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Fungal hydrolysis in submerged fermentation for food waste treatment and fermentation feedstock preparation

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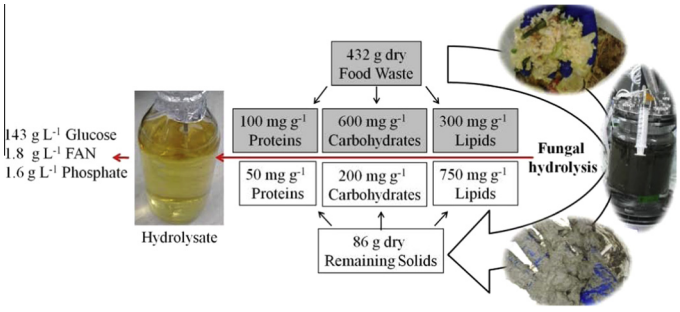
HIGHLIGHTS

- Fungal hydrolysis of food waste in submerged fermentation was investigated.
- Maximum solid-to-liquid ratio tested was 43.2% (w/v).
- A hydrolysate with 143 g L^{-1} glucose, 1.8 g L^{-1} FAN and 1.6 g L^{-1} phosphate was obtained.
- 80–90% of initial dry weight of food waste was diminished.
- Remaining solids were rich in lipids.

GRAPHICAL ABSTRACT



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ABSTRACT

Potential of fungal hydrolysis in submerged fermentation by *Aspergillus awamori* and *Aspergillus oryzae* as a food waste treatment process and for preparation of fermentation feedstock has been investigated. By fungal hydrolysis, 80–90% of the initial amount of waste was reduced and degraded within 36–48 h into glucose, free amino nitrogen (FAN) and phosphate. Experiments revealed that 80–90% of starch can be converted into glucose and highest concentration of FAN obtained, when solid mashes of *A. awamori* and *A. oryzae* are successively added to fermentations at an interval of 24 h. A maximal solid-to-liquid ratio of 43.2% (w/v) of food waste has been tested without a negative impact on releases of glucose, FAN and phosphate, and final concentrations of 143 g L^{-1}, 1.8 g L^{-1} and 1.6 g L^{-1} were obtained in the hydrolysate, respectively. Additionally, fungal hydrolysis as an alternative to conventional treatments for utilization of food waste is discussed.

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1. Introduction

More than one billion tonnes of food is wasted globally per year and needs to be treated with methods that do not pose human health risks, do not harm the environment and enable a recycling of organic waste material (Arvanitoyannis et al., 2008; Kim and Kim, 2010; FAO, 2011; Gustavsson et al., 2011, 2013; Takata et al., 2012). In Hong Kong, more than 3000 tonnes of industrial and commercial as well as domestic food wastes are produced and disposed in landfill sites per day. The capacity for waste disposal of landfill sites in Hong Kong, however, is estimated to be reached in 2018. Therefore, it raises concerns about how to deal with the enormous amounts of food waste in the near future. Incineration and anaerobic digestion could be alternative processes providing energy and heat (Zhang et al., 2007; Pirotta et al., 2013). However, food is produced under excessive consumption of energy, water and nutrients (Cuéllar and Webber, 2010; FAO, 2011). Therefore, the whole potential of waste organic matter should be exploited beyond the use as an energy source.

Even when wasted food is not edible anymore due to hygienic issues and considered to have no value, it remains a source of sugar

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monomers, amino acids, fatty acids and phosphate. Recent studies in our group have introduced opportunities to recycle carbohydrates, proteins and phosphate present in food waste as nutrient sources in microbial cultivations (Leung et al., 2012; Pleissner et al., 2013; Zhang et al., 2013). Bread, food and bakery wastes were hydrolyzed using enzymes actively secreted *in situ* by the fungi *Aspergillus awamori* and *Aspergillus oryzae*. The resultant hydrolysate that is rich in glucose, free amino nitrogen (FAN) and phosphate was used in cultivations of bacteria and heterotrophic microalgae.

Even though fungal hydrolysis of daily appearing food waste in submerged fermentation is fast and efficient, the potential as a waste treatment has only been briefly demonstrated in previous studies conducted by our group (Leung et al., 2012; Lam et al., 2013; Pleissner et al., 2013; Zhang et al., 2013). Other reported studies dealing with fungal hydrolysis focus on agro-waste, wheat straw and waste streams from food processing in submerged and solid state fermentations (e.g. Jin et al., 2005; Wang et al., 2008). The aim of these studies, however, was predominantly to produce enzymes and organic chemicals rather than the development of an efficient organic waste treatment process. For instance, Wang et al. (2008) used food waste slurry for the production of glucoamylase by *Aspergillus niger*. The reduction in food waste matter was only 10% (w/v) making this process only attractive for enzyme production but not for food waste degradation. However, an efficient degradation and utilization of waste matter as fermentation feedstock may help to overcome the mounting food waste problem in Hong Kong and other densely populated areas in a sustainable way.

