



Removal of various sources of odours from food wastes by *Saccharomyces cerevisiae* and *Bacillus subtilis*

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Abstract

Odour reduction from food waste was studied using *Bacillus subtilis*. and *Saccharomyces cerevisiae*. The microorganisms selected were tested for their effectiveness on reduction of odours produced by food wastes. The microorganisms tested were *Saccharomyces cerevisiae*, *Bacillus subtilis*, and the mixture of the two microorganisms. The 500g of food waste sample was tested for each selected microorganism. For each microorganism, 10% of the pre-cultured strains was added to the food waste sample and observed for 15 days during the fermentation at room temperature. Thereafter, sensory evaluation was made by the panel test and the odour detection devices. The gaseous odour components analyzed were hydrogen sulphide, methyl-mercaptane and dimethyl disulfide. There was no significant difference between *Saccharomyces cerevisiae* and *Bacillus subtilis* for the effects on reduction of various odour components. However, each strain was more efficient than the control group ($P<0.05$) for all the sources of odours. Gas chromatography results revealed that the combination of bacteria reduced the gases to the zero level. This study revealed that the combination of both these bacteria is very effective against the odour of waste food.

Keywords: Food waste; odours; microorganisms; hydrogen sulphide; methyl-mercaptane; dimethyl disulfide

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Introduction

A world-wide environmental pollution, also threatening human health, has long been of critical concern and needs a tremendous cost to recover the polluted environments. The sources of pollutions are several in that they are contaminating air, lands and oceans. Chemical and biological pollutions are both affecting our daily lives. Out of all the sources of pollutions, food waste treatment is a major concern of our daily living conditions. It was reported by FAO (2011) that “about one third of the food produced in the world for human consumption every year - approximately 1.3 billion tons - gets lost or wasted”. The report also says that the problem of food waste is the same phenomenon for both developed and developing countries in terms of the percentage of food waste. Up to date, most of food waste treatments in Korea and other developed countries are conducted

mainly by chemicals at commercial levels. The food waste produced per year is approximately 5.6 million tons in Korea and the cost for treatment of the food waste is about 150,000 Won/ton (US\$ 140) (Yun, 2005). The problems are not just the costly expenses for the food waste treatment but more seriously are the environmental pollution during the process of the food waste treatment at commercial levels (Kinya et al., 2005). Even during the collection and transportation of food waste, liquid leakage from the collection trucks could be a big potential threat for human health because those untreated liquid and residues dripping during the transportation are full of harmful and even fatal microbes. Not only treatment of food waste but also the way of transporting them must be cautiously handled. Food wastes contain various sources of organic components such as cereals, vegetables, oils and processed fish, meat products and etc. Most of them are low calorific values and high moisture contents, which

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is not suitable for incineration and has to be buried under the ground. The mishandling of food wastes could create side effects to environment such as bad smell and contaminated ground water and no more available fill-up land. Korean governments have allowed no more fill-up land since 2005 (Kuo et al., 2005). The odours from food wastes are mostly unpleasant smells caused by hydrogen sulphide, ammonia, hydrogen sulphide, methyl-mercaptane, dimethyl disulfide, amines etc. These odours are caused by various chemicals and create sensitive responses to human such as mental and physiological stresses, headache, hard-breathing, eating disorder and various allergic reactions. Odours are in general gaseous substances of discomfort and even cause the problems of nervous systems such as sense of hearing, smelling and tasting. Methods for evaluation of odours are to quantitatively analyze the odour causing materials with its concentration level and the sensory measurement method by the panel test with no mechanical devices. The methods for odour treatments are physical, chemical and biological treatment (Kinya et al., 2005). The physical treatment to remove odours is that they can be washed off with water or use activated carbons, zeolite, and many other minerals with large absorption surface areas. The physical treatment is relatively expensive compared to other relevant methods. The chemical method can be divided into the masking and the neutralization. However, the chemical treatment needs an excessive consumption of chemicals which could secondary contamination and costly operation drawbacks. Therefore, the biological method for odour removal is highly needed for environmental conservation. In this study, two types of microorganisms were used for the removal of odours generated from the food waste and were tested for their effectiveness.

