Isolation and Screening of Potential Bacteria Responsible for Biodegradation of Kitchen Waste in the Winter Season

Akansha Shukla[1] Sunita Mishra[*]

Department of Food and Nutrition, Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow

Abstract:

Kitchen waste is the waste that comes out from homes, hostels, hotels, restaurants, and places where food is processed. From 20 to 80% of the mass of municipal solid waste (MSW) is made from Kitchen waste [23]. This waste can cause serious health issues if not handled properly. Such waste comes in huge amounts due to which they occupy a large land area. Nowadays land is the major issue because of a growing population. This waste can be degraded easily as they contain a large number of microbes which causes degradation and can also lead to compost formation. In this study, samples were collected from the Yashodhara Girls hostel mess of Babasaheb Bhimrao Ambedkar (A Central University) University, Lucknow India. Various bacteria were isolated on plant count agar medium by studying their colonies, and morphological structure along with doing biochemical tests. The bacteria isolated were Bacillus megaterium, Bacillus subtilis, Pseudomonas aeruginosa, E.coli, Staphylococcus aureus. Kitchen waste was studied at different temperatures (35°C, 40°C, and 45°C) and different pHs (5.7, 6.6, and 7.0). Acid treatment, heat treatment, and cold treatment were used to study the degradation pattern of kitchen waste.

***Keywords*: KW: Kitchen waste, MSW: Municipal Solid Waste, OD: Optical density, PCA: Plate count agar, BOD: Biological Oxygen Demand**

1. INTRODUCTION

Kitchen waste generally comes from the house, restaurants, hostel mess, and other eating places. Kitchen waste is undesirable food that is discarded from a kitchen they can be prepared food, vegetable peels fruit peels, spoiled vegetables, and fruits. According to a survey performed by Toxic links New Delhi in May 2002, about 0.1 million tons of Municipal Solid Waste is produced daily in India, resulting in 6.5 million tons of solid waste yearly[1].

The old method of using kitchen waste was feeding them to the animals but it cannot control the excess generation of kitchen waste that comes from urban and rural areas [2]. As it is seen that MSW is increasing day by day rapidly. Food squander (waste) is a worldwide issue of state [24]. This condition is causing many problems in managing these wastes as it occupies larger land areas. Because of their improper handling, it is causing a lot of infection and pollution in the areas where they are kept.

Municipal Waste collected is 70-80% out of which only 22-28% get treated and processed. The reaming is deposited in dumping yards.

Kitchen waste's major portion comes from the kitchen mainly containing vegetable peels, unwanted cooked food, rotten fruits, and vegetables. The environmental load can also be managed if organic waste material is managed properly. If these wastes are managed properly it can decrease the human health hazards caused by various microbes present in wastes coming from different areas [3]. Due to the high moisture content in kitchen waste, it is very difficult to process [4]. Wastes that come from the kitchen are containing fatty acids, and their esters, amino acids, peptides, and carbohydrates [5,6].

Different organic waste can be recycled due to the biodegradable properties of kitchen waste. 95% of kitchen waste is biodegradable and suitable for anaerobic digestion resulting substantial source of organic matter [7].

Microbial growth and their enzymatic reaction are very fast due to moisture present in kitchen waste which helps in metabolizing the waste into simple organic compounds. In the bioconversion of kitchen waste bacteria and fungi play a major role.

Kitchen waste has high moisture content and degrading ability suitable for anaerobic digestion with biogas production [8].

Disruption of the cell wall of food leading to the release of intercellular and cell walls in the medium can be taken up by microorganisms [9].

2. MATERIALS AND METHODS

2.1. Experimental set-up

Kitchen waste of the winter season was collected from Yashodhara Girls Hostel Mess of Baba Saheb Bhim Rao Ambedkar University Campus Lucknow, India. For one-week kitchen waste was collected in a tustbin whose length, width, and height is 31 x 15 x 12 Centimetres respectively. Waste is filled in the trustbin in such a way that it is 70% filled and 30% free. Degradation of the waste is studied under various parameters. After 25 days the degraded material was taken for microbial study.

Fig.a **fig. b**

Figure 1. In (a) Trust-bin is shown (b) degraded kitchen waste of the winter season is seen.

2.2. Colony-forming unit test

A colony-forming unit test was first done to know the bacterial count present in the winter season kitchen waste. Serial dilution blanks were marked sequentially from 10^{-1} to 10^{-8}. After dilution streaking was done by the pour plate method. Plate count agar media was prepared and poured on petri-plates under the laminar hood then placed overnight so that if there is any contamination caused while making media can be seen easily. The next day after serial dilution streaking was done by pouring 20µl on PCA plates. The Petri-plates were placed on a BOD incubator for 24hours. The colony formed of plates was counted with the help of a hemocytometer.

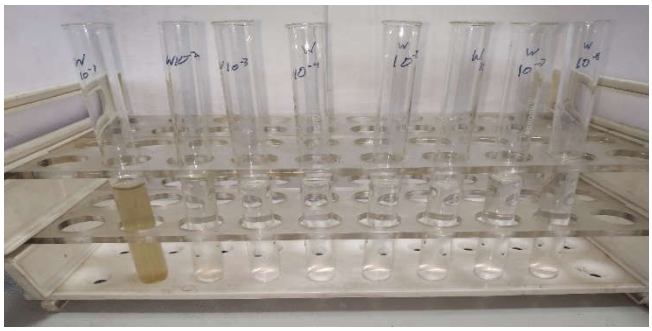


Fig 2 showing serial dilution of kitchen waste leachant

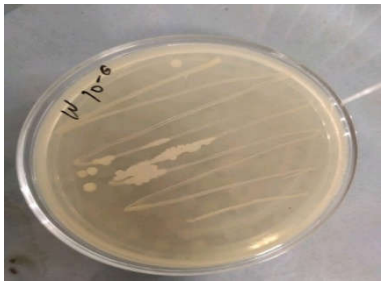


fig. a **fig. b**

Fig.3(a) colony seen after spreading on bacteria at 10^{-7} dilution and (b) secondary culture of bacteria after streaking

2.3. Potential Biodegrading Bacteria Isolation

Bacterial samples were spread on a plate count agar medium. Plates were placed in a BOD incubator at 37°C for 24hrs. After 24 hours of incubation mixed growth of the colony was seen on Petri-plates. Secondary culture of bacteria was done to get the pure culture of bacteria on plate count agar medium. Incubated pure culture at 37°C.

2.4. Identification of isolated bacteria

Isolated bacteria were identified on the basis of colony morphology, shape, surface pigmentation, size, and surface on plate count agar medium. Gram staining and spore staining had been performed to analyze the

shape, spore-producing bacteria, and cell arrangement. Under a microscope morphology of bacteria was seen with the help of gram staining [10].

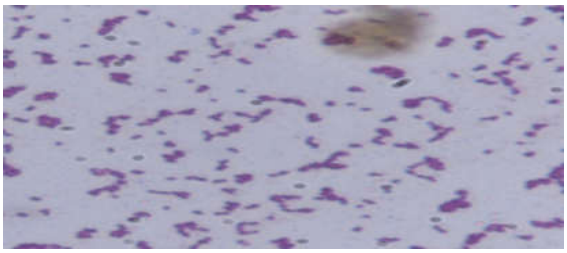


Fig. 5 microscopic view of bacteria after gram staining

2.5. Metabolic characteristic Determination

Isolates were streaked on different mediums to determine the metabolic characteristic of bacteria to degrade food waste. Media like Skim milk agar, MacConkey agar, cellulose Congo red agar, and starch agar were used to identify the enzymatic property of bacteria are protease & lipase, enteric lactose fermenting bacteria, cellulose, and amylase producers, respectively. Plates were incubated in BOD incubator at 30°C for 2 days. Zone clearing was observed around each bacterial isolate. Flooded the plates with iodine for starch agar to get the zone clearing around bacterial isolates.

2.6. Screening of Cellulose-Degrading Bacteria

Congo red dilution assay was used to check the cellulose-degrading ability of bacterial isolates. LB medium plate containing1% CMC with a few drops of Congo red dye solution had been taken to streak the bacterial isolates [11].

2.7. Formation Of Consortia

The nutrient broth was taken in tested to inoculate the isolated bacteria. The nutrient broth was kept for 3 days at 37°C with the addition of 0.1% starch, cellulose, and casein consortia were made. The optical density of the broth was measured using a spectrophotometer at 600nm. *Bacillus* strains and *Pseudomonas* were selected for consortium [12].

2.8. Kitchen Waste Treatment

Autoclaved kitchen waste of winter was subjected to the following treatment.

2.8.1. Acid Treatment

500g of raw kitchen waste of winter season was taken and mixed with HCl (10N) at room temperature (18 ± 2°C) until pH 2, checked the value after 24 hrs. Raw waste mixed with HCl and pH were measured in different spots of containers [13].

2.8.2. Heat Treatment

500gm of raw kitchen waste of the winter season was autoclaved at 121°C at 15 psi following operational cycle: 30 min. pre-heating at 121°C + 30 min. autoclaving at 121°C 15 psi +30 min. cooling to room temperature (18 ± 2°C)[13].

2.8.3. Cold Treatment

500g of raw kitchen waste of the winter season was kept at -4°C in an ultra-low temperature freezer. After 24 hours, the frozen winter season kitchen waste was gradually placed in a hot oven at 55±2°C for 30 minutes.

2.9. Optimization Of pH And Temperature

The kitchen waste of the winter season was analyzed at different pH ranges (5.7, 6.5, and 7.5) with respective different temperatures (37°C, 40°C, and 45°C). Degradation of kitchen waste is observed based on color, change in texture, physical appearance, and odor. Pre-treated kitchen waste was mixed with 5ml consortia solution to pH optimization of pH and temperature.

3. Results

Various bacterial colonies were analyzed on a plate count agar medium. Afterward, their gram staining was done along with some biochemical tests. Five different bacteria were isolated from the kitchen waste one from the *Pseudomonas* genus, two from the Bacillus genus and two from Enterobacteriaceae. Zone clearance on Congo red dilution assay was observed showed the presence of *Pseudomonas.* Bacillus species showed positivity towards the casein (protein) hydrolysis, starch hydrolysis.

TABLE 1

PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF STRAIN

Isolates	Morphology	Amylase	Protease	Cellulase	Lipase	Bacteria
Iso 1	(+) rods	+ve	+ve	-ve	-ve	*Bacillus subtilis*
Iso 2	(+) cocci	-ve	-ve	-ve	-ve	*Staphylococcus aureus*
Iso 3	(-)rods	+ve	+ve	+ve	-ve	*Pseudomonas aeruginosa*
Iso 4	(+)rods	+ve	+ve	-ve	-ve	*Bacillus megaterium*
Iso 5	(-) cocci	-ve	-ve	-ve	-ve	*E.coli*

TABLE 2

The optical density of incubated bacterial isolates

Days	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Control**
1	0.092	0.96	0.128	0.077	0.059	0.006
2	1.159	0.947	1.085	0.804	1.013	0.008
3	1.128	0.040	0.03	0.058	0.068	0.008

The optical density of the sample increased rapidly in 48 hours. A sudden decrease in optical density was seen after 72 hours of bacteria died. OD was measured by spectrophotometer of all the species which were isolated for 3 days. Those bacteria were selected for the consortium and revealed the enzymatic property of bacteria which helps them to degrade the organic matter with enzymes namely amylase, cellulose, and casease.

Kitchen waste was undergone in various treatment like heat, cold and acid treatment. A reduction in the weight of kitchen waste was observed in these above mention treatments. 62g, 103.8g, and 4g was measured by heat, cold and acid treatment respectively in 24 hours.

Fig. 6 Weight decreased in kitchen waste at 45°C after 24 hours of heat treatment

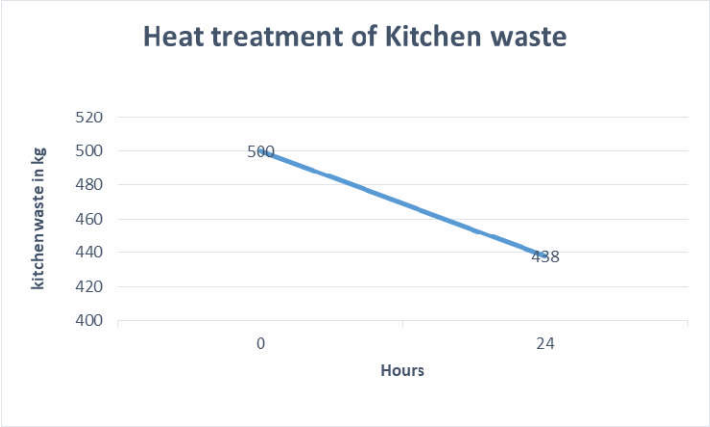


Fig. 7 Weight reduction in kitchen waste at 37°C after 24 hours of acid treatment

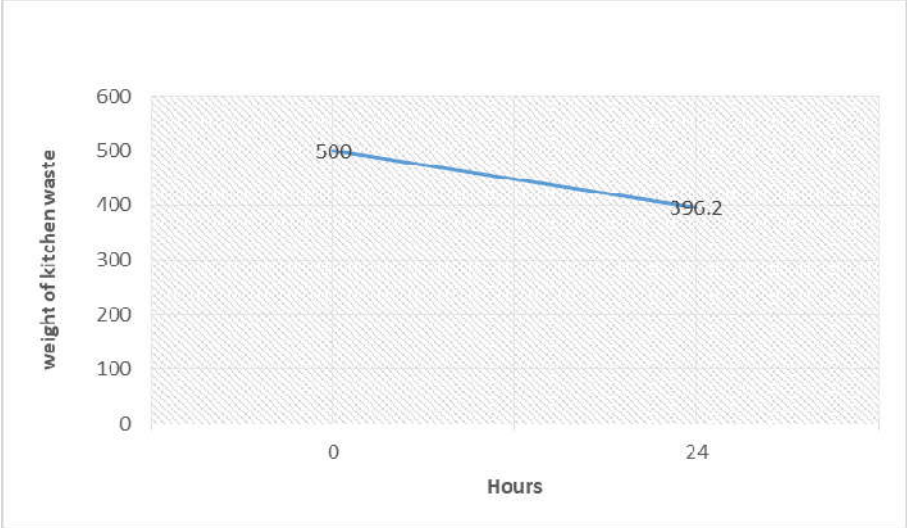
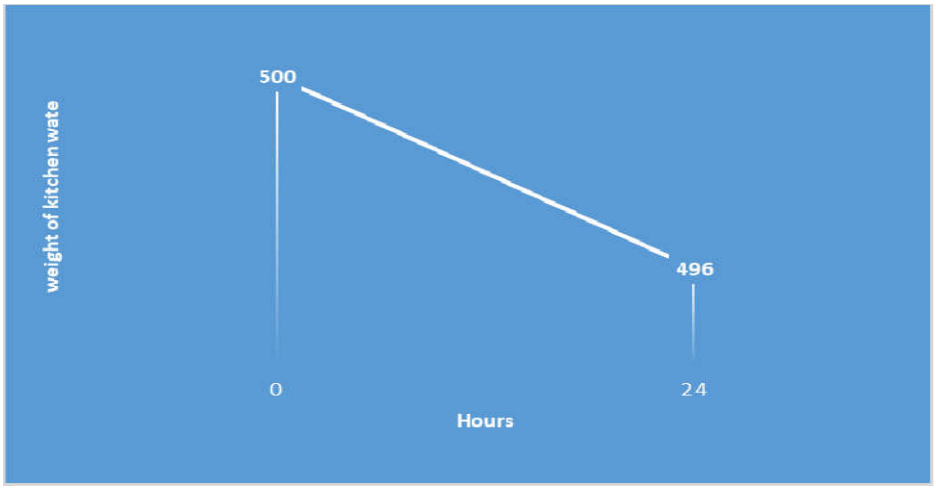


Fig. 8 Weight reduction after freeze method at -4 °C within 24 hours



4. DISCUSSION

This study is concerned with the Isolation and screening of potential bacteria responsible for the biodegradation of kitchen waste in the winter season. The comparison was made between different isolates and their ability to degrade the winter season kitchen waste, and various effects of heat, cold and acid treatment on kitchen waste were also observed. The optical density of the consortia was observed and pH optimization was also done.

Plate count media was preapared for 8 petriplates and poured in them under laminar air flow. Left overnight under the laminar hood to know if there is any contamination in media or not. Serial dilution of the kitchen waste was done by taking 9ml distilled water in 8 test tubes. Then 1ml of the liquid in kitchen waste was mixed with the help of a micropipette in 1 test tube having 9 ml distilled water then 1ml sample was taken from the first test tube following the other 7 test tubes. 20 µl of the 10^{-1} dilution sample was taken with the help of a micropipette under the laminar hood and spread with the spreader. This process is done till 10^{-8} dilutions. Petri plates were placed in the BOD incubator for hours to see the colonies of the bacteria present in kitchen waste during winter season. Colonies were seen and counted after 24 hours. Then secondary culture of the bacteria was done by streaking single colony on plate count agar and again media was placed in a BOD incubator for 24 hours. The pure culture for the bacteria was obtained. Characterization of the isolated strain was performed on the basis of morphology, colony, and biochemical tests as described by Saha*et al.* [14]. Investigated parameters were colony characteristic, shape, spore, gram staining mortality test, urease, and catalase production. Isolates of *Bacillus* for different species were identified by performing gram staining, spore staining, catalase, starch hydrolysis, and MR test. Species of the *Bacillus* family have thick peptidoglycan layer around them, catalase and amylase enzymes. According to Logan and Berkeley, some strains of bacilli were variable for MR tests [15].

Indole, oxide anand d sugar fermentation, MR, VP, citrate tests were done for enteric isolates. William *et.al.* [16] all the biochemical tests were performed, which represent the different bacteria at genus and species levels belonging to Enterobacteriaceae.

Various media like starch agar, skim milk agar and Congo red agar medium were used to check the ability of isolates to biodegrade zone clearance around the bacterial growth was seen. An alpha-amylase enzyme is secreted by *Bacillus* species which hydrolyses starch into simpler compounds that can be consumed by bacteria easily. *Pseudomonas aeruginosa* secretes casease enzyme which degrades casein protein showing proteolytic activity along with cellulase degradation into glucose by cellulase enzyme when on skim milk agar and Congo red agar medium respectively. When casein protein is hydrolyzed it results in a halo formation around the growth. Results were found by that of Usha*et al.* [17] and Behera*et al.* [18]. Usha*et al.* [17] performed the screening of Bacillus, Pseudomonas, and Streptomyces sp. by observing the clear zone around the colonies due to enzyme secretion after 15-20 days of incubation. The degrading ability of bacteria showed similarity during the incubation period which caused disagreement with that of Usha*et al.* [17] as a result after two days of the incubation period. The cellulos degrading ability of *Pseudomonas sp.* was mentioned by Behera*et al.* [18]. Due to various enzyme production zone clearance was observed on Congo red agar medium.

Isolated bacteria was inoculated in the nutrient agar broth for bacterial consortium. Nutrient broth was incubated for 3 days at 37°C for 24 hours by adopting the method of Sarkar *et al.* [12]. Optical density was measured after different intervals of times i.e. 24 hours, 48 hours, and 72 hours. The bacterial growth curve was observed clearly as after 24 and 48 hours bacterial density increased the after 72 hours it decreased rapidly. As incubation time increase bacterial growth decrease because of a lack of nutrient and oxygen for aerobic bacteria.

Treatments like heat, cold, and acid treatment prior to consortia addition, aimed to enhance biodegradation. 62 g, 103.8 g, and 4 g reduction in kitchen waste out of 500 g weight was measured by heat, acid, cold method respectively in 24 hours. The disintegration of cell membrane, solubility, and biodegration in cell is seen due to thermal treatment as described by Bien *et al.* [19]. Due freezing the cell disruption in intracellular ice crystals formation causing damage of cell membrane, but not complete destruction as explained by Stabnikov*et al.* [20]. High degradation is seen acid treatment as compared to thermal and cold treatment without consortium. pH of the medium decrease when acid is added and high level of undissociated acid damage macromolecules [21]. