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Effect of leachate recycling and inoculation on the biochemical characteristics of municipal refuse in landfill bioreactors

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Abstract: Activity development of key groups of enzymes involved in municipal refuse decomposition was measured in laboratory landfill bioreactors with and without leachate recycling and inoculation for about 210 days. The results showed that the enzymes (amylase, protease, cellulase, lipase and pectinase) were present in fresh refuse but at low values and positively affected by leachate recycling and refuse inoculation. The total average of cellulase activity in digesters D3 operated with leachate recycling but no inoculation, D4 and D5 operated with leachate recycling and inoculation was much higher than that in digesters D1 and D2 without leachate recycling and inoculation by 88%—127%, 117%—162% and 64%—98%. The total average of protease activity was higher in digester D4 than that in digesters D1, D2, D3 and D5 by 63%, 39%, 24% and 24%, respectively, and the positive effect of leachate recycling and inoculation on protease activity of landfilled refuse mainly was at the first two months. The total average of amylase activity was higher in digesters D3, D4 and D5 than that in digesters D1 and D2 by 83%—132%, 96%—148% and 81%—129%. During the early phase of incubation, the stimulatory effect of inoculation on lipase activity was measured, but refuse moisture was the main factor affecting lipase activity of landfilled refuse. The inoculation, initial and continuous inoculation of microorganisms existing in leachate, was the mainly stimulatory factor affecting pectinase activity of landfilled refuse.

Keywords: municipal refuse; landfill bioreactor; leachate recycle; inoculation; enzyme activity; biochemical characteristics

Introduction

The disposal of waste to landfill is the dominant method of disposal for domestic and most other waste streams. However, nonlandfilling which has previously been the method of choice for many cities is at a crisis point for the reasons of shorter life, space availability, cost and pollution, in particular, landfilling of domestic solid waste gives rise to a highly polluting liquor known as leachate. The attainment of sustainable waste management and the development of sustainable landfilling practices will be an important step in sustainable development recognizes.

Understanding the microbiological processes occurring in landfill site is of paramount importance both to controlling pollution problems resulting from the accumulation of carboxylic acids and inorganic ions and to the commercial exploitation of landfill bioreactors(Barlaz, 1989). Substrate decomposition to methane in sanitary landfill follows the patterns established for habitats such as sediments(Abram, 1978; Jones, 1982), sludge digestion and the rumen, can be regarded as being concerned first with hydrolysis and fermentation of polymers such as starch, protein, cellulose, hemicellulose and lignin to volatile fatty acids (Bryant, 1977). In the absence of nitrate and sulphate, the terminal products of refuse decomposition are carbon dioxide and methane, which represents a usable but underutilized source of energy. But research on enhancement of methane production has not led to an understanding of refuse decomposition adequate to predict and increase methane yields in sanitary landfills(Barlaz, 1987; Kinman, 1987; Buivid, 1981). Enzyme activity determinations may be a measure of the potential of landfill site to produce biogas (Jones, 1983). Pohland and Gould(Pohland, 1986) used leachate recycle to accelerate refuse decomposition, thus reducing the time required to assess the effects of co-disposal of industrial waste sludge with municipal refuse. However, leachate recycle is not typically practiced in full-scale landfill sites and the effects of leachate recycle on microbial and biochemical characteristics of refuse are little known.

The objective of this paper was to compare and evaluate biochemical characteristics of refuse, incubated with and without leachate recycle and inoculation in laboratory-scale landfill bioreactors.

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1 Materials and methods

1.1 Materials

According to the constituent of modern municipal refuse, synthetic municipal solid waste mixture was man-made in our laboratory; composition of this material is shown in Table 1.

Table 1 Composition of the mixed refuse

Components	Vegetables	Fish	Meat	Fruit	Paper	Plastics and leather rubber	Cellulose textile	Brick sand and soil	Metals and glasses	Woods
Percent by wet weight	45.0	2.50	1.00	8.95	7.47	11.92	3.60	8.46	7.57	3.53

The refuse mixture was shredded into 2 to 4 cm in length or width; the water content of mixture was about 50% of the water holding capacity.

1.2 Experimental equipment

Refuse was incubated in 60-liter simulated bioreactor landfills (Fig.1). A polyethylene male adapter (about 0.8 cm) was installed in the bottom of each container as a leachate drainage port. Two such adapters were installed in the lid of each container for leachate recycle and gas collection. Adapters were held in place with wax to provide a gas-tight system.

1.3 Experimental design

The objective of this study was to study and compare biochemical characteristics of refuse during the refuse decomposition, incubated with and without leachate recycle and inoculation. Thus, five containers were employed, among them, the first one (D1) was used as control, the second (D2) was adjusted to 75% moisture (wet weight) and no leachate recycle, the third (D3) was adjusted to 75% moisture and initiated with leachate recycle but no inoculation, the fourth (D4) was adjusted to 75% moisture and initiated with leachate recycle and inoculated with high-effective microorganisms selected from old refuse in general landfill, the fifth (D5) was nearly the same as the fourth but double inoculation. The refuse mixture was compacted in each of equipments until a specific weight of 0.5 ton per cubic meter was attained. All leachate was not neutralized and recycled through the top of the bioreactor on a daily basis. And all stimulated landfill bioreactors were incubated at 28 ± 1℃.

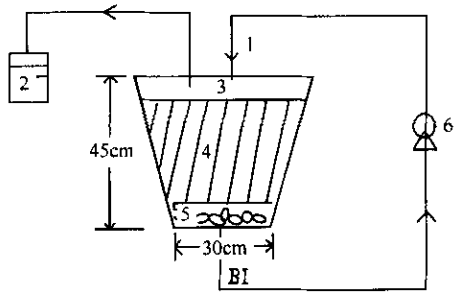

Fig.1 Schematic representation of digesters used during the experiment
1. water injection port; 2. gas bag; 3. sand layer; 4. refused (solid waste); 5. stone; 6. pump

1.4 Procedure for container sampling and enzyme activity determination

Approximately 100g of the refuse mixture were withdrawn periodically from several sites of each bioreactor. The refuse sample was immediately placed in a plastic bag, which was closed, and all free air was removed by squeezing. Each 5.0g of the refuse sample in the bag was used to determine amylase, protease, cellulase, lipase and pectinase activities. The remaining was used to monitored for moisture content by measuring the loss of sample weight after drying in an oven at 105℃ for approximately 16—24h to a constant weight.

1.5 Enzyme activity determinations

1.5.1 Amylase activity

The reaction mixture in 100 ml volumetric flask, containing 5.0g of the refuse sample, 10 ml of 0.1% starch, 10 ml of 0.07 mol/L phosphate buffer (pH 5.6) and 1 ml toluene, was incubated at 30 ±

1℃ for 48 hours, then filtered. The concentration of reducing sugar in the filtrate was determined spectrophotometrically at 551 nm. Amylase activity was expressed in the unit corresponding to the amount of glucose liberated from per gram of refuse in one day.

1.5.2 Cellulase activity

Cellulase activity was assayed by the same method as amylase activity, but the reaction mixture was different, containing 5.0g of the refuse sample, 10 ml of 0.2 mol/L acetate buffer(pH 5.5), 1.0 ml toluene and 10 ml of 1.0% CMC solution, and incubated at 30 ± 1℃ for 72 hours.

1.5.3 Lipase activity

Lipase activity was assayed using a reaction mixture containing 5.0g of the refuse sample, 2.0 ml toluene, 5.0 ml distilled water, 5 ml of 0.2 mol/L phosphate buffer(pH 7.0) and 2.5 ml sunflower oil. After incubation at 30 ± 1℃ for 72 hours, 10 ml of 96% enthol was added to stop the reaction and dissolve the oil. Then the mixture was filtered. The fatty acid liberated into the filtrate was measured by the titration of 0.1 mol/L KOH enthol standard solutions. Lipase activity was expressed in the corresponding to the amount of the fatty acid liberated from per gram of refuse in one day.

1.5.4 Protease activity

The reaction mixture in 100 ml volumetric flask, containing 5.0g of the refuse sample, 1.0 ml toluene, 2.0 ml of 1.0% casein solution and 10 ml of 0.2 mol/L phosphate buffer(pH 7.4), was incubated at 30 ± 1℃ for 48 hours, then filtered. 5.0 ml filtrate was mixed with 20 ml alcohol in 50 ml flask, after standing 5 min filtered again. The concentration of glycocoll in the filtrate was determined spectrophotometrically at 560 nm. Protease activity was expressed in the unit corresponding to the amount of glycocoll liberated from per gram of refuse in one day.

1.5.5 Pectinase activity

The reaction mixture in 100 ml volumetric flask, containing 5.0g of the refuse sample, 1.0 ml toluene, 1.0 ml of 1.0% pectin from tangerines and 10 ml of 0.2 mol/L actate buffer(pH 5.0), was incubated at 30 ± 1℃ for 48 hours, then filtered. 5.0 ml filtrate was mixed with 2.5 ml of 1.0 mol/L Na_2CO_3 solution and 5.0 ml of 0.05 mol/L I_2 solutions in 250 ml flask, put in the dark cabinet for 20 min, then 5.0 ml of 1 mol/L H_2SO_4 was added. Immediately the solution was titrated by 2.5 mmol/L $Na_2S_2O_3$. Pectinase activity was expressed in the unit corresponding to the amount of galactoaldehyde acid liberated from per gram of refuse in one day.

2 Results and discussion

According to the procedures described in materials and methods, several hydrolytic enzymes (amylase, protease, cellulase, lipase and pectinase) activities were measured in all digesters incubated under laboratory conditions during 210 days.

2.1 Cellulase activity

Many bacterial species and fungi are able to synthesize cellulase, which includes three types of enzymes: cellobiohydrolase, endoglycanse and β-glucosidase. They are all hydrolytic enzymes and act either in a sequential manner or simultaneously.

2.1.1 General trend of cellulase activity

Cellulase activity was measured in different digesters during refuse decomposition, which was buried for 210 days. The results are presented in Fig.2. The cellulase activity was first detected in each digester on day 6. At the time the amount of anaerobic cellulolytic bacteria was small in all digesters, suggesting that cellulase activity of aerobic organisms only was detected or dominant. The prevalence of aerobic condition and the presence of oxygen in refuse could support the growth of fungi during the early stage of refuse incubation. However, once the oxygen present initially was depleted(anaerobic condition prevailed

in refuse ecosystem), the growth of aerobic microorganisms would be inhibited. Maybe it was the reason that the cellulase activity decreased between days 6 and 13. But the cellulase activity in digesters D4 and D5 increased due to the effect of inoculation of refuse. Between days 13 and 29 cellulase activity slowly decreased in digsters D4 and D5, which was probably attributed to the feedback inhibition of an accumulation of volatile fatty acids (VFA) arising from organic matter including cellulase hydrolysis. It slowly increased between days 88 and 118, which is corresponded to a 10^1—10^2 increase in the most probable number (MPN) of anaerobic cellulolytic bacteria in digesters D4 and D5(Shen, 2001). Then, with the degradation of refuse, the cellulase activity of the refuse in digesters D4 and D5 deceased gradually.

2.1.2 Effect of leachate recycle and inoculum on cellulase activity

During the incubation of refuse, the different in the cellulase activity between different digesters suggested that moisture content, leachate recycle and inoculation were beneficial to increase cellulase activity, particularly, leachate recycle (Fig.2). However, the cellulase activity in digester D5 was smaller than that in digesters D4 and D3, which may be too much the inoculum of protein-decomposing microorganisms, which also degraded cellulase protein.

The total average of cellulase activity in digesters D3, D4 and D5 was much higher than that in digesters D1 and D2 by 88%—127%, 117%—162% and 64%—98% (Fig.3). This explained that inoculation and leachate recycle accelerated cellulose decomposition into simple organic matter which could degraded by SRB (sulfate-reducing bacteria), MPB (methane-producing bacteria) and other fermentative bacteria, and decreased refuse volume.

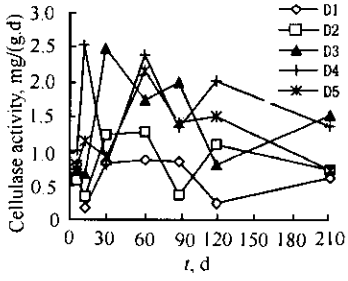

Fig.2 Variation of cellulase activity in different digesters during refuse

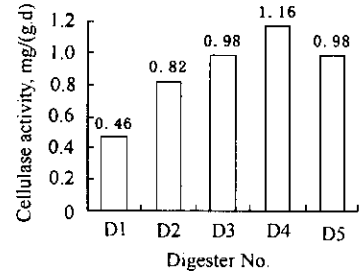

Fig.3 Total average of cellulase activity during refuse decomposition

2.2 Protease activity

The proteinases are highly complex group of enzymes, exhibiting a wide range of varying physico-chemical and catalytic properties. They are produced both extracelluarly and intracellularly. The major role of extracellular proteinases, in common with other extracellular depolymerizing enzymes, in nutrition, is to hydrolyze large polypeptide substrates into smaller molecular entities which cell can absorb in.

2.2.1 General trend of protease activity

Protease activity was measured in different digesters during the incubation of refuse (Fig.4). The results showed that protease activity was lower in fresh refuse, but with the incubation of refuse, the protease activity rapidly reached to its peak between days 6 and 13, then sharply decreased until day 60 nearly to the the same level of day 6 in all digesters. From days 60 to 118, the protease activity increased a little, then slowly decreased until day 210 to the near depletion with the degradation of refuse.

2.2.2 Effect of leachate recycle and inoculum on protease activity

Although the trends of protease activity were similar in all digesters, but there were differences in the maximum of protease activity between different digesters (Fig.5). In digesters D3, D4 and D5 the maximum of protease activity was higher than that in digesters D1 and D2. And the maximum of protease

activity in digesters D4 and D5 was reached on day 14, which was earlier than that in digesters D2 and D3 on day 30. These differences suggested that the positive effect of leachate recycle and inoculation on protease activity of landfilled refuse mainly was at the first two months.

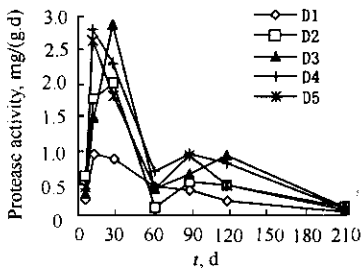

Fig.4 Variation of protease activity in different digesters during refuse

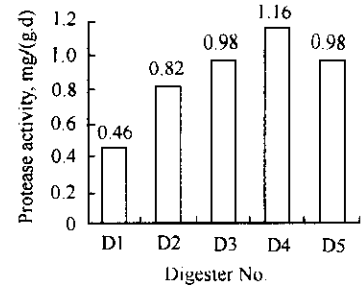

Fig.5 Total average of protease activity during refuse decomposition

During the whole incubation period, the total average of protease activity of refuse was higher in digester D4 than that in digesters D1, D2, D3 and D5 by 63%, 39%, 24% and 24%, respectively, which further confirmed the positive effect of leachate recycle and inoculating on protease activity.

2.3 Amylase activity

Amylase includes α-amylase and β-amylase. α-amylase is distributed widely in microorganisms. Hydrolysis of amylose by α-amylase causes its conversion into maltose and maltotriose, then maltotriose or amylopectin may be slowly hydrolyzed into glucose and maltose. β-amylase occurs widely in higher plants and was crystallized from a number of sources. Action of β-amylase on amylase results in 50%—60% conversion to maltose and formation of a β-amylase dextrin.

2.3.1 General trend of amylase activity

The amylase activity was measured in all digesters during the incubation of refuse. The results are shown in Fig.6. It was a common trend that the amylase activity was clearly detectable in all the digesters after 6 days of incubation, and it was higher in digesters D3, D4 and D5 compared with that in digesters D1 and D2. Amylase activity in digesters D4 and D5 showed an increase between days 6 and 60 with a small decrease(days 13—29). It may be the reason that the pH of refuse decreased, inhibiting amylase activity. Then, with the degradation of refuse, the amylase activity in digesters D3, D4 and D5 decreased gradually until day 210 to the lower lever. The amylase activity in digesters D1 and D2 increased a little during the early stage of refuse incubation, but it was low relative to the others.

2.3.2 Effect of leachate recycle and inoculum on amylase activity

The amylase activity in digesters D3, D4 and D5 was higher than that in digesters D1 and D2 during the whole incubation period. It might be that digesters D3, D4 and D5 operated with leachate recycle or inoculation, which may increase the refuse moisture and the amount of the microorganisms producing amylase. The high activity of amylase in digester D4 compared with that in digesters D1, D2 and D3 may be due to the effect of inoculation. However, too much the inoculum of protein-decomposing microorganisms, which also degraded amylase protein, would inhibit amylase activity (D5). The amylase activity in digester D2 was higher than that in digester D1 between days 6 and 60, which might be due to the effect of moisture, with the incubation of refuse in digesters and no leachate recycle, refuse moisture in digester D2 decreased, which may inhibit the growth of microorganisms and amylase activity.

The total average of amylase activity was higher in digesters D3, D4 and D5 than that in digesters D1 and D2 by 83%—132%, 96%—148% and 81%—129%, respectively (Fig.7), which confirmed that leachate recycle might be the main factor affecting amylase activity.

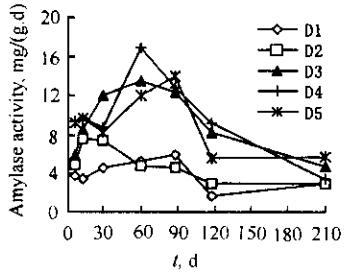

Fig.6 Variation of amylase activity in different digesters during refuse

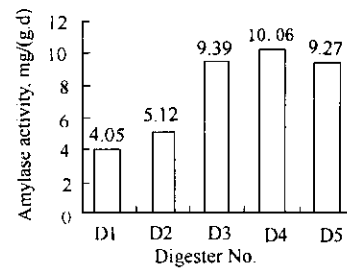

Fig.7 Total average of cellulase activity during refuse decomposition

2.4 Lipase activity

The natural substrates of lipase are triglycerides of long-chain fatty acids, which are insoluble in water. And lipase is characterized by the ability to rapidly catalyze the hydrolysis of ester bonds of the interface between the insoluble substrate phase and the aqueous phase in which the enzyme is soluble. Thus, lipase can catalyze the hydrolysis of a wide range of insoluble fatty acid esters, although glycerides are normally substrate.

