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Isolation and Screening of Biodegrading Fungi from Kitchen Waste and Optimization of Physico-chemical Conditions for Enhanced Biodegradation

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Abstract

Kitchen waste impose precarious effects to the human health as well as to environment. The principal determinants of kitchen waste generation include rapid increase in human population and urbanization. The highly biodegradable kitchen waste must be carefully handled and disposed-off to reduce the risk of pollution. Biodegradation of kitchen waste is usually carried out by various types of microorganisms including fungi. Fungi can produce extracellular enzymes that can degrade industrial, agricultural and municipal solid waste. The present study has emphasized the isolation and screening of indigenous fungal species from kitchen waste. Furthermore, optimum conditions to enhance biodegradation of kitchen waste was observed through physiochemical processes. The kitchen waste samples were collected to isolate biodegrading fungi directly from the dustbins of University of Agriculture Faisalabad. Fungal isolation was performed by inoculating the sample on selective media and were further screened by Congo red plate method. Six isolates of fungi were identified at specie level, while different parameters were optimized like acid pretreatment, thermos-acid pretreatment, optimization of temperature, pH and preparation of fungal consortia for enhanced biodegradation of kitchen waste. Out of six fungal isolates four were identified as *Trichoderma harzianum Aspergillus niger, Aspergillus flavus* and *Penicillium expansum*, exhibiting biodegrading ability and formed clear zone around their growth. The influence on degradation of kitchen waste by different parameters such as acid pretreatment, thermos-acid pretreatment, freeze thaw pretreatment, temperature (25°C, 37°C and 45°C) and pH (5.7, 6.5 and 7.5) resulted in weight loss. The optimization parameter has indicated more weight loss (66%) at 25°C temperature as compared to other parameters. Thus, 25°C was the optimum temperature for enhanced biodegradation of kitchen waste.

Key Words: Biodegradation, isolation, biodegrading fungi, kitchen waste, physico-chemical

Introduction

Unwanted, discarded and useless material having no adequate value and is thrown away by the owner is termed as waste. Huge quantity of waste is generated every day in developed and developing countries. In rapidly growing cities of developing countries, urban solid waste is one of the major problem. The various types of waste have an adverse effect on environment and living organisms (Jebapriya *et al*., 2013).

Each municipal resident generates about 0.35 to 1.0 kg solid waste every day. This investigation emphasis on biodegradable kitchen waste handling and consumption of restaurant and catering facility (Park *et al.*, 2008). Household leftover along with a minor share of marketable leftover collected is biggest group of waste existing on this earth causing ecological contamination. Kitchen waste consists more than half of the total part of municipal solid waste produced in form of fruit and vegetable peelings, non-consumable rotten or ripened vegetables and fruits discharged from restaurants, public catering rooms and homes. Those wastes having more carbohydrate content can be used for energy recovering by fermentation process and are available all over the world (Han and Shin, 2004). Kitchen waste (KW) is the dumped and surplus organic matter from households, lodges and restaurants (Li *et al*., 2009).

Previously, the most common methods such as composting and reutilization as animal feed were implemented to dispose-off kitchen waste (Ma *et al*., 2011). Most of the kitchen waste has been disposed along with other wastes which results in environmental pollution such as foul smell, discharge of toxic gases, waste of land, vermin attraction and contamination of ground water (Shin *et al*., 2001). The abundance of kitchen waste generation has been reported in highly populated areas. It is difficult to process kitchen waste by standard means due to high moisture content (Kuo and Cheng, 2007). Kitchen waste organic bulk consists of amino acids, peptides,

proteins, carbohydrates and fatty acids (Braun and Grasmug, 2003).

A diverse group of fungi utilize cellulose as a source of carbon and to meet their energy requirements. *Trichoderma, Aspergillus, Chaetomium, Fusarium, Curvularia, Phomo, Memoniella* and *Thielavia* are represented by species of genera having strongly cellulose degrading ability (Vries and Visser, 2001).

Biodegradation of kitchen waste through indigenous fungi can be enhanced by implementing certain strategies. Optimization of pH, temperature, moisture and pre-treatment of kitchen waste by acids, heat and thermos-acid treatment can lead to maximum degradation. Disruption of cell releases all the intracellular and cell wall constituents in the medium which can easily be taken up by microorganisms (Bien *et al*., 2004).

Materials and Methods

Sample collection

A total of 15 kitchen waste samples were collected directly from dustbins of Afzal Hall mess, University of Agriculture Faisalabad. Sample waste consists of fruits and vegetables peeling, rice, chicken curry, potato cutlets, mayonnaise, butter and bread. The samples were collected in pre-sterilized screw cap glass vials. These samples were brought to laboratory for isolation of biodegrading fungi.

