Isolation and screening of biodegrading bacteria from kitchen waste and optimization of physiochemical conditions to enhance degradation

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Abstract—Kitchen waste is the left over organic matter of unwanted raw or cooked food and major part of domestic waste with a potential to cause health issues if not handled properly or left for natural degradation of several years. Keeping in view enzymatic secretion of microorganisms, biodegradation is considered as an important strategy to overcome waste related problems. In the present study, samples were collected from waste receiving dustbins of Fatima hall mess, UAF, Pakistan. Eight different bacterial isolates were identified on nutrient agar media by morphological, colonial and biochemical characterization. Biodegrading ability of these isolates was screened by measuring zone of clearance and halo formation around growth on specific media. Amylase, protease and cellulase secretion of isolated bacterial species indicated their ability for kitchen waste degradation. 500g Kitchen waste was used in five different trials separately at different pH (5.7, 6.5 and 7.0) and temperature (37ºC, 40ºC and 45ºC). Acid, heat and freeze-thaw methods were used to enhance degradation. 55g (11%), 100.9g (20.18%) and 3g (0.6%) weight reduction was calculated without consortia by thermal, acid and freeze-thaw pre-treatment respectively. 59.29g (11.85%), 39.91g (7.98%) and 37.63g (7.5%) weight reduction was observed within 7 days in three different trials with consortium. 7.0 pH and 45ºC temperature was found optimum for biodegradation by consortium of *Serratia spp.,* *Pseudomonas aeruginose, Bacillus cereus, Bacillus subtilis* and *Bacillus megaterium.*

Keywords: KW: Kitchen waste, MSW: Muncipal Solid Waste, OD: Optical Density,

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1 INTRODUCTION

IN most developing countries, Municipal Solid Waste (MSW) management is an extremely ignored part of the whole environmental management. Improper handling of MSW with its consequences is now a serious concern of developing nations [1]. At present, the world wide MSW generation is approximately 1.3 billion tons per annum and estimated to reach 3 billion tons by 2025 [2]. In Europe, kitchen waste (KW) production is about 2.5 billion tons per year representing an incredible prospective of exploitable biomass. Composting and reutilization as animal feed remained ways of disposal of KW in past [3]. As an estimate 0.35-1.0 kg of solid waste is generated by each urban resident [4].Kitchen waste production is growing progressively as the time is passing through with an outburst of residential areas, urbanization and consumption practices of human beings that are getting

extra sumptuous. Its production and changeability rely on the demeanor of inhabitants, their consumption practices, their earnings, and climate situations in a specific region [5].

In Pakistan, more than 90% of collected waste is managed by MSW using primary and secondary collection and open dumping. Only 60% of the waste is managed while remaining is left as thrown in the public places including roads, streets, railway lines, open plots, open sewerage lines in overall urban area. In Lahore, 5000 tons of solid waste is produced per day [6].Community discontinues observing objects as throwaway and starts noticing it as reusable and important reserves and energy, the substances are wasted. A judgment of need to alter this viewpoint is rising for solid waste managers because municipal solid waste is increased by 40% from 1980 to 1997 and is expected to raise more upto 40% by 2020 [7].

Kitchen waste is the dumped and surplus organic matter from households, lodges and restaurants [8]. Kitchen waste forms a major part of domestic waste. Unwanted raw or cooked food is dumped during or after food preparation considered as food waste [9]. The management of organic waste material is necessary to lessen environmental load, decrease hazards to human health and minimize resource

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depletion [10]. Kitchen waste production is in tons in highly populated areas. Due to high moisture contents, it is difficult to process kitchen wastes by standard means, like incineration[11]. The bulk of organic kit of KW consists of carbohydrates, amino acids, peptides, fatty acids and their esters [12], [13].Kitchen waste comprises considerable quantity of processed food as well as paper and plastics. People give attention towards recycling of paper, plastic and glass; organic waste can be recycled into compost. Biodegradation of Kitchen waste can be used for recycling of different organic constitutes. Upto 95 percent of biodegradable fraction of kitchen waste is appropriate for anaerobic digestion which makes it an affluent source of organic material[14].

Enzymes are fascinating subject matter of study all over the world; believing their vast series of physiological, industrial and analytical applications; particularly from microbes because of their wide biochemical variety, viability of mass culture and relieve of genetic handling [15]. The optimization of physical and chemical factors for degradation is important due to their effect on the financial system and achievability of the procedure [16].

