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Screening on oil-decomposing microorganisms and application in organic waste treatment machine

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Abstract: As an oil-decomposable mixture of two bacteria strains (*Bacillus* sp. and *Pseudomonas* sp.), Y3 was isolated after 50 d domestication under the condition that oil was used as the limited carbon source. The decomposing rate by Y3 was higher than that by each separate individual strain, indicating a synergistic effect of the two bacteria. Under the conditions that $T = 25—40℃$, pH = 6—8, *HRT* (Hydraulic retention time) = 36 h and the oil concentration at 0.1%, Y3 yielded the highest decomposing rate of 95.7 %. Y3 was also applied in an organic waste treatment machine and a certain rate of activated bacteria was put into the stuffing. A series of tests including humidity, pH, temperature, C/N rate and oil percentage of the stuffing were carried out to check the efficacy of oil-decomposition. Results showed that the oil content of the stuffing with inoculums was only half of that of the control. Furthermore, the bacteria were also beneficial to maintain the stability of the machine operating. Therefore, the bacteria mixture as well as the machines in this study could be very useful for waste treatment.

Keywords: oil-decomposing microorganism; screening; application; organic waste treatment machine; decomposing rate

Introduction

The BOD(biochemical oxygen demand) of fats and oils derived from animal, plant or mineral, is about 1.8 mg/L, indicating that fats and oils may be easilier decomposed than the most other organic compounds. After being introduced into the environment, however, fats and oils can form compact films, which can prevent the transfer of O_2 across the interfaces between different media, and causes harm to the aerobic decomposition by other organisms. Therefore, treatments of solid/liquid waste containing fats and oils have being increasingly concerned.

The conventional techniques of separating fats and oils from wastewater depend on gravity and floatation such as grease-trap. Unfortunately, there are always some restaurants that produce a great deal of wastewater containing too much fat and oil to be treated by such equipments. Furthermore, solid waste with fats and oils is difficult to be treated wastewater. In China, bigger-scale restaurants produce lots of kitchen-waste with a high concentration of fats and oils, which cannot be used to feed livestock according to the Chinese. The organic waste treatment machine is a kind of novel equipment relying on microorganisms to degrade organic waste, which has been developed and applied often in many cities in China. However, there still remain some limitations, especially during the treatment of kitchen-waste containing lots of fats and oils, the anaerobic condition and gooey derived from accumulated fat/oil will baffle the machine from well-balanced running. It is well known that a number of natural microorganisms, including 28 species of bacteria, 4 species of actinomycetes, 10 species of yeasts, can decompose fat/oil readily. Therefore, it is important to use microorganisms to decompose effectively fats and oils in the waste.

The aim of this work was to deal with domestication by a mixture of two bacteria with a high decomposing rate of fat/oil, and its application in the organic waste treatment machine. The characteristics of the bacterial culture and the effect of this culture on the performance of machine treatment are also described.

1 Materials and methods

1.1 Samples for screening

Five sludge samples collected from four restaurants and a Waste Water Treatment Plant in Shanghai City were used in this experiment. The samples were kept at 25℃ in the aseptic laboratory before used.

1.2 Organic waste treatment machine

The organic waste treatment machine (YFL001) was provided by Taiwan Yao Feng Company.

1.3 Screening on the microorganisms from samples

About 5 g of each sludge sample was put into 250 ml Erlenmeyer flasks with 45 ml sterilized distilled water and glass balls. They were thoroughly mixed by shaking on an oscillator at 300 r/min for 10 min. Then 10 ml diluents were pipetted into 90 ml enrichment medium in 250 ml Erlenmeyer flasks. The flasks were then incubated at 40℃ and shaken at 200 r/min. The composition of the enrichment medium (g/L distilled water) was: NaCl, 5.0; peptone, 5.0; KH_2PO_4, 0.3; $MgSO_4 \cdot 7H_2O$, 0.1; K_2HPO_4, 0.3; $(NH_4)_2SO_4$, 1.0; peanut oil, 10 ml, and the initial pH was 7.0. One ml peanut oil was then pipetted into the flask in which oil could be decomposed well every day. Ten ml cultures were pipetted into 90 ml fresh enrichment culture with 2% peanut oil every 5 d to improve microbial ability of decomposing fat/oil. The domestication process lasted 50 d. After enrichment, the fat/oil-decomposing microorganisms were screened on isolation plates (the enrichment medium without peptone but 2% agar) with Neutral red, Bromocresol purple (Yu, 1990), or blank isolation plates. The colonies were picked up individually, and inoculated into liquid medium in flasks to ascertain their abilities to decompose fat/oil. The composition of the liquid medium (g/L distilled water) was: NaCl, 5.0; peptone, 10.0; beef extract, 5.0; KH_2PO_4, 0.3; $MgSO_4 \cdot 7H_2O$, 0.5; K_2HPO_4, 0.5; $CaCl_2$, 0.02; peanut oil, 10 ml; pH 7.0.