Lipase activity was measured in different digesters during refuse decomposition, which was buried for 210 days. The results are presented in Fig.8. Lipase activity, first detected in all digesters on day 6, was higher in digesters D4 and D5 compared with that in digesters D1, D2 and D3, suggesting the stimulatory effect of inoculation during the early phase of inoculation. And it increased in all digesters between days 6 and 13. From day 13 the lipase activity decrease in all the digesters except digesters D3 until day 60. The near depletion of lipase activity in digesters D1 on day 30 may be due to the lower moisture content. The difference of the total average of lipase activity in digester D1 from the others (Fig.9), suggested that refuse moisture was the main factor affecting lipase activity of landfilled refuse during the whole landfilling period.

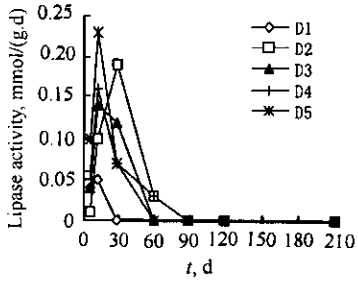

Fig.8 Variation of lipase activity in different digesters during refuse

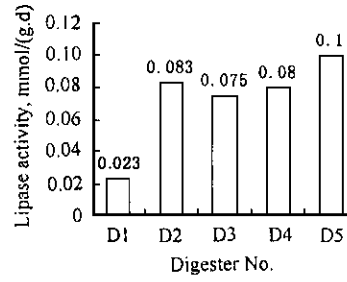

Fig.9 Total average of lipase activity during refuse decomposition

2.5 Pectinase activity

Pectin, originating from fruits and plants, is a natural compound different to be degraded by natural microorganisms. Pectinase includes four types of pectic hydrolase: endopolygalacturonases, exopolygalacturonases, oligogalacturonases and endopolymethylgalacturonases.

Pectinase activity was measured in different digesters during refuse decomposition, which was buried for 210 days. The results are showed in Fig.10. On day 13 pectinase activity was higher in digesters D3, D4 and D5 compared with digesters D1 and D2. Then pectinase activity rapidly decreased in all digesters until day 118 and kept lower level during the last stage of refuse incubating.

The total average of pectinase activity was higher in digesters D3, D4 and D5 than that in digesters

D1 and D2 (Fig. 11), which suggested that leachate recycle and initial inoculation might stimulate the pectinase activity of landfilled refuse. But the almost same activity of pectinase in digesters D1 and D2 suggested that refuse moisture seemed not to be an important factor. Therefore, it might be concluded that inoculation, initial and continuous inoculation of microorganisms existing in leachate, was the mainly stimulatory factor affecting pectinase activity of landfilled refuse.

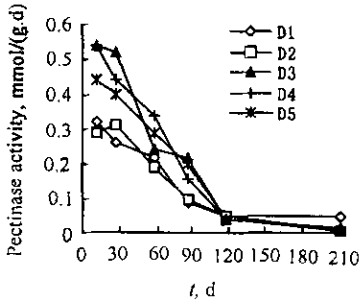

Fig. 10 Variation of pectinase activity in different digesters during refuse

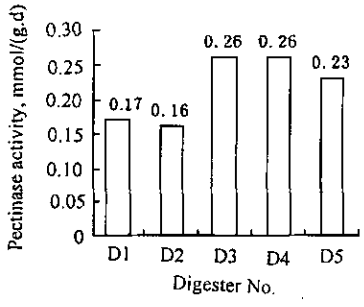

Fig. 11 Total average of pectinase activity during refuse decomposition

3 Conclusions

The key groups of hydrolytic enzymes including amylase, protease, cellulase, lipase and pectinase were present in fresh refuse but at low values and positively affected by leachate recycle and refuse inoculation.

The refuse inoculation mainly increased the amounts of selective microorganisms producing hydrolytic enzymes, which were essential in degradation of refuse constituent. And leachate recycle increased refuse moisture and caused a well-mixed soluble constituent pool which were atypical of both laboratory-scale lysimeters operated in the absence of water flux, and field-scale landfills. If the effects can be addressed, then leachate recycle and inoculation of selective microorganism is a useful technique for acceleration of refuse decomposition.

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