.