Moisture provides favorable environment for microbial growth which metabolizes waste into simpler compounds important for soil fertility, plant growth and well balanced natural ecosystem. Bacteria and saprobic fungi play a key role in optimal agricultural and Kitchen waste bioconversion known as biodegradation.

Kitchen waste is suitable for anaerobic digestion with biogas production due to its high moisture contents and degrading ability [17]. Degradation of kitchen waste can be enhanced by adopting some strategies. By forming bacterial consortia, biodegradation can be enhanced as consortia contain different bacteria with different enzymatic makeup.

Optimization of pH, temperature, and pre-treatment of KW by acids, heat and freeze-thaw methods can lead to maximum degradation. Disruption of cells in food commodities, releases all the intracellular and cell wall components in the medium which can easily be taken up by microorganisms [18]. Improper handling of KW can lead to serious health problems with slow process of degradation. This study aimed to investigate optimum pH and temperature for enhanced KW biodegradation by utilizing biodegrading bacteria isolated from kitchen waste. This study has been designed with objectives of isolation and screening of biodegrading bacteria and optimization of conditions to enhance biodegradation of Kitchen waste.

2 MATERIALS AND METHODS

2.1 Isolation of Biodegrading Bacteria
Samples were streaked on nutrient agar plates and incubated at 37°C for 24hrs. Next day mixed growth was observed and each different colony was purified on separate nutrient agar plates which were incubated for 24hrs at 37°C.

2.2 Identification of Bacterial isolates
Bacterial isolates were identified macroscopically by examining colony morphology; surface pigmentation, shape, size, margin and surface on nutrient agar plates. Microscopic examination was done including Gram staining and spore staining to identify shape, cell arrangement and spore producing bacterial isolates. Morphological characteristics of bacteria were determined under microscope after Gram staining [19].

2.3 Determination of metabolic characteristics
The metabolic ability of the isolates to degrade food waste was determined by observing individual isolates on different media. MacConkey agar, Skim milk agar, cellulose Congo red agar and starch agar were used to identify the enteric lactose fermenting bacteria, protease & lipase, cellulase and amylase producers, respectively. All plates were incubated at 30°C for 2 days and checked for a zone of clearing around each bacterial isolate. For starch agar, the zone of clearing was observed after flooding the plates with iodine.

2.4 Screening of Cellulose degrading bacteria
Cellulose degrading ability of bacterial isolates was checked by Congo red dilution assay. Bacterial isolates were streaked on LB medium plates containing 1% CMC with few drops of Congo red dye solution [20].

2.5 Consortia Formation

Consortium was made by inoculating isolates in 10ml nutrient broth test tubes separately at 37°C for 3 days with addition of 0.1% starch, casein and cellulose. Optical density was measured by Elisa Reader at 650nm. *Bacillus* strains and *Pseudomonas* were selected for consortium [21].

2.6 Waste Treatment

Autoclaved Kitchen waste was placed in 4 flasks, 3 were subjected to following pre-treatments and 1 was used as control.

2.6.1 Acid pre-treatment
500 g of raw mixed KW was acidified with HCl (10 N) at room temperature (18 ± 2°C) until pH 2, checking this value after 24 hours of contact time. During the pre-treatment, the KW was continuously mixed for the well distribution of the HCl and the pH was measured at different spots in the container [22].

2.6.2 Thermal pre-treatment
500 g of raw mixed KW was autoclaved at 121°C (1 bar) with the following operational cycle: 30 min pre-heating to 121°C + 30 min autoclaving at 121°C + 30 min cooling to room temperature (18 ± 2°C) [22].

2.6.3 Freeze–thaw pre-treatment
500 g of raw mixed KW was frozen to -4°C in an ultra-low temperature freezer. After 24 hours, the frozen KW was thawed in a thermal oven at 55 ± 2°C for 30 min [22].

2.7 Optimization of pH and temperature

Kitchen waste was subjected to different pH ranges (5.7, 6.5 and 7.5) in different trials with respective temperature ranges (37oC, 40oC and 45oC). Kitchen waste was subjected to these conditions and degradation was observed on the basis of physical appearance, change in texture, color, and odor. 5ml consortia solution was mixed with pre-treated waste prior to pH optimization of pH and temperature.

2.8 Weight reduction analysis

Weight reduction of kitchen waste due to biodegradation was measured by weighing balance. Fresh weight was noticed on day 1 prior to any pre-treatment and after 15 days weight was measured. 3 trials were conducted for 7 days after pre-treatments.