Materials and Methods

Sample collection

The food wastes for the experiment were collected from a local restaurant and were drained for 1 to 2 hrs. Liquid in the food wastes then was removed by the juice extraction machine.

Microorganisms used

Strains of *Saccharomyces cerevisiae* and *Bacillus subtilis* were isolated from the food waste and were tested in the experiment. In comparisons among different microorganisms for their effects, the control groups were treated with no microbes (control group) and the other two of the microbes commercially sold for the odour removal in liquid type (Offensive remover 1(OR1, Super-fresh[®], Jane-Dare World Co. Ltd, Korea) and offensive remover 2 (OR2, Bioblast[®], Jane-Dare World Co. Ltd, Korea). For pre-cultivation media, Sabourard Dextrose agar (Difco, USA) and Tryptic Soy Broth (Difco, USA)

Table 1: Measured scores of odour in food waste

odour score	Degree of odour
0	None
1	Threshold (low odour)
2	Moderate
3	Strong
4	Very strong
5	Over strong

Table 2: Conditions of gas-chromatography for analysis of hydrogen sulphide, methyl-mercaptane and dimethyl disulfide

Description	Condition
Instrument	HP 5890 series II Gas chromatograph, Hewlett Packard, USA
Injector	150 °C
Column	Glass Packed Column, 1,2,3-TCEP/ID: 2.6mm, L:2m
Column flow	N ₂ , 10.1ml/min
Oven Temp.	60 °C(0min) → 20°C /min → 130°C (1min)
Detector	FPD, 150 °C

were used. The treatment groups were treated with *Saccharomyces cerevisiae*, *Bacillus subtilis*, and the mixture of the two strains. A total of 6 treatment groups including the control group were tested in this study.

General components of food wastes

The general components of the food wastes were quantitatively analyzed by Association of Official Analytical Chemists (AOAC) method. The moisture content was measured by the heat and pressure drying method. Crude fat was analyzed by Soxhlet's extraction method and Mojonnier method. Crude protein content was determined by Kjeldahl method and crude ash by the direct burning method at 550°C. Sugar content was analyzed by Dinitrosalicylic acid (DNS) method.

Fermentation

Each of the two pre-incubated strains and the mixture of the two strains were injected with 10% of 300g of the food waste sample and were stored in sterile bags. The bags were kept at the temperatures appropriate for each of the 3 strains treatment groups for 10 days.

Isolation of microorganisms

The 9 ml of sterile water was mixed with 1 ml of the food waste leachate. Then, they were agitated and centrifuged for 5 min. at 3000 rpm. They were also stepwise-diluted, spread onto NA medium for 48 hrs at 37°C and then, each of the colonies was isolated as a single colony.

The separated colony was incubated in the media added with ammonium sulphate and the strains were isolated step-wise based on their ammonia nitrogen oxidation.

Table 3: Composition of food wastes

Composition	Water content	Crude protein	Crude lipid	Crude ash	Crude fiber	Reducing sugar (g/l)
Content (%)	75.5	20.2	7.42	0.7	12.24	2.99

Table 4: Panel test results from sensory evaluation for the odour reduction by different microorganisms used in the analysis

Microorganism	Panel test for sensory evaluation
<i>Saccharomyces cerevisiae</i>	4.33±0.33 ^a
<i>Bacillus subtilis</i>	3.93±0.42 ^a
The mixture of two strains	1.72±0.12 ^d
Offensive (1)	2.20±0.18 ^c
Offensive (2)	2.76±0.13 ^c
Control	3.45±0.14 ^a

Different subscripts in the column significantly different at P=0.05

Table 5: Analysis of odour components in food waste by gas chromatography

Analysis sample	Concentration (ppm)	
	A	B (control)
Hydrogen sulphide	0.00	0.21
Methyl mercaptane	0.00	1.25
Dimethyl disulphide	0.00	0.20

A=*Saccharomyces cerevisiae* + *Bacillus subtilis*, B=without microorganisms

Panel test

The panel test was performed by experienced panels and the odour measurement was done by the Korea standard manual of environmental pollution process. The criteria for the panel test were shown in Table 1.

Analysis by Gastech® device

Hydrogen sulphide was measured by the Gastech® (KORINS co., Korea) using the gas detection test tubes ranging from 0.25~120 ppm to 0.2~2 ppm.

