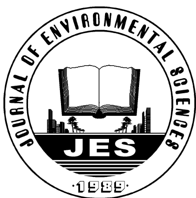

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Effect of leachate inoculum on biopretreatment of municipal solid waste by a combined hydrolytic-aerobic process

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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 inoculum addition 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

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Introduction

Nowadays, the biopretreatment of municipal solid waste (MSW) is becoming an increasing option before either landfilling 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-Garcła *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

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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 m^3 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. Raw MSW sample 32 kg was loaded into each column and the leachate inoculum was sprayed on the MSW feedstock during the loading. LWRs varied for different trials were 1%, 5%, 7%, 10% (*W/W*, wet weight), respectively. In addition, a trial without leachate inoculation was set-up as a control.

Table 1 Initial characteristic of sampled wastes and inoculating leachate

Parameter	Sampled wastes	Inoculating leachate
Amylum (g/g TS)	0.42 ± 0.028	–
Protein (g/g TS)	0.12 ± 0.013	–
Lipid (g/g TS)	0.12 ± 0.009	–
Cellulose (g/g TS)	0.15 ± 0.009	–
Hemicellulose (g/g TS)	0.01 ± 0.001	–
Lignin (g/g TS)	0.05 ± 0.007	–
Ash (g/g TS)	0.13 ± 0.008	–
TOC (mg/L)	–	1.80×10^4
Bacteria (CFU/(g DM or mL))	1.91×10^9	7.00×10^9
Celluloses degrader (CFU/(g DM or mL))	2.18×10^6	1.00×10^7

TS: total solid; TOC: total organic carbon; CFU: colony forming units; DM: dry materials.

1.4 Experimental monitoring

Temperature, O_2 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 O_2 concentration (CYS-1, Xuelian Co., Shanghai, China) was set at the central point along the longitudinal section of the waste body to measure O_2 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 detected 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 amylums, the air-dried solid sample was first digested by aether, 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

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Bernfeld (1955). The CMCase activity was determined according to the method introduced by Nakamura and Kitamura (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 microbes 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 biopretreatment

Under a given ventilation interval and air-flow rate, the O_2 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 O_2 concentrations before ventilation and pH of the leachate in the hydrolytic stage. After a first rapid drop, the O_2 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 O_2 concentrations before ventilation, followed by the 7% and 5% trials. The lowest O_2 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 maintained a plateau value of 35°C due to the limited O_2 supply, whereas during the aerobic stage, the temperatures underwent three phases: heating phase, thermophilic phase and cooling phase. Furthermore, the temperatures inversely varied corresponding to the O_2 concentrations before ventilation during this stage (Figs. 1 and 2).

The lower O_2 concentrations before ventilation or higher temperatures during the aerobic stage implied a higher biological activity or higher organics degradation level.

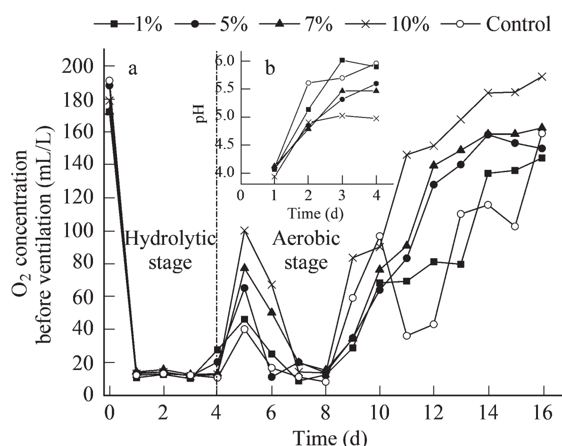

Fig. 1 Temporal evolution of O_2 concentration in the free space of the columns before ventilation during biopretreatment and pH of the leachate in the hydrolytic stage. (a) O_2 concentration; (b) pH.

2.2 Microbial populations and enzyme activities during biopretreatment

The substrates were degraded by microorganisms which were active in the hydrolysis of macromolecular substrates 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 environmental conditions (such as pH, O_2 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 corresponded 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

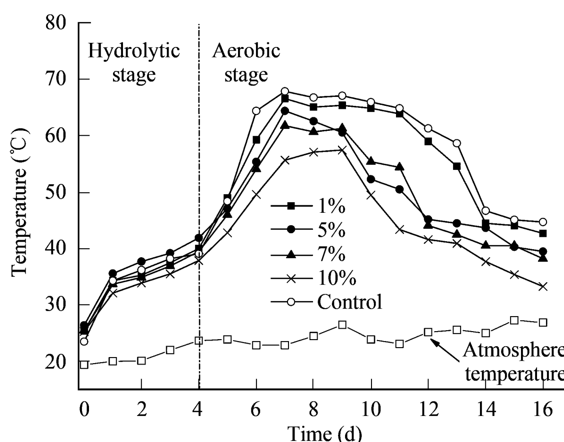

Fig. 2 Temporal evolution of temperature during biopretreatment.

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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%, at 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 trials 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. Although

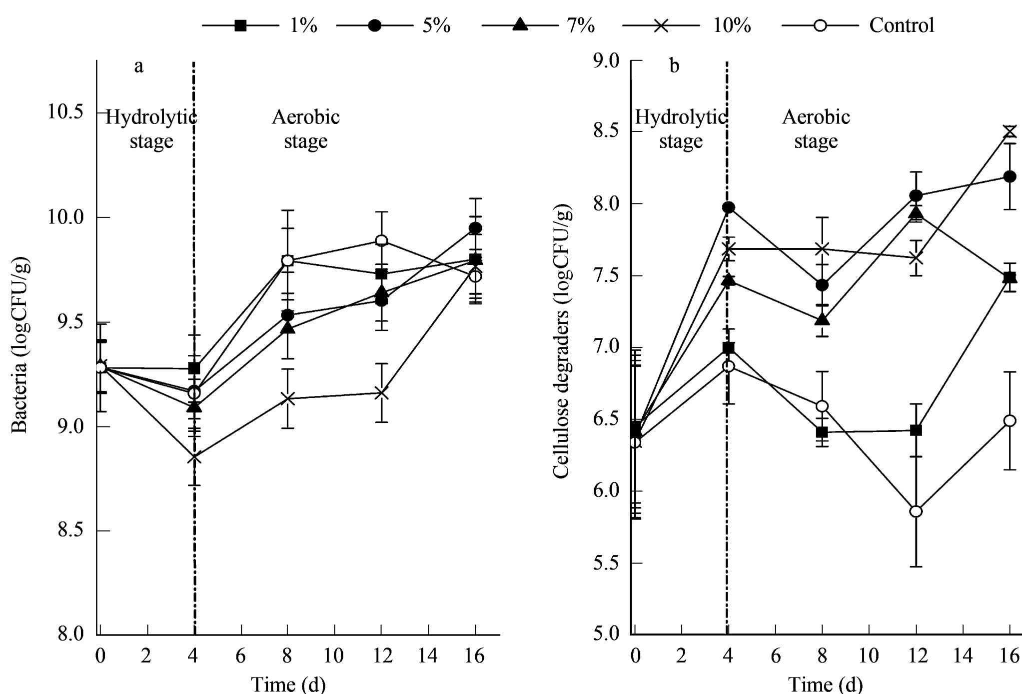

Fig. 3 Evolution of the numbers of bacteria and cellulose degraders during biopretreatment. (a) bacteria; (b) cellulose degraders.