1.4 Culture conditions and fat/oil biodegradability test of Y3

Various tests about Y3 were carried out to ascertain their effectiveness, and to investigate the influence of various culture conditions including initial pH of medium, incubation

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temperature, treatment time, O_2 concentration, different kinds of fat/oil as carbon sources and different nitrogen sources, on fat/oil biodegradability of Y3. About 2 ml of each inoculum was inoculated into 50 ml medium in the 250 ml Erlenmeyer flasks and incubated under different conditions according to the experimental design. Control sets without inoculum were also incubated and analyzed at the same time. After incubation, the samples were collected for fat/oil analysis using the method described below.

1.5 Application in organic waste treatment machine and the analysis

Activated inoculums were put at a certain rate into the machine operated under usual conditions. At the same time, control machines also ran under the same conditions comparatively. The machine is able to treat 1.5 kg organic waste per day. Therefore, leaching into each machine every day put 1 to 1.5 kg dried kitchen waste. The stuffing in each machine was analyzed every 7 d, and a set of data of fat/oil percentage, pH, temperature, C/N ratio, humidity, organism, total N, etc. were obtained.

1.6 Estimation of residual fat/oil

The method for estimating residual fat/oil in liquid culture was different from that for solid stuffing. Residual fat/oil in liquid culture was estimated using GB50096, which is the standard method released by Chinese government. The residue fat/oil in stuffing was extracted by Soxhlet Extraction for 24 h with petroleum ether(30—60℃), and the remained petroleum ether was evaporated by a rolling-evaporation equipment. The dried fraction was put into an air-oven and dried to a constant weight at 105℃ before extraction. The amount of decomposed fat/oil was estimated by the loss of weight attributed to the inoculums.

1.7 Stuffing sampling and analysis

The stuffing samples were collected every 7 d from 5 different sites in the machines. The pH of the samples were determined at a ratio of 1∶2.5(soil: water). Humidity was measured by drying samples to a constant weight in an air-oven at 105℃ for 2 h. Temperature was measured in the stuffing at 5 different sites every day. Volatile organics were determined in muffle at 550℃. Ammonium and organic nitrogen(TKN) were measured with dry combustion using an automated N analyzer(Gerhardt).

1.8 Identification of microorganisms

On the basis of the biochemical and morphological characteristics, isolates were identified according to the descriptions in the "Berggy's Manual of Systematic Bateriology".

2 Results and discussion

2.1 Screening on fat/soil-decomposing microorganisms

Seven colonies, Y1—Y7, were found on the plate in the first isolation. It was clear through flask tests that Y3 had the highest ability to decompose fat/oil, which can increase the OD value at 540 nm of the liquid culture to 5.046(taking the culture without inoculums as blank one), and had no any separation between water and oil after 48 h incubation. However, when Y3 was separated further into two kinds of bacteria, Y3.1 and Y3.2, the ability to decompose fat/oil decreased obviously. Y3.1(a kind of bacteria) could not survive for more than one month alone, while had a higher ability of decomposing fat/oil than Y3.2. Therefore, it could be concluded that there existed a association between both kinds of bacteria for their surviving and decompose of fat/oil.

Three kinds of indicators, Neutral red, Bromocresol purple and $CuSO_4$ were used during the isolation. Neutral red and Bromocresol purple could be served for indicating whether or not pH reducing was caused by fatty acids production from the decompose of fat/oil. While $CuSO_4$ indication is based on the deposited → opposite reaction caused by $CuSO_4$ and fatty acids produced by decomposing of fat/oil.

2.2 Changes in pH of liquid medium with different initial pH

It is important to maintain an initial pH before the incubation and degradation of fat/oil cultures. Results show that the culture grew the best at the pH ranging from neutral to alkaline with a fat/oil degradation rate higher than 85%, poor at pH > 9 or pH < 5 with a fat/oil degradation rate lower than 50 % (Fig.1). It is also observed that no matter what the initial pH was, pH values around pH 7.5 could be observed after 24 h incubation(Fig.2). It was well known that the microbial degradation of fat/oil only occurs at the oil-water interface(Brock, 1991), which means that adequate mixing and dispersion benefit fat/oil degradation. Alkali pH conditions result in a better emulsification in liquid medium than acidic pH conditions, therefore the fat/oil in kitchen waste could be easilier and better degraded by microorganisms. However, it is also necessary to consider the microbial activity as well as emulsification because microbial growth can be restrained at pH > 9. In addition, no significant decrease of pH value caused by fatty acid accumulation in the liquid medium was observed.

