Effect of leachate inoculum on biopretreatment of municipal solid waste by a combined hydrolytic-aerobic process

ZHANG Dongqing, HE Pinjing*, SHAO Liming

State Key Laboratory of Pollution Control and Resources Reuse, Key Laboratory of Yangtze River Water Environment, College of Environmental Science and Engineering, Tongji University, Shanghai 200092, China. E-mail: xhpjk@tongji.edu.cn

Received 21 August 2008; revised 30 September 2008; accepted 20 October 2008

Abstract

The biopretreatment of municipal solid waste by a combined hydrolytic-aerobic process has become of great interest for biostabilization or biodrying. The study aimed at investigating the effect of leachate inoculum on the biopretreatment. During the hydrolytic stage, the inoculum addition at the ratio of leachate to waste (LWR) of 5% and 7% stimulated the start-up of hydrolysis and enhanced the hydrolysis rate by 27.4% and 24.2%, respectively; whereas the inoculation at LWR of 1% had almost no effect on the hydrolysis rate and the inoculation at LWR of 10% reduced the hydrolysis rate by 12.7%. During the subsequent aerobic stage, the inoculations at LWRs greater than 5% decreased organics degradation rate. As a whole, compared with none inoculation, the total degradation rates of organics for inoculating trials at LWRs of 5%, 7% and 10% decreased by 14.5%, 14.3% and 32.7%, respectively. Correspondingly, their net water removal rates were reduced by 4.4%, 5.8% and 19.0%. The inoculation at LWR of 1% could not significantly affect the biopretreatment. The inoculation at LWR of 5% and 7% could shorten hydrolytic stage and thus accelerate the whole combined process. Moreover, the inoculations at LWRs greater than 5% were favorable for lignocelluloses degradation.

Key words: biopretreatment; hydrolysis-aeration; inoculation; leachate; municipal solid waste

DOI: 10.1016/S1001-0742(08)62397-9

Introduction

Nowadays, the biopretreatment of municipal solid waste (MSW) is becoming an increasing option before either landfiling or combustion (Adani et al., 2002; Norbu et al., 2005; Calabro et al., 2007; Nguyen et al., 2007; Rada et al., 2007). For MSW with a high ratio of water content to biodegradable organics, a combined hydrolytic-aerobic process has been proposed (Bezama et al., 2007; Zhang et al., 2008a). The combined process is characterized by supplementing a hydrolytic stage prior to the aerobic degradation, so that during this stage, lots of cell-contained water will be released with less organics consumption due to the destruction of cell wall or membrane. In this way, the ratio of water content to biodegradable organics is expected to be lowered during the hydrolytic stage, which will be favorable for the degradation of organics during the subsequent aerobic stage.

In order to initiate and/or accelerate the process of aerobic degradation or anaerobic digestion of organics, inocula were usually applied (Staley et al., 2006; Vargas-Garcia et al., 2007; Wei et al., 2007; Wang and Wu, 2008) and the effectiveness depended on the ecology of inoculating microorganisms. Many studies have found that the inoculation dosing is a controlling factor on the processes (Fernandez et al., 2001; Barrena et al., 2006; Raposo et al., 2006; O’Sullivan et al., 2008). For the combined process, Zhang et al. (2008a) has shown that the addition of biopretreatment products had almost no positive effect on the biopretreatment performance. Nevertheless, the leachate generated in the hydrolytic stage of the combined process is rich in indigenous microorganisms with high hydrolysis ability. These microbes can be inoculated to strengthen the hydrolysis during the hydrolytic stage for biopretreatment. However, for MSW with high water content, it is unclear how the addition of leachate inoculum influences the hydrolytic stage and subsequent aerobic stage of the combined process, and it is needed to find a suitable ratio of leachate to waste (LWR).

This study investigated the effects of the addition of leachate inoculum on the organics degradation and water removal during biopretreatment. The activities of enzymes and quantity of microorganisms were monitored to evaluate the performance of biopretreatment from the points of microbial metabolism.

1 Materials and methods

1.1 Characteristics of MSW feedstock and inoculating leachate

The MSW feedstock was sampled from a residential...
area in Shanghai, China. It comprised 60% (W/W, in wet weight) of kitchen waste, 23% (W/W) of paper, 11% (W/W) of plastics and 6% (W/W) of the others. The initial water content was 72% (W/W). Plastics, glasses and metals were removed from wastes. The biochemical composition and the number of microbial populations of samples are shown in Table 1. The inoculating leachate was generated from the hydrolytic stage of the combined process in the former experiment and stored at 4°C before use (within 7 d).