3 RESULTS

Different colonies of bacteria were obtained on nutrient agar plates which were further screened by Gram staining and biochemical tests. Eight different bacterial isolates were obtained from kitchen waste. Four isolates were from Enterobacteriaceae, three were from Bacillus genus and one was from genus *Pseudomonas.* Positivity of *Bacillus* species towards starch hydrolysis, protein (casein) hydrolysis, showed their ability to produce alpha-amylase and casease enzyme. *Pseudomonas* was found to be cellulolytic after observing zone of clearance by Congo red dilution assay. Moreover *Pseudomonas* showed casein hydrolysis on skim milk agar along with *Serriatia*spp.

TABLE 1
PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OFSTRAINS.

Isolates	Morphology	Amylase	Protease	Cellulase	Lipase
Iso1	(+) rods	+ve	+ve	-ve	-ve
Iso2	(-)	-ve	-ve	-ve	-ve
Iso3	(+) cocci	-ve	-ve	-ve	-ve
Iso4	(-) rods	+ve	+ve	+ve	-ve
Iso5	(+) rods	+ve	+ve	-ve	-ve
Iso6	(-) cocci	-ve	-ve	-ve	-ve
Iso7	(+) rods	+ve	+ve	-ve	-ve
Iso8	(-)	-ve	-ve	-ve	-ve

Isolates 1, 2,3,4,5,6,7,8 represent *Bacillus subtilis, Serratia spp., Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus megaterium, E.coli, Bacillus cereus, Salmonella*

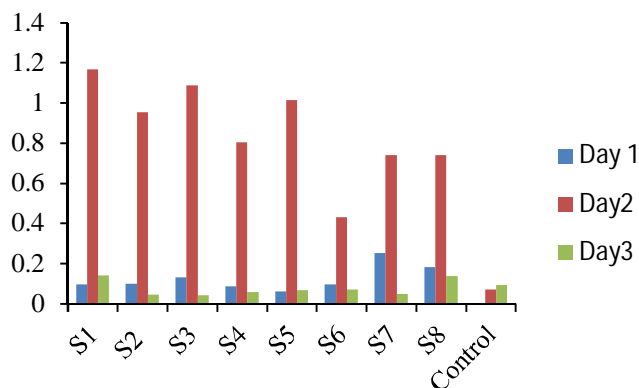*enterica*respectively with their specific enzyme secretion ability.

Fig. 1. Graph representing OD of eight different isolates incubated at 37oC for 24, 48 and 72 hours.

TABLE 2

OD OF INCUBATED BACTERIAL ISOLATES

Days	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Control
1	0.094	0.097	0.131	0.086	0.061	0.095	0.251	0.181	0.007
2	1.165	0.952	1.088	0.803	1.012	0.43	0.739	0.738	0.009
3	0.138	0.044	0.04	0.055	0.066	0.068	0.046	0.137	0.009

In table 2 OD increased continuously for 48 hours and decreased after 72 hours.OD of all isolates were measured for 3 days and Bacillus species, Pseudomonas spp and Serratia spp. were selected for consortium and their enzymatic production revealed their ability to degrade organic matter of waste by specific enzymes (amylase, casease, cellulose).
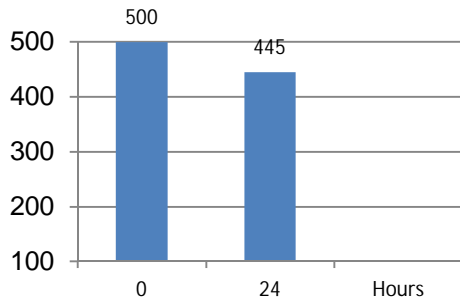
Fig. 2. Weight reduction of Kitchen waste after 24 hour at 45°C with heat pre-treatment.
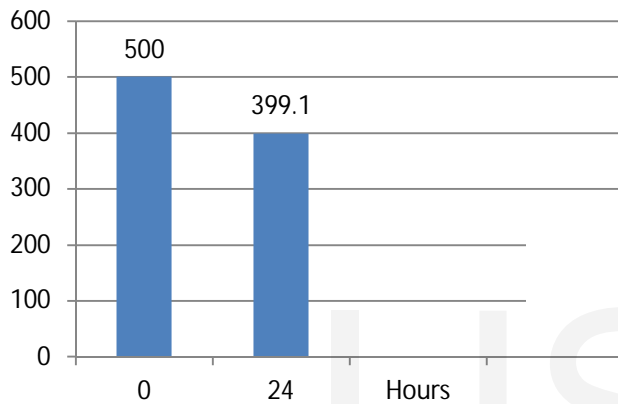
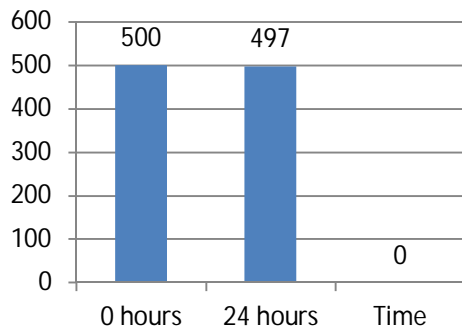Fig. 3. Weight reduction in 24 hours during acid pre-treatment 37°C.