Isolation of fungi

All the kitchen waste samples were processed within 3 hours of collection. For isolation, dilution plate method was used. One gram fresh weigh sample was placed in 10 ml of sterile water and shaken for proper mixing at room temperature. Then, 100 µl portions of the suspensions were inoculated onto plates containing potato dextrose agar (PDA). The plates were incubated at 25°C to 28°C for 4-8 days. After incubation, different morphological characteristics including shape, size and color were observed on the plates and further transferred to fresh media to obtain pure cultures. Aseptic inoculation of fungal colonies was performed to get pure fungal cultures.

Identification of isolated fungi

The technique of James and Natalie (2001) was adopted for identification of the unknown fungal isolates using cotton blue in lactophenol stain. The identification was achieved by placing a drop of lactophenol stain on a clean slide with the aid of amounting needle followed by adding a small portion of mycelium from the fungal cultures. The mycelium was spread very well on the slide with the aid of the needle. A cover slip was gently applied with little pressure to eliminate air bubbles. The slide was then mounted and observed with x10 and x40 objective lenses under the microscope respectively.

Screening of biodegradable fungus

The fungal isolates were screened to check their ability to degrade kitchen waste through the Congo red plate screening method. The isolates were grown on PDA medium supplemented with 1% CMC and incubated at 27ºC for 5 days. After incubation, the petri plates were flooded with Congo red solution (0.1%), after 15 mins the Congo red solution was discarded and the plates were washed with 1M NaOH solution. The washing solution could stand for 15–20 minutes. The clear zone of inhibition was observed after the enzymatic utilization of the cellulose (Khokhar *et al.*, 2012).

Waste Treatment:

After screening of fungal isolates kitchen waste was subjected to acid pretreatment, thermos-acid pretreatment, freeze thaw pretreatment, temperature (25ºC, 37ºC and 45ºC) and pH optimization in different trials. Kitchen waste was subjected to the above-mentioned conditions and weight loss was measured by following formulae:

Dry weight calculation

Weight loss (%) = W- W1/W X100

W=Initial weight

W1=Final weight after 45 days of incubation.

Results

Isolation of fungi from kitchen waste sample:

Different colonies of fungi having different color and shape were obtained by inoculation of the kitchen waste sample on potato dextrose agar. The cultural characteristics of six fungal isolates on PDA are shown in Fig 1-6 (a). Moreover, the macroscopic examination of these fungal isolates comprising of colony morphology is described in Table 1. These isolates were further screened by microscopic examination shown in Fig 1-6 (b).

Screening of biodegrading fungi

Among six fungal isolates such as *Aspergillus niger, Aspergillus flavus, Penicillium expansum, Trichoderma harzianum, Rhizopus* and *Alternaria*, first four isolates showed biodegradation activity and formed clear zone around the colony while *Alternaria* and *Rhizopus* did not show any biodegradation activity.

Waste treatment

After screening of fungal isolates, the kitchen waste was subjected to acid pretreatment, freeze thaw pretreatment, temperature (37°C and 45°C) and pH (5.7, 6.5 and 7.5) optimization parameter. The weight loss was observed to be 55%, 50%, 44%, 40%, 58%, 54% and 49% respectively while more weight loss was analyzed in thermos-acid pretreatment and at 25°C temperature. Under these

conditions, the weight loss was observed to be 60% and 66% after inoculating the flasks with 5% fungal suspension and then giving them incubation for 45 days (Fig 7).

Discussion

During the process of handling and removal, highly biodegradable waste of kitchen poses a lot of pollution problem. Due to different formations of restaurants, kitchen waste cookeries and cafeterias, different contagious agents spread (Shin *et al.,* 2001). Raw material, temperature, pH and certain other conditions are responsible for the uncertainty of microorganisms due to which it has become difficult to continue persistent degradation (Eriksson *et al*., 2012).

Different parameters like temperature, pH, acid pretreatment and thermal pretreatment were optimized for enhanced biodegradation (Bien *et al.,* 2004). Ishii *et al.* 2007 isolated twelve polyethylene degrading fungi from various soil environments. Isolated species were assessed for their ability to degrade polyethylene. In present study, the different fungi were isolated and identified by examining morphological, colonial and microscopic characteristics. From different sources with 1% Congo red fungal species like *Trichoderma viride, Trichoderma harzianum, Trichoderma koningii, Aspergillus nidulans, Aspergillus japonicus, Penicillium expansum, Penicillium lanosum* and *Penicillium oxalicum* were screened and they have depicted highest cellulase activity (Khokhar *et al.* 2012). In the present study six fungal isolates were isolated, identified and screened from kitchen waste sample, out of which four isolates *Aspergillus niger, Aspergillus flavus, Penicillium expansum* and *Trichoderma harzianum* have exhibited the cellulolytic activity.

Rahman *et al*. (2009) stated that in kitchen waste decomposition the fungal strain *Trichoderma harzianum* was found to be the most effective. It provided weight (30.80%) losses and highest volume (31.80%) in treated waste with suspension of spore in their studies. Present study reveals that weight loss at temperature 25°C was more than that of previous studies and was recorded as 66% due to fungal consortia.