1.2 Experimental equipment

The trials were performed in column reactors, as previously described by Zhang et al. (2008b). Each column was 1200 mm in height and 400 mm in internal diameter. The outer wall of the column was wrapped with 100-mm-thick hollow cotton for thermal insulation. A 100 mm high layer, filled with crockery balls (about 5 mm in diameter), was placed at the bottom of each column for leachate drainage and air distribution. Above the balls, a perforated baffle (2-mm mesh) was placed to support the waste and to facilitate aeration. Two layers, straw and cotton, covered the waste to avoid heat loss and vapor condensation. A whirlpool pump (XGB-8, Penghu Co., Shanghai, China) and a gas-flow meter (LZB-10, Shanghai Instrument Co., Shanghai, China) were used for aeration.

1.3 Experimental setup and operation

The whole experiment lasted for 16 d and was separated into two stages, i.e., the hydrolytic and aerobic stages. The air-inflow rate was fixed at 0.056 m³ per kg wet wastes per hour during the whole experiment. During the hydrolytic stage (0–4 d), the ventilation interval was 10 min run/230 min stop. During the aerobic stage (5–16 d), the ventilation intervals of all trials were enhanced to 7 min run/23 min stop and the fed wastes were manually turned every 2 d. During the aerobic stage, the ventilation interval was reduced to 7 min run/7 min stop. Every 2 d when the fed materials were turned, samples of about 300 g were collected according to quarter method for analysis. The analyses for all samples were carried out in triplicates with standard deviations less than 10%. Moreover, the calibration curves were established each time when the activities of extracellular enzyme were determined.

1.4 Experimental monitoring

Temperature, O₂ concentration and leachate quantity were measured 6 times per day. Temperature was monitored by a thermometer (WMY-01C, Huachen Co., Shanghai, China) with sensor probes located at the top, middle and bottom points along the longitudinal axis of the column, and the average value was reported. A probe for measuring the O₂ concentration (CYS-1, Xuelian Co., Shanghai, China) was set at the central point along the longitudinal section of the waste body to measure O₂ concentration before ventilations. The leachate produced from the column was collected and weighed.

1.5 Sampling and analytical methods

Every 2 d when the fed materials were turned, samples of about 300 g were collected according to quarter method for analysis. The analyses for all samples were carried out in triplicates with standard deviations less than 10%. Moreover, the calibration curves were established each time when the activities of extracellular enzyme were determined.

1.5.1 Physicochemical analysis

The pH and total organic carbon (TOC) of the collected leachate were determined using a pH meter (pHS-2F, Shanghai Precision Scientific Instrument Co., Ltd., Shanghai, China) and a total carbon/total nitrogen analyzer (multi N/C 3000, Analytikjena, Germany).

The water content of the wastes was determined by air-drying at 70°C for 48 h. Volatile solid was analyzed by loss on ignition at 550°C to the constant weight. The determination of celluloses, hemicelluloses and lignins was based on the measurement of neutral detergent fiber, acid detergent fiber and ash contents of the samples (Faithfull, 2002). For the measurement of amyloins, the air-dried solid sample was first digested by aether and ethanol and boiled HCl solution (6 mol/L) in sequence, and then titrated with alkaline copper tartrate (Faithfull, 2002). The lipids concentration was determined gravimetrically after Soxlett extraction with petroleum ether (DLUT, 1994). The proteins content was determined using the Kjeldahl nitrogen (KN). The measured KN was multiplied by 6.25 to obtain the proteins content (APHA, 1998).

1.5.2 Assay of extracellular enzyme activities

For the determination of the extracellular enzyme activities, 15 g of the fresh solid sample was immersed in 15 mL of a 0.9% NaCl solution and centrifuged twice at 5000 × g for 30 min. The supernatant was collected. The pellet was immersed by suspension in 30 mL potassium dihydrogen phosphate buffer (pH 7.2, 0.1 mol/L) for 1 h and then centrifuged at 5000 × g for 30 min to get the supernatant again. Both of the collected supernatants were mixed for the assay of enzyme activities (Zhang et al., 2007).