Gas chromatography analysis

The analytic conditions for GC analysis were shown in Table 2. For the sample with the highest odour reducing effects and the control sample, hydrogen sulfide, methyl-mercaptane, and dimethyl disulfide were analyzed. The food waste was put in a 6L plastic bag filled with high-purity nitrogen gas for 24 hours at room temp and hydrogen sulfide was analyzed by GC/PFPD.

Statistical analysis

The analysis was made for the mean comparison among the treatment groups by Tukey test using SAS (SAS version 9.1, 2009).

Results

General component analysis

Results from the composition analysis for food waste are shown in Table 3. The food waste in this study contained 20.2% of protein content in solid form of food

waste, which is relatively low in comparison with the 23% of the average crude protein content in general food waste. The 7.42% of crude oils and 0.07% crude ash resulted from this study is relatively lower than average crude ash in food waste in general. The result of sensory evaluation is given in Table 4. It showed that odour reduced significantly when the combination of both of the bacteria was used.

Gas Chromatography Analysis

The typical odour compounds from food wastes such as hydrogen sulphide, methyl-mercaptane, and dimethyl disulphide were analyzed by using the gas chromatography. The samples treated with the mixture of *Saccharomyces cerevisiae* and *Bacillus subtilis* for sensory evaluation with the lowest odour and odour-reducing effects were analyzed. The analysis for hydrogen sulphide, methyl mercaptane, and dimethyl disulfide are shown in Table 5. In the treated groups, the gas production was eliminated compared to the control. The most significant effect was found when used the mixture of the two strains. In the control group, hydrogen sulphide level was much higher than the required level by law.

Discussion

In Korea, the average amount of daily food waste is around 11.000 tons which contains mostly organic matters and water. The collection and treatment process generates large amounts of odour compounds. The general usage of treated food wastes was composting and recycling of food wastes, fertilizers, animal feeds, and aerobic methane gas production (Lee et al., 1989). However, during the process of collecting and transporting food wastes, odours generated during the treatment process is becoming the main issue of complaints. Thus, more effective and hygienic method of odour removal development is urgently needed. The chemical and physical methods for reducing odours are costly and the secondary emerging problems are of environmental contamination (Cho et al., 1992). Not getting rid of the actual odour components but a rather self-absorption, or masking effect is in a great risk. However, biological treatment processes are eco-friendly and cost-efficient. In this study, an effective biological method was studied by using the strains that were screened for recycling of food wastes (Kuo et al., 2005). Strains selected and screened were yeast and *bacillus sp.* These microorganisms produce a variety of metabolites such as organic acids and alcohol, which increase palatability and storage period of animal feed additives. The experiment was repeated to confirm the effects of the odour removal from the strain selected in this study (ISO 6326-4, 1994).

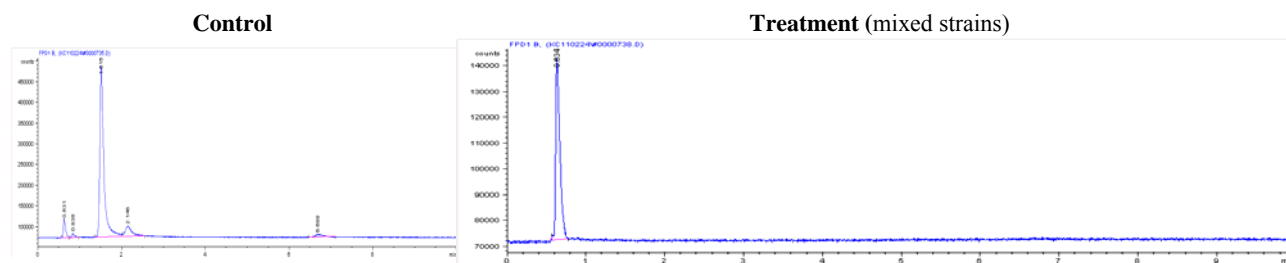


Fig. 1: Gas chromatogram of odour compounds from food waste treated with mixed strains (*Saccharomyces cerevisiae* + *Bacillus subtilis*)

These results indicated that the combination of these bacteria is very effective against the odour production of the waste food materials.

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