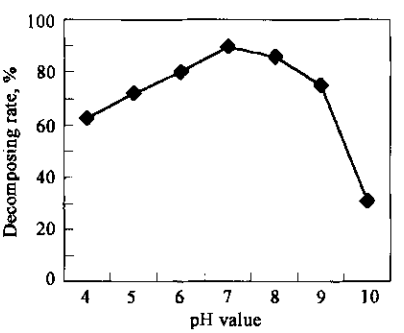

Fig.1 Effects of original pH on decomposing rate

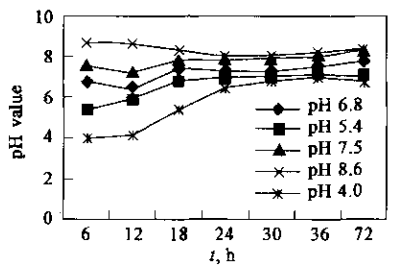

Fig.2 Curve of pH

2.3 Effects of temperature and shaking speed on the decomposing rate

The culture decomposed fat/oil effectively at temperatures ranging from 25 to 45℃ (Fig.3), the highest decomposing rate reached 87.2% at 30℃. It should be noted that both incubation and mixing of water and fat/oil could be optimized within the temperature range as Zobbel (Zobbel, 1969). The shaking speed and temperature can affect two factors influencing decomposing rate of fat/oil, first is the dissolved O_2, the another is the adequate mixing of water and fat/oil. Therefore, there are three different culture conditions that can affect the mixing kitchen waste in machine directly or indirectly(Table 1). The adequate mixing can be regarded as the precondition of microbial decomposition. There would not be any microbial decomposition of fat/oil without adequate mixing and dispersing of fat/oil, water and microorganisms. It was observed that the fat/oil decomposition was a strict O_2-demanding biochemical reaction, as similar to previous reports(Zhang, 1996). There was a saturated tendency with the increase of shaking speed.

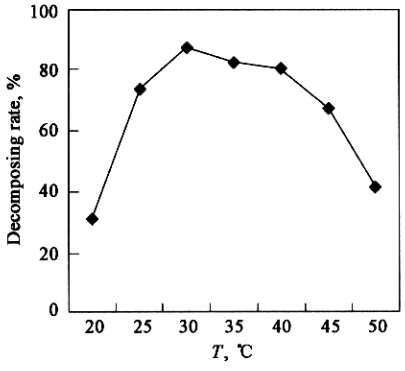

Fig.3 Effects of temperature on decomposing rate

Table 1 Effects of shaking speed on decomposing rate

	Continuous shaking						
Shaking speed, r/min	150	175	200	225	250	Intervals	Fixed
Decomposing rate, %	63.9	74.4	79.4	81.6	82.4	39.7	7.1

2.4 Effects of treating time on the decomposing rate

The degraded amount of fat/oil increased with treating time, when fat/oil concentration was 1% (Fig.4). However, after a 36 h treatment this tendency was no longer obvious, indicating the inhibition of the lipase by fatty acids(Smith, 1996). Considering the economic factor, 33 to 36 h for treatment was proposed. The treatment time should be adjusted according to the various fat/oil concentration of the waste.

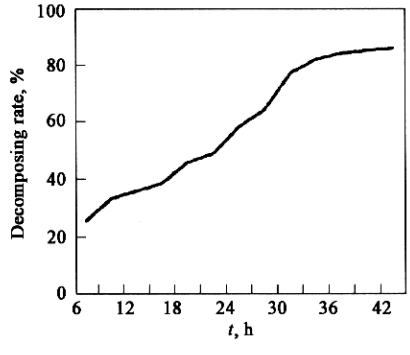

Fig.4 Effects of HRT on decomposing rate

2.5 Effects of carbon sources on decomposing rate

The different fats/oils were added as carbon resource on the basis of the liquid medium to examine the effects caused by individual. It shows that the decomposing rate of fat/oil from plants was higher than the from animals. Usually, fats from animals have a higher melting point than that from plants, leading to form conglomerations, having resistance to the decomposition in the liquid medium. The antimicrobial activities against some bacteria by certain fatty acid esters reported by Kwaku *et al*.(Kwaku, 1999) were not observed in this study. The waste fat/oil from kitchen waste was a mixture of plant- and animal-original fat/oil, so the degradability of waste was higher than the latter but lower than that the former. After a 48 h treatment, the decomposing rate reached 74% (Fig.5).

