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Development of a novel compound microbial agent for degradation of kitchen waste

Kaining Zhao[a], Rui Xu[a], Ying Zhang[a], Hao Tang[a], Chuanbin Zhou[b], Aixin Cao[b], Guozhu Zhao[a,*], Hui Guo[a,*]

[a] Beijing Forestry University, College of Biological Sciences and Biotechnology, Beijing, China
[b] Chinese Academy of Sciences, Research Center for Eco-environmental Sciences, State Key Laboratory of Urban and Regional Ecology, Beijing, China

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ABSTRACT

Large quantities of kitchen waste are produced in modern society and its disposal poses serious environmental and social problems. The aim of this study was to isolate degradative strains from kitchen waste and to develop a novel and effective microbial agent. One hundred and four strains were isolated from kitchen waste and the 84 dominant strains were used to inoculate protein-, starch-, fat- and cellulose-containing media for detecting their degradability. Twelve dominant strains of various species with high degradability (eight bacteria, one actinomycetes and three fungi) were selected to develop a compound microbial agent "YH" and five strains of these species including H7 (*Brevibacterium epidermidis*), A3 (*Paenibacillus polymyxa*), E3 (*Aspergillus japonicus*), F9 (*Aspergillus versicolor*) and A5 (*Penicillium digitatum*), were new for kitchen waste degradation. YH was compared with three commercial microbial agents—"Tiangeng" (TG), "Yilezai" (YLZ) and EM (Effective Microorganisms), by their effects on reduction, maturity and deodorization. The results showed that YH exerted the greatest efficacy on mass loss which decreased about 65.87% after 14 days. The agent inhibited NH_3 and H_2S emissions significantly during composting process. The concentration of NH_3 decreased from 7.1 to 3.2 ppm and that of H_2S reduced from 0.7 to 0.2 ppm. Moreover, E_4/E_6 (Extinction value$_{460nm}$/Extinction value$_{665nm}$) of YH decreased from 2.51 to 1.31, which meant YH had an obvious maturity effect. These results highlighted the potential application of YH in composting kitchen waste.

Introduction

Kitchen waste is the most common type of anthropogenic organic waste and includes many types of discarded food residue. The major chemical components of kitchen waste are starch, protein, fat, cellulose, and others. Due to continuous urbanization and population growth in many countries, the production of kitchen waste has increased annually, and in many districts, its leachate is discharged directly into

* *Corresponding authors at*: College of Biological Sciences and Technology, Beijing Forestry University, No. 35 Tsinghua East Road, Haidian District, Beijing 100083, China.
E-mails: zhaogz@im.ac.cn (G. Zhao), guohuiya@126.com (H. Guo).
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the sewer system. This represents a waste of environmental resources.[1] The deterioration of kitchen waste produces large amounts of toxins and foul odors, such as NH_3 and H_2S. Ammonia (NH_3) has a strong, pungent odor and can cause serious burns to the skin, eyes and respiratory tract.[2] Reducing NH_3 emission during kitchen waste composting is important for environmental protection and safety. Hydrothion (H_2S) is an acidic flammable gas that has an odor reminiscent of rotten eggs and is highly toxic to humans.[3] These gases cause serious water and air pollution. Therefore, the efficient and environmentally responsible disposal of kitchen waste is important.

Because of its high organic matter content, comprehensive nutrient profile and abundant microorganisms, compost enables kitchen waste to be degraded effectively. Using biological composting technology, organic fertilizer can facilitate the safe and non-polluting recycling of nutrient resources. During composting, the strain selection is key for the preparation of effective, complex microbial agents.[4] Optimal efficacy requires the fermenting and culturing of various microorganisms in appropriate proportions to prevent antagonism. During the past forty years, many microbial agents such as EM (University of the Ryukyus in Japan) and ABS-GC (AquaticBioScience Company),[5] have been studied and developed for purification of domestic sewage, treatment of industrial wastewater, and degradation of organics and garden waste.[6,7] EM has been widely used in more than ninety countries in applications including agricultural production, garden waste treatment and soil fertility improvement.