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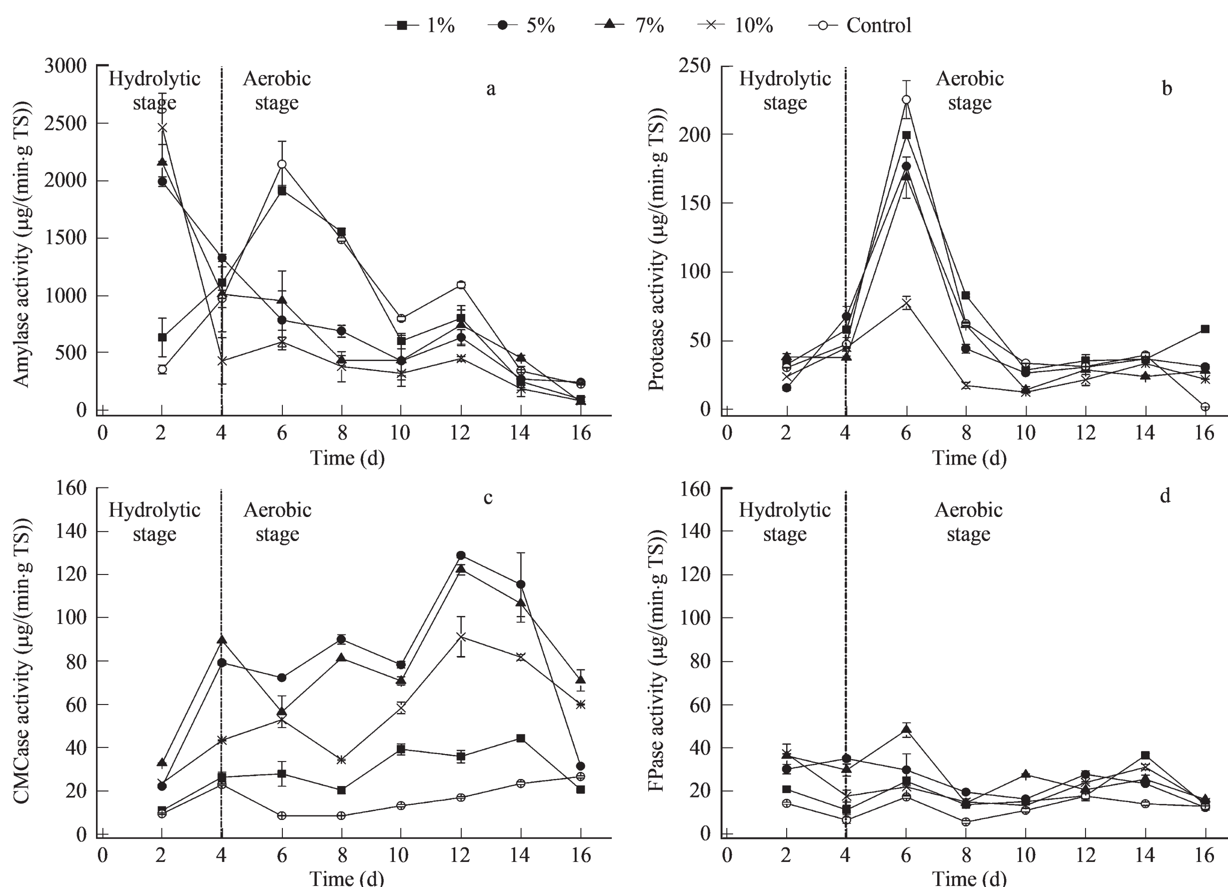

Fig. 4 Temporal evolution of amylase, protease, CMCase and FPase activities during biopretreatment. (a) amylase; (b) protease; (c) CMCase; (d) FPase.

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.