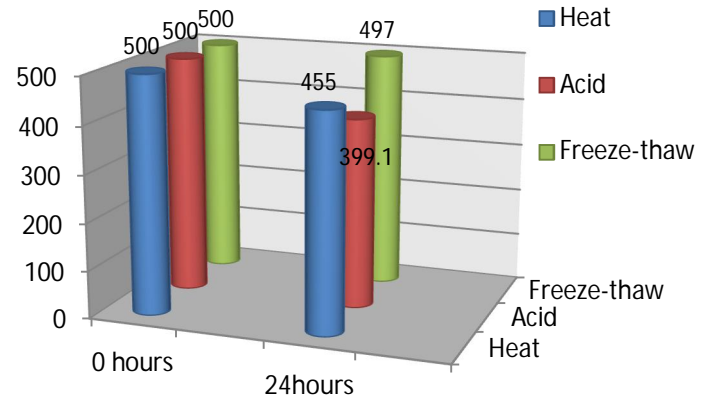Fig. 4. In this illustration reduction in weight after freeze-thaw method within24hours at -4°C is shown.

Fig. 5. Comparison of different pre-treatment in weight reduction of KW without consortium
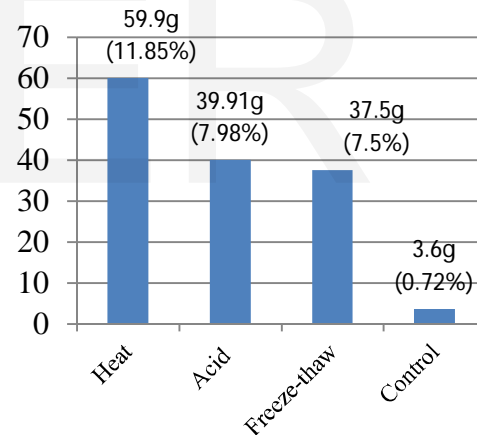
3.1 Biodegradation Analysis

Fig. 6. Graph representing weight reduction of three different pretreated waste samples by microbial consortium after 7 days.

Kitchen waste was subjected to heat, acid and freeze thaw pre-treatment prior to consortium addition, aimed to enhance biodegradation. 55g, 100.9g and 3g reduction in kitchen waste out of 500 grams was measured by heat, acid and freeze-thaw method respectively in 24 hours.

4 DISCUSSION

Present study concerns with the isolation and screening of biodegrading bacteria from kitchen waste, comparative analysis of isolated species for their ability to degrade organic kit of Kitchen waste, effect of pre-treatments and consortia on Kitchen waste degradation, role of OD in

consortia formation and optimization of pH and temperature for their effect to enhance biodegradation of Kitchen waste.

Kitchen waste samples were inoculated on general purpose media to isolate different bacteria. Morphological, colonial and biochemical characterization of the isolated strains were performed as described by Saha*et al.* [23]. The bacterial isolates were presumptively identified by means of morphological examination and some biochemical characterisation. The parameters investigated include colony characteristics, shape, spore, motility, Gram's reaction, catalase production, urease production, Voges-Proskauer (V-P) reaction, Indole production, Nitrate reduction, citrate utilization, carbohydrate metabolism (acid-gas production), starch hydrolysis, hydrolysis, Casein hydrolysis, Growth at different pH and temperature were carried out following the standard methods described in Bergey's Manual of Determinative Bacteria. Isolates from genus Bacillus were differentiated from other isolates on the basis of spore staining, gram staining, catalase, starch hydrolysis and MR test. As all the *Bacillus sp.* contain thick peptidoglycan, Catalase and amylase enzyme. Some of strains of bacilli were found to be variable for MR tests as described by Logan and Berkeley [5].

Enteric isolates were examined by MR, VP, Indole, citrate, oxidase and sugar fermentation test. Biochemical tests were performed in laboratory as performed by William *et al.* [24], who presented tabular differentiation among different bacteria at genus and species level belonging to Enterobacteriaceae.