Amylase, filter paper cellulase (FPase) and carboxymethyl cellulase (CMCase) activities were analyzed by testing the generation rate of glucoses from enzymolysis under different incubation conditions. The amylase activity was measured using the modified method of

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Initial characteristic of sampled wastes and inoculating leachate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td>Sampled wastes</td>
</tr>
<tr>
<td>Amylum (g/g TS)</td>
<td>0.42 ± 0.028</td>
</tr>
<tr>
<td>Protein (g/g TS)</td>
<td>0.12 ± 0.013</td>
</tr>
<tr>
<td>Lipid (g/g TS)</td>
<td>0.12 ± 0.009</td>
</tr>
<tr>
<td>Cellulose (g/g TS)</td>
<td>0.15 ± 0.009</td>
</tr>
<tr>
<td>Hemicellulose (g/g TS)</td>
<td>0.01 ± 0.001</td>
</tr>
<tr>
<td>Lignin (g/g TS)</td>
<td>0.05 ± 0.007</td>
</tr>
<tr>
<td>Ash (g/g TS)</td>
<td>0.13 ± 0.008</td>
</tr>
<tr>
<td>TOC (mg/L)</td>
<td>–</td>
</tr>
<tr>
<td>Bacteria (CFU/g DM or mL)</td>
<td>1.91 × 10⁹</td>
</tr>
<tr>
<td>Celluloses degrader (CFU/g DM or mL)</td>
<td>2.18 × 10⁶</td>
</tr>
</tbody>
</table>

TS: total solid; TOC: total organic carbon; CFU: colony forming units; DM: dry materials.
Bernfeld (1955). The CMCase activity was determined according to the method introduced by Nakamura and Kita-
tamura (1988). The FPase activity was measured following the
method of Ghose (1987). The protease activity was
measured as described by Lowry et al. (1951).

1.5.3 Microbiological assay
The number of bacteria and celluloses degraders was
analyzed using the standard dilution-plating method (Wei
et al., 2007). To obtain the leached solution, 10 g fresh
samples were added into 90 mL sterile water and shaken for
20 min. Subsequently, a serial 10-fold dilution was
made for determination. Each suitable dilution (1.0 mL)
was cultivated on yeast peptone glucose agar for bacteria
and Congo red medium for celluloses degraders. All mi-
crobes were incubated to enumerate the colony forming
units (CFU) at 30°C (2 and 9 d for bacteria and celluloses
degraders, respectively).

1.6 Statistical analysis
All statistical analyses were performed using SPSS 16.0
(SPSS, Inc., Chicago, USA). The statistically significant
difference between the two groups was compared by t-
test. The differences presented were confirmed at a 95%
confidence level.

2 Results and discussion

2.1 Oxygen concentration and temperature during bio-
pretreatment
Under a given ventilation interval and air-flow rate, the
O2 concentration in the free space of the columns just
before ventilation could indicate oxic degradation level of
organics, to some extent (Zhang et al., 2008a). Figure 1
presents the temporal evolution of the O2 concentrations
before ventilation and pH of the leachate in the hydrolytic
stage. After a first rapid drop, the O2 concentrations of all
five trials remained at a low level (< 30 mL/L) during the
hydrolytic stage, since the air input was greatly lower than
the quantity required for sufficient aerobic degradation.
During the aerobic stage, the 10% trial had the highest O2
concentrations before ventilation, followed by the 7% and
5% trials. The lowest O2 concentrations before ventilation
were observed for the 1% trial and the control. In the
hydrolytic stage, pH of the leachate increased and followed
a decreasing order of the control and 1% trial > 5% and 7%
trials > 10% trial (Fig. 1b).

As the same, the temperature was also an important
indicator of the organics degradation level. During the
hydrolytic stage, the temperatures of all five trials main-
tained a plateau value of 35°C due to the limited O2
supply, whereas during the aerobic stage, the temperatures
underwent three phases: heating phase, thermophilic phase
and cooling phase. Furthermore, the temperatures inverse-
ly varied corresponding to the O2 concentrations before
ventilation during this stage (Figs. 1 and 2).

The lower O2 concentrations before ventilation or higher
temperatures during the aerobic stage implied a higher
biological activity or higher organics degradation level.

2.2 Microbial populations and enzyme activities during biopretreatment
The substrates were degraded by microorganisms which
were active in the hydrolysis of macromolecular sub-
strates and in the degradation of monomer hydrolytes.
The metabolism of microorganisms was related not only
to the quantity and their ability to degrade corresponding
substrates, but also to substrates availability and environ-
mental conditions (such as pH, O2 and water content)
for microbial growth. Bacteria were mainly responsible
for the degradation of putrescible substrates (Haug, 1993;
Sole-Mauri et al., 2007). The celluloses degraders referred
to those microorganisms degrading lignocelluloses, e.g.,
most of fungi, actinomycetes, and so on. Amylase and
protease could indicate the microbial ability to hydrolyze
putrescible substrates; whereas FPase and CMCase corre-
sponded to the hydrolysis of lignocelluloses.