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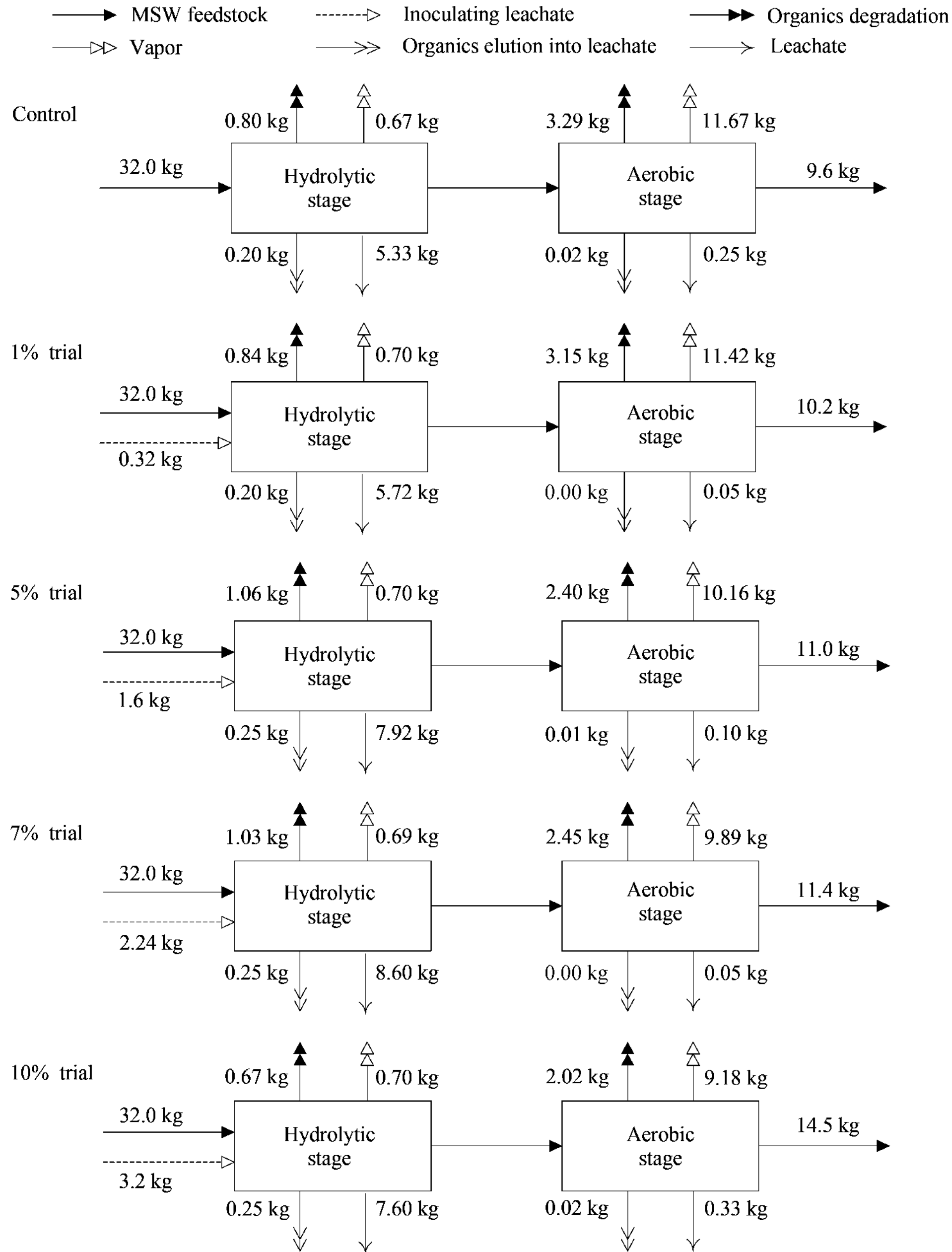

Fig. 5 Balance of organics and water losses during biopretreatment.

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Appendix

The water losses at time t (W^t_{loss}, kg) are calculated by:

$$W^t_{\text{loss}} = (\text{WM}_0 \times w_0) - (\text{WM}_t \times w_t) \quad (1)$$

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 ($\text{TO}^t_{\text{loss}}$, kg) are given by:

$$\text{TO}^t_{\text{loss}} = \text{WM}_0 - \text{WM}_t - W^t_{\text{loss}} \quad (2)$$

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

$$\text{LO}^t_{\text{loss}} = \text{accumulated TOC}_t / C_t \quad (3)$$

where, accumulated 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.

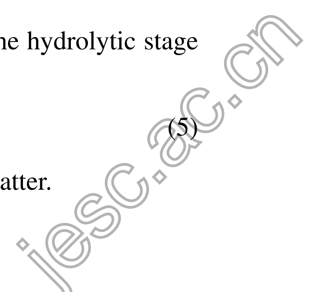
The organics degradation losses at time t ($\text{DO}^t_{\text{loss}}$, kg) are calculated by:

$$\text{DO}^t_{\text{loss}} = \text{TO}^t_{\text{loss}} - \text{LO}^t_{\text{loss}} \quad (4)$$

The hydrolysis rate (R_{h}, %) during the hydrolytic stage is calculated by:

$$R_{\text{h}} = \text{TO}^t_{\text{loss}} / \text{OM}_0 \quad (5)$$

where OM_0 (kg) is the initial organic matter.



The degradation rate (R_d) during the aerobic stage is calculated by:

$$R_d = (TO_{loss}^{16} - TO_{loss}^{4})/OM_0 \quad (6)$$

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:

$$R_{total} = TO_{loss}^{16}/OM_0 \quad (7)$$

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