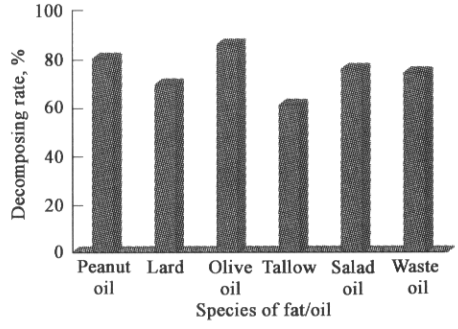

Fig.5 Effect of different fat/oil as carbon sources on the decomposing rate

2.6 Effects of nitrogen sources on the decomposing rate

The effects of different kinds of organic and inorganic nitrogen resources on the decomposing rate of fat/oil in the liquid medium were studied. The decomposing rate could reach over 80% with organic nitrogen resources, while decreasing to below 50% with inorganic nitrogen resources, such as $(NH_4)_2SO_4$ and NH_4Cl (Fig.6). From this point, the inoculums are suitable to treat the waste containing lots of natural organic nitrogen sources from restaurant.

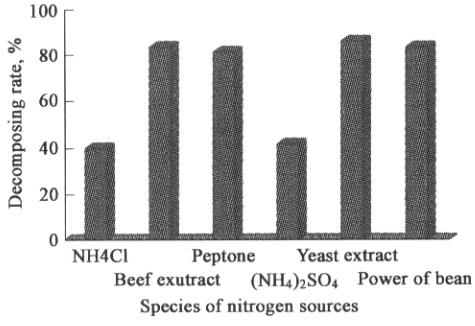

Fig.6 Effects of different nitrogen sources on the decomposing rate

2.7 Variation in fat/oil concentration of the stuffing in the organic waste treatment machine

According to the variation in fat/oil concentration of the stuffing, the information about the situation of microbial degradation in the organic waste treatment machine could be obtained. Compared with the machine without inoculum, a lower accumulation of fat/oil was observed in the machine with inoculum. The fat/oil concentration of the stuffing in the inoculated machine did not vary obviously during the whole

treatment. However, there was an obvious increase of fat/oil in the control machine without inoculums. On the day 48, the fat/oil content in the inoculated machine was only a half of that in the control machine (Fig. 7). The initial fat/oil concentration was not zero because certain amount of wood-scraps containing fat/oil was added into the organic waste treatment machine as stuffing to adjust the operation conditions, including C/N ratio, dissolved O_2, etc. in the machine.

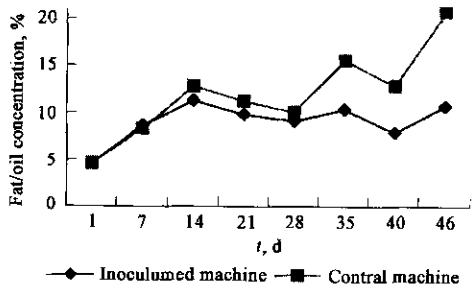

Fig.7 Curve of fat/oil concentration of the stuffing in the machine

2.8 Variation in temperature of the stuffing in the organic waste treatment machine

Temperature is always one of the most important factors to influence the treatment efficiency of waste. High temperature can reduce insect eggs and pathogens associated with organic waste, as well as the inoculums, and damage to the machine at some degree. Therefore, it is very important to maintain optimized temperature, above 55—65℃, in the machine. Fig.8 shows that the temperature in the inoculated machine could be maintained 6℃ higher than that in the control one. It can be concluded that the inoculum could not only improve the degradation of fat/oil but also maintain the stability at a relatively high temperature in the machine.

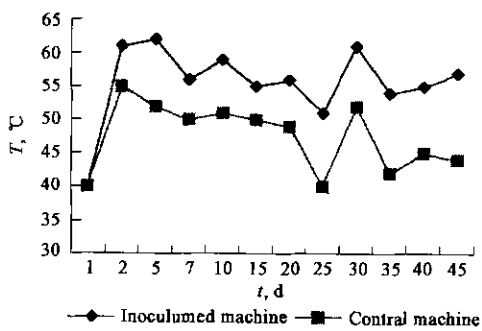

Fig.8 Curve of temperature in the waste treatment machine

2.9 Variation in humidity of the stuffing in the organic waste treatment machine

It is essential to maintain a certain humidity of the stuffing in the machine for inoculums. It is well known that ability of microorganisms obtaining nutrition will attenuate when the humidity decrease to below 30%. When the humidity increases to over 65%, almost all of the pores in the stuffing can be filled by water, preventing O_2 from transport(Smith, 1996), leading to the formation of odorous metabolite. It is observed in Fig.9 that the humidity in the inoculated machine could be maintained in the range of 30%—40%. However, the humidity could not be maintained stable in the control machine. On the day 14, the humidity in the control machine declined to below 30%. It is also observed that the humidity continued to decrease, even after adding 300 ml distilled water.