Figures 3 and 4 indicate the temporal evolution of the
microbial populations and enzyme activities during
biopretreatment. The addition of leachate inoculum for
the 1% trial could not lead to a significant variation of
both microbial populations and enzyme activities during
the whole process, due to a small quantity of inoculating
microorganisms. During the hydrolytic stage, the 5% and
7% trials had the amount of bacteria similar to that of
the control \((p > 0.05)\); however the inoculation for these two trials obviously enhanced the amylase activities at day 2 (Fig. 4a), suggesting that the addition of leachate inoculum at LWRs of both 5% and 7% stimulated the commencement of amylums hydrolysis. Nevertheless, the inoculation for the 10% trial evidently reduced either the bacteria number or the activities of amylase and protease during the hydrolytic stage. This could be attributed to the serious acid inhibition. At the end of the hydrolytic stage, pH was only 4.97 for 10% trial; in contrast, it was 5.95 for control. The serious acid environment was unfavorable for the proliferation of the hydrolysis microorganisms and therefore inhibited the hydrolysis during the hydrolytic stage (Vavilin et al., 2008). During the subsequent aerobic stage, the amount of bacteria and the activities of amylase and protease were both affected by the properties of substrates and by the environmental conditions at the end of hydrolytic stage. For the inoculating trails at LWRs greater than 5%, the end of hydrolytic stage, unsuitable FAS (i.e., free air space) due to the addition of leachate and lower pH values (5.59 for 5% trial and 5.46 for 7% trial) would further inhibit the aerobic metabolism during the subsequent aerobic stage. Thereby, it was no wonder that during the aerobic stage, both the numbers of bacteria and the activities of enzymes for the inoculating trails at LWRs greater than 5% were lower than those for control. It is worth mentioning that the inoculation for 5% and 7% trials could not enhance protease activities significantly during the whole biopretreatment, as presented in Fig. 4b.

Interestingly, all the inoculating trials except 1% trial evidently stimulated the proliferation of cellulose degraders and enhanced the activities of cellulase (especially CMCase), indicating that the inoculation was favorable for the degradation of recalcitrant organics. The possible interpretation was that the leachate inoculum was rich in indigenous cellulose degraders with intensive ability of hydrolyzing lignocelluloses (Table 1). Moreover, there were similar quantity of cellulose degraders and activity of cellulase for the inoculating trails at LWRs greater than 5%, suggesting that the lignocelluloses degradation was no longer limited by the amount of cellulose degraders at these higher LWRs.

### 2.3 Balance of water and organics losses

The balance of organics and water losses is presented in Fig. 5. The calculation equations are listed in the appendix. As a whole, the net water removal rate (excluding the amount of leachate added into the reactor) varied correspondingly to the organics degradation rate. During the hydrolytic stage, the cell walls or membranes of putrescible organics were destructed to release the cell-contained water in the form of leachate, thus the water removal rate was mostly determined by the organics hydrolysis rate. During the aerobic stage, the water was mainly removed by evaporation and the removal capacity was determined by the heat energy produced by the aerobic degradation of organic matters under the given ventilation interval and air-flow rate.

The inoculation for 1% trial had almost no effects on the organics hydrolysis and net leachate production rates during the hydrolytic stage and then on the degradation rate during the subsequent aerobic stage. For 5% and 7% trials, the addition of leachate inoculum stimulated the start-up of hydrolysis, indicated by the fact that their hydrolysis rates at day 2 were 54.4% and 58.1% higher than that for the control, respectively. Moreover, at the end of the hydrolytic stage, the hydrolysis rate was enhanced by 27.4% for 5% trial and 24.2% for 7% trial. Correspondingly, the net leachate production rates were improved by 19.1% and 20.1%, respectively.

![Fig. 3](https://example.com/fig3.png)

**Fig. 3** Evolution of the numbers of bacteria and cellulose degraders during biopretreatment. (a) bacteria; (b) cellulose degraders.
the inoculation was also favorable for the start-up of hydrolysis of specific substrates (Figs. 4a, 4c, and 4d), the organics hydrolysis rate for the 10% trial was reduced by 12.7% during the hydrolytic stage, causing the net leachate production rate lowered by 16.6%. For these trials at LWRs greater than 5%, the inoculation was favorable for the degradation of recalcitrant organics, but this could not offset the adverse effects on the degradation caused by the leachated. Therefore, during the subsequent aerobic stage, lower degradation and water removal rates were obtained for these trials. As a whole, the total organics degradation rates for 5%, 7% and 10% trials were reduced by 14.5%, 14.3% and 32.7%, respectively. Correspondingly, their net water removal rates were descended by 4.4%, 5.8% and 19.0%, respectively.

In summary, the inoculum addition at a suitable LWR could stimulate the start-up of hydrolysis and enhance the hydrolysis rate during the hydrolytic stage. This could shorten the hydrolytic stage and then accelerate the whole process. However, the addition of leachate inoculum was unfavorable for the degradation and water removal during the subsequent aerobic stage, since the aerobic metabolism was inhibited. Therefore, the future work should focus on improving the properties of substrates and the environmental conditions at the end of the hydrolytic stage.

3 Conclusions

Leachate inoculations had various effects on the organics hydrolysis rate during the hydrolytic stage of the combined hydrolysis-aerobic process, which strongly depended on LWR. Compared with none inoculation, the inoculation at LWR of 1% had almost no effects on the improvement of hydrolysis and total degradation rates of organics. The addition of leachate inoculum at LWR of 5% or 7% stimulated the start-up of hydrolysis and thus enhanced the hydrolysis rate by 27.4% and 24.2%, respectively. The inoculation at LWR of 10% reduced the organics hydrolysis rate by 12.7%. Nevertheless, all the inoculating trials at LWRs greater than 5% reduced the organics degradation rate during the subsequent aerobic stage, although the degradation of recalcitrant organics would be strengthened. As a whole, the total organics degradation rates for inoculating trials at LWRs of 5%, 7% and 10% were decreased by 14.5%, 14.3% and 32.7%, respectively. Correspondingly, their net water removal rates were descended by 4.4%, 5.8% and 19.0%. Therefore, the addition of leachate inoculum could shorten the hydrolytic stage and then accelerate the whole process. The improvement of the properties of substrates and environmental conditions at the end of the hydrolytic stage should be concerned in the future work.
Acknowledgments

This work was supported by the National Key Technology R&D Program of China (No. 2006BAC06B04, 2006BAC02A03) and the Key Grant Project of Shanghai Committee of Science and Technology (No. 06dz12308).

Appendix

The water losses at time $t$ ($W_{\text{loss}}^t$, kg) are calculated by:

$$W_{\text{loss}}^t = (WM_0 \times w_0) - (WM_t \times w_t)$$

where, $WM_0$ (kg) and $WM_t$ (kg) are the wet materials at the initial and time $t$, respectively, $w_0$ (%) and $w_t$ (%) are water contents at initial time and time $t$, respectively.

The total organics losses at time $t$ ($\text{TO}_{\text{loss}}^t$, kg) are given by:

$$\text{TO}_{\text{loss}}^t = WM_0 - WM_t - W_{\text{loss}}^t$$

The organics degradation losses at time $t$ ($\text{DO}_{\text{loss}}^t$, kg) are calculated by:

$$\text{DO}_{\text{loss}}^t = \text{TO}_{\text{loss}}^t - \text{LO}_{\text{loss}}^t$$

The hydrolysis rate ($R_h$, %) during the hydrolytic stage is calculated by:

$$R_h = \frac{\text{TO}_{\text{loss}}^t}{\text{OM}_0}$$

The organics losses into leachate ($\text{LO}_{\text{loss}}^t$, kg) are calculated by:

$$\text{LO}_{\text{loss}}^t = \text{accumulated TOC}_t/C_t$$

where, accumulated $\text{TOC}_t$ (kg) is the accumulated total organic carbons in leachate at time $t$, $C_t$ (%) is the carbon content of MSW at time $t$.

The water losses at time $t$ are calculated as follows:

- **Control:** $32.0$ kg to $2.02$ kg
- **1% trial:** $32.0$ kg to $2.40$ kg
- **5% trial:** $32.0$ kg to $2.45$ kg
- **7% trial:** $32.0$ kg to $2.45$ kg
- **10% trial:** $32.0$ kg to $2.02$ kg

Fig. 5  Balance of organics and water losses during biopretreatment.
The degradation rate ($R_d$) during the aerobic stage is calculated by:

$$R_d = \frac{{(TO_{loss}^{16} - TO_{loss}^4) / OM_0}}{OM_0}$$

(6)

where, $TO_{loss}^{16}$ (kg) and $TO_{loss}^4$ (kg) are the total organics losses at day 16 and day 4, respectively.

The total degradation rate ($R_{total}$, %) of organics is calculated by:

$$R_{total} = \frac{{TO_{loss}^{16} / OM_0}}{OM_0}$$

(7)

References