Conclusion

It can be concluded from the present study that the that out of six fungal isolates obtained from kitchen waste source, four isolates of fungi: *Aspergillus niger, Aspergillus flavus, Penicillium expansum* and *Trichoderma harzianum* had biodegradation ability. Kitchen waste biodegradation was observed maximum at temperature of 25ºC having weight loss (66%) and at thermos-acid pretreatment (60%) by isolated fungal consortia. The present study activity would contribute towards bioprocess technology to reduce pollutants toxicity.

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Fig 1: Cultural (1a) and Microscopic (1b) examination of isolated *Aspergillus niger* from kitchen waste on Potato Dextrose Agar

Fig 2: Cultural (2a) and Microscopic (2b) examination of isolated *Aspergillus flavus* from kitchen waste on Potato Dextrose Agar

Fig 3: Cultural (3a) and Microscopic (3b) examination of isolated *Penicillium expansum* from kitchen waste on Potato Dextrose Agar

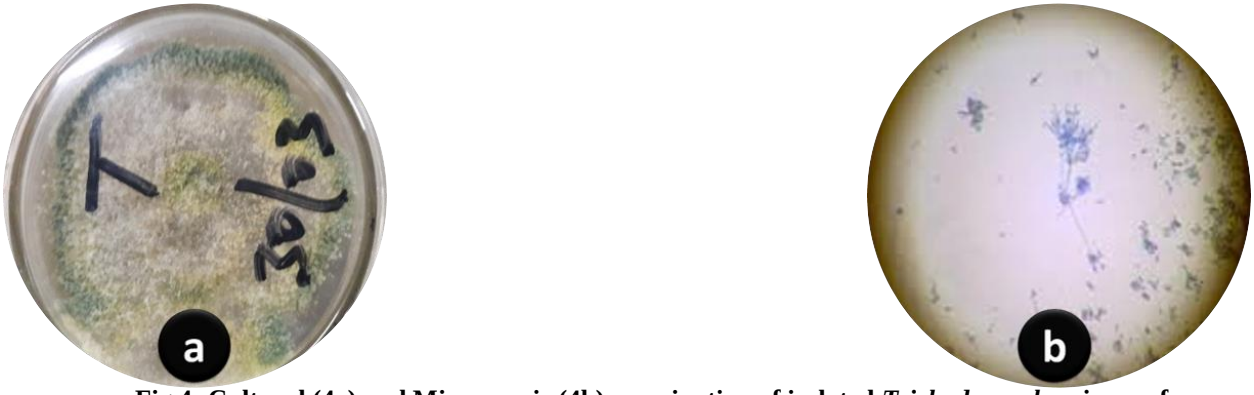

Fig 4: Cultural (4a) and Microscopic (4b) examination of isolated *Trichoderma harzianum* from kitchen waste on Potato Dextrose Agar

Fig 5: Cultural (5a) and Microscopic (5b) examination of isolated *Rhizopus* from kitchen waste on Potato Dextrose Agar

Fig 6: Cultural (6a) and Microscopic (6b) examination of isolated *Alternaria* from kitchen waste on Potato Dextrose Agar

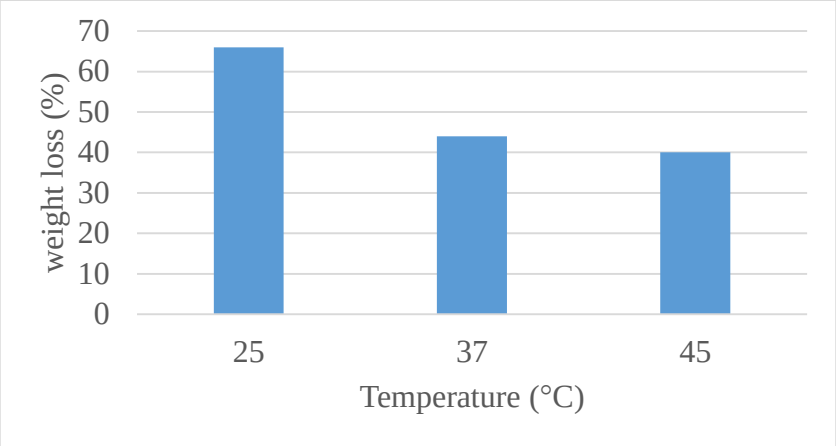

Fig 7: Biodegradation of kitchen waste at different temperatures

Table 1: Macroscopic identification of fungi isolated from kitchen waste sample

Fungi	**Colony Characteristics**		
	Texture	**Surface color**	**Reverse color**
Aspergillus flavus	Woolly to cottony	Green	Cream
Aspergillus niger	Soft and velvety	Initially white, further becomes black	Pale yellow
Penicillium expansum	Velvety	Green having sterile white margin	Dirty white to cream
Trichoderma harzianum	Wooly	Green	Bright orange
Rhizopus	Cottony	Initially white colonies then turn grey to brown	Pale yellow
Alternaria	Downy to wooly	Black or greyish	Brown to black