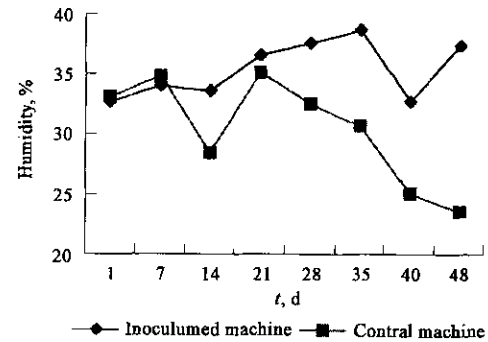

Fig.9 Curve of humidity during the treatment

2.10 pH variation of the stuffing in the organic waste treatment machine

Initial pH of stuffing was greatly affected by adding wood-scraps in the organic waste treatment machine. The pH of wood-scraps was measured to be about 5.1 to 5.4. The pH value of hogwash also varied in the range of 4.0 to 6.0. The inoculated machine with Y3 could reach a neutral pH after a 20-day running, which was beneficial to the waste treatment (Fig. 10), while relatively great variations of pH in the control machine were observed. Therefore, there is no need to adjust the initial pH in the machine, although the acidic condition of stuffing seemed to be a disadvantage for the operation.

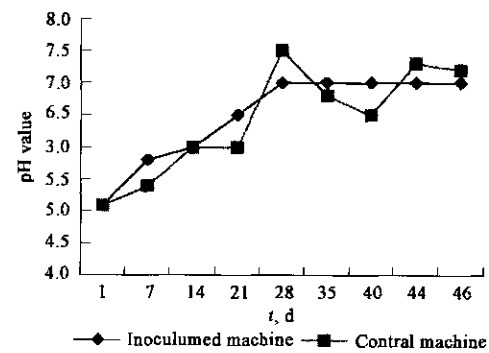

Fig.10 Curve of pH value during the treatment

2.11 Variation in C/N rate of the stuffing in the organic waste treatment machine

The principles of the organic waste treatment in machine are different from that of traditional or mechanical combustion. The C/N rate of combustion has to be adjusted and the materials of combustion need to be added before fermentation. However, a certain amount of stuffing, such as wood-scraps, was needed for the organic waste treatment machine before the treatment to adjust C/N ratio and the humidity. After that, the kitchen waste was put into the machine at intervals with the amount no more than 1.5 kg/d. The organic waste treatment machine works like a continuous culture process of microorganisms. Wood-scraps were added at the beginning so that it could constantly be consumed before the loss of nutrition balance. The C/N ratio decreased

sharply in the first week, and then kept at a relatively stable level within 40 d after starting machine(Fig.11). There was no significant difference between the C/N ratios and the material combustions. In the inoculated and control machines, although the C/N ratio of the material combustions in the inoculated machine appeared more stable than that in the control machine. It was suggested that wood-scraps could be used as the adjustor and nutrition should be compensated for their consumption during the operation.

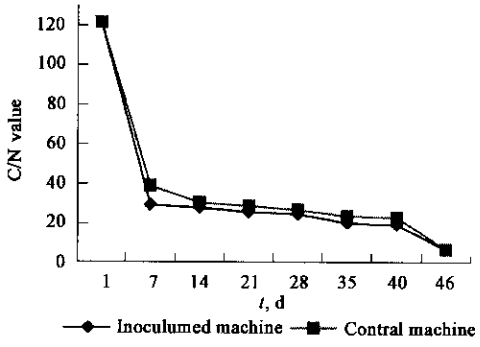

Fig.11 Curve of C/N rate

3 Conclusions

After half a year's domestication and screening, Y3, a bacterial mixture with a high fat/oil decomposing ability was screened. A series of tests were carried out to verify its ability and optimize cultural conditions for decomposition of fat/oil. The inoculums were applied into an organic waste treatment machine. It was concluded that the inoculum Y3 was able to effectively decompose fat/oil, and kept the machine in a good operation. The application of the organic waste treatment machine in treating solid waste is innovative. The machine was successfully applied into the domesticated inoculum. It was proved not only the high fat/oil decomposing ability of the inoculum, but also applicability of the machine and the technology. In addition, with regard to the wide range of nutrition and cultural conditions, the inoculum can also be used for the treatment of wastewater containing a high concentration of fat/oil.

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