In this study, the process being introduced involves submerged fermentation of daily appearing food waste by hydrolytic enzymes of the fungi *A. awamori* and *A. oryzae* for waste reduction and fermentation feedstock preparation. Fungal hydrolysis of food waste was investigated regarding maximal releases of glucose, FAN and phosphate when (i) only *A. awamori* or *A. oryzae* was added, (ii) when both fungi were added simultaneously as well as (iii) successively to fermentations. In addition, different solid-to-liquid ratios were studied in order to assess the maximal solid loading that can effectively be treated in order to obtain a concentrated hydrolysate. Furthermore, the carbohydrate, protein and lipid contents in total suspended solids (TSS) were quantified before and after fermentation. The process was further compared to the conventional food waste treatment processes: composting, anaerobic digestion, disposal in landfill sites and incineration.

2. Methods

2.1. Handling of food and bakery wastes

Food and bakery wastes were collected from canteens located in Hong Kong Science Park and Starbucks, Shatin, Hong Kong. These wastes were immediately blended in the laboratory by using a domestic kitchen blender, respectively. The blends were stored at 4 °C until use, but for no more than two weeks. Due to the limited availability of food waste in Hong Kong Science Park, two different food wastes (A and B) consisting of rice, noodles, meat and vegetables were collected at different dates from the same canteen and successively used in experiments. The compositions of Food wastes A and B are shown in Table 1.

Table 1
Compositions of bakery and food wastes used in this study.

	Bakery waste	Food waste A	Food waste B
Carbohydrates (mg g^{-1})	654.5	738.4	470.3
Starch (mg g^{1})	316.7	612.3	361.5
Proteins (mg g^{-1})	98.2	57.9	99.3
Lipids (mg g^{-1})	265.8	73.8	373.7

2.2. Fungi and solid state fermentation

A. oryzae was isolated from a soy sauce starter provided by the Amoy Food Ltd., Hong Kong (Leung et al., 2012). *A. awamori* ATCC 14331 was purchased from the American Type Culture Collection (Rockville, MD, USA). Spore solutions of both fungi were produced as described earlier (Lam et al., 2013).

Solid state fermentation was performed in a petri dish to which 10 g (8.5 g dry weight) of bakery waste, consisting of cake, bun and pastry, were added. The bakery waste was then inoculated with 1 mL of spore solution of *A. awamori* (4.6×10^5 spores mL^{-1}) and 1 mL of spore solution of *A. oryzae* (6.3×10^5 spores mL^{-1}), respectively, and incubated for 7 days at 30 °C (Leung et al., 2012; Pleissner et al., 2013). Fungal solid mash refers to the fungal biomass obtained after solid state fermentation.

2.3. Submerged fermentation

Blended food waste was inoculated with fungal solid mashes of *A. awamori* and/or *A. oryzae* grown in solid state fermentation, and mixed together. The total volume of the blend was adjusted to 1 L by adding demineralized water. Fermentation was performed in a 2.5 L bioreactor (Brunswick) at 55 °C and pH 4.0–4.5, without aeration and stirred at 1400 rpm. No pH control was needed as pH remained at 4.0–4.5 during fermentation found to be appropriate for enzymes of *A. awamori* and *A. oryzae* to be active (Wang et al., 2007; Lam et al., 2013). Samples were taken regularly for pH, glucose, FAN, phosphate and TSS measurements. Samples and final fermentation broth were centrifuged at 11,500*g* for 30 min, and the supernatant was filtered through Whatman No. 1 filter paper. Pellet and filter residue were mixed together, lyophilized and represent the TSS after fermentation. The resultant hydrolysate and TSS were kept frozen at −20 and −80 °C, respectively.

2.3.1. Addition of fungal solid mashes

Addition of fungal solid mashes was investigated regarding maximal releases of glucose, FAN and phosphate. Food Waste A (Table 1) was incubated for 48 h with (i) either fungal solid mashes of two petri dishes of *A. awamori* or *A. oryzae*, (ii) with one fungal solid mash of each fungi simultaneously and in different combinations: (iii) first with one fungal solid mash of *A. awamori* for 24 h followed by addition of one fungal solid mash of *A. oryzae* and incubation for another 24 h, and (iv) vice versa. In addition, fermentation without fungal solid mashes was performed as control. Fermentations were carried out in duplicate at an initial 7.6 ± 0.5% (w/v) solid-to-liquid ratio, which is defined as the ratio in percentage of gram dry food waste and fungal solid mash per milliliter liquid.

Diminished and reduced solids refer to the difference of initial TSS dry weight (food waste and fungal solid mash) and dry weight of TSS after fermentation.

2.3.2. Solid-to-liquid ratio

Maximal solid loading that can effectively be treated by fungal hydrolysis in submerged fermentation in order to achieve constantly high yields of glucose, FAN and phosphate was investigated at 12.8%, 16.8%, 22.4%, 24.4% and 43.2% (w/v) solid-to-liquid ratios using Food Waste B (Table 1). Experiments were performed according to the findings of Section 2.3.1. Enzyme-to-solid ratio was kept constant by adding 8.5 g (dry weight) of solid mashes of each of *A. awamori* and *A. oryzae* per 100 g (dry weight) food waste.

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