Biodegrading ability of isolates was screened by measuring zone of clearance around their growth on starch agar, skim milk agar and Congo red agar medium. Bacillus species secreted exoenzyme (alpha-amylase) which hydrolysed starch into simpler compounds which were taken up by bacteria easily, *Pseudomonas aeruginosa* showed proteolytic activity by degrading casein protein with the secretion of casease enzyme alongwith cellulose degradation into glucose by cellulase enzyme when inoculated on skim milk agar and Congo red agar medium respectively. White color of milk is due to casein protein and its hydrolysis results in halo formation around growth. Results were found in accordance with that of Usha*et al.* [25] and Behera*et al.* [26]. Usha*et al.* [25] performed the screening of *Bacillus*, *Pseudomonas* and *Streptomyces* sp. by observing the clear zone around colonies due to enzyme secretion after 15-20 days of incubation. Smilaritylies in the degrading ability of bacteria while incubation period was found to be the cause of disagreement with that of Usha*et al.* [25] as the results were obtained after two days of incubation in present study. Behera*et al.* [26] mentioned the cellulose degrading ability of *Pseudomonas* sp. alongwith with fifteen other cellulolytic bacteria by observing zone of

clearance on Congo red agar medium with enzyme production ranging from 2.471 to 98.253 U/ml/m.

Bacterial consortium was prepared by inoculating and incubating isolates in nutrient broth for 3 days at 37°C for 24 hours by adopting the method of Sarkar *et al.* [21]. Although the incubation time was not according to the followed protocol but results were somewhat similar and optical density was measured after 24 hours, 48 hours and 72 hours. Optical density of each isolate increased after 24 and 48 hours indicating bacterial growth. After 72 hours, there was a gradual decrease in optical density of each isolate. Findings related to OD after 72 hours were contradictory to that of Carvalho*et al.* (2002) who reported the gradual increase of bacterial OD after 96 hours. As incubation time increases, bacterial growth tends to decrease due to nutrient depletion and lack of oxygen for aerobic bacteria. Moreover, optical density is directly proportional to cell mass not cell number. Larger cells absorb and scatter more light while shrunken cells due to nutrient depletion scatter low light ultimately results in low optical density as incubation time increases.

Kitchen waste was subjected to heat, acid and freeze thaw pre-treatment prior to consortia addition, aimed to enhance biodegradation. 55 g, 100.9 g and 3 g reduction in kitchen waste out of 500 g weight was measured by heat, acid and freeze-thaw method respectively in 24 hours. Thermal hydrolysis with an ability to disintegrate the cell membranes, enhance solubility and biodegradation as described by Bien *et al.* [18]. Freezing the material at low temperature aggravates cell disruption due to intracellular ice crystals formation causing damage of cell membrane, but not the complete destruction as explained by Stabnikova*et al.* [28]. Acid pre-treatment showed high degradation as compared to thermal and freeze thaw pre-treatment without consortium. Acid decreases pH of the medium and high levels of undissociated acids damage macromolecules [29]. The acid pretreatment may break down the polymers into monomers or oligomers, which allow an increase in the rate of digestion by microbes [30].

Weight loss of waste samples was observed after 7 days' trial with heat, acid and freeze-thaw pre-treatments. Weight reduction after consortia addition was 59.29 g, 39.91 g and 37.5 g out of 500 g by heat, acid and freeze-thaw method respectively and 3.6 g weight was reduced in case of control without consortia. Sarkar *et al.* [21] reported 55-65% weight reduction in kitchen waste due to microbial consortia in 21 days whereas 7.98%, 11.85% and 7.5% of weight reduction was recorded in present study over a period of 7 days by acid, heat and freeze-thaw pre-treatments with microbial consortium. Control sample was reduced due to decrease in moisture contents as kitchen waste contains 64% moisture. After addition of consortium, reduction in weight was more significant during heat pre-treatment as compared to any

other method subjected to Kitchen waste in 7-day trial. Results related to weight reduction with time were similar to those observed by Andrea *et al.* [31], who measured a weight loss of 29% in one month, and Gautam *et al.* [3] who observed 30.2 % weight loss at pH 7.0 and temperature was maintained at 35-40°C over a 45-day period.

5 CONCLUSION

Present study showed that microbial consortium is an effective way to degrade Kitchen waste. 45°C temperature and pH 7.0 among all the trails resulted in highest weight reduction. Present study showed effectiveness of consortium for enhanced degradation of kitchen waste and optimization of parameters like temperature and pH supported high reduction rate of Kitchen waste.

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