Several studies on substance conversion in kitchen waste compost have been conducted, including those discussing the effect of inoculation on composting[8] and improvement of the agent application method.[9] However, reports on the selection of effective degradative strains from kitchen waste and development of novel microbial agents are rare. For kitchen waste disposal, reduction is the most important aim and the optimal indicator of efficacy. Maturity is a useful indicator of environmental protection.[10] Deodorization is also significant for environment protection,[11] due to the emission of byproducts discharged by kitchen waste during storage and composting. Therefore, the objectives of this study were (i) to identify microbial strains that degrade kitchen waste effectively, (ii) to develop a new microbial agent, and (iii) to compare this new agent's efficacy with that of other commercially available agents in treating kitchen waste, in terms of reduction, maturity and deodorization (NH_3 and H_2S emission mitigation).

Materials and methods

Kitchen waste materials

Kitchen waste was obtained from the dining rooms of Beijing Forestry University (Beijing, China). The initial water content of this waste was 62.6%, due to the waste's high contents of fresh vegetables and meat. The initial pH value was 5.28. Kitchen waste was stored for 3 days prior to the experiment, during which period microorganisms were fermenting the nutrients, producing some organic acids, such as acetic acid and butyric acid. Therefore, the pH value of the kitchen waste was mostly lower than 7.

The proportions (wet mass) of the main components were as follows: staple food, 27.9%; fruits and vegetables, 31.5%; bone and eggshell, 9.1%; meat, 0.8%; peels, 28.2%; and shells and pits, 2.5%. The bone and eggshell composition of the Kitchen waste materials was relatively high. $CaCO_3$ the major component of bone and eggshells, can react with acidic materials to adjust the pH value. When the concentration of H^+ in the system reached a certain level, the carbonate adsorbed the H^+, causing the pH value to trend increasingly toward neutral, which was beneficial for microbial growth.[12]

Strain isolation and identification

Ten gram samples of fresh kitchen waste were weighed and placed into flasks filled with 90 mL sterile water, maintained at 30 °C and shaken at 160 r/min for 30 min. The supernatant obtained was diluted with sterile water and a 10-fold series of dilutions (10^{-1}–10^{-6}) was carried out. Then, microbial strains were isolated by spreading the dilutions onto agar plates. The agar plates used were LB medium (tryptone 1%, yeast power 0.5%, NaCl 0.5%, agar 15%, H_2O 1000 mL) and PDA medium (potato 20%, sucrose 2%, H_2O 1000 mL, pH 6.0–7.0) (w/v).

Microbial strains were identified using *Bergey's Manual of Systematic Bacteriology (Ninth Edition)* and the *Fungal Identification Manual* by Jingchao Wei (1979.9).

Molecular identification of microbes was achieved by means of DNA sequencing. DNA was extracted from bacteria using the TIANamp DNA Kit (Tiangen) and from fungi using a fungal DNA kit (Omega). Then, PCR amplification of the 16S rRNA gene (bacteria and actinomycetes) and 5.8S-ITS region (fungi) sequences was performed and the products were subjected to purification and sequencing.[1] Partial or nearly full-length nucleotide sequences were compared by BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Closely related strains were retrieved from NCBI for further analysis.

Screening of strains with high degradative activity

The dominant strains were inoculated into starch-, fat-, protein- and cellulose-containing media. The starch medium (1000 mL) comprised soluble starch 2%, NaCl 0.5%, peptone 0.5%, and agar 2%; the fat medium (1000 mL) comprised peptone 1%, NaCl 1%, $CaCl_2{\cdot}7H_2O$ 0.01%, Tween-80 1%, and agar 2%, pH 7.4–7.8; the protein medium (1000 mL) comprised nonfat dried milk 5% and agar 1.8%; and the cellulose medium (1000 mL) comprised K_2HPO_4 0.05%, microcrystalline cellulose 0.188%, $MgSO_4$ 0.025%, gelatin 0.2%, Congo red 0.02%, and agar 1.4% (w/v).[13] The presence of "clearance zones" surrounding the colonies was taken to indicate degradative activity. Moreover, iodine was added to the starch medium and neutral red dye to the fat medium to increase the color contrast. In triplicate, we measured the degradative activity and subsequently calculated the mean. Strains with the greatest degradative activity were selected to comprise the new agent named YH (abbreviation from its Chinese name "Yuanhui").

Optimization of fermentation conditions

The selected strains (1%, v/v) were each inoculated in mixed-fermenting medium (potato 20%, sucrose 1%, glucose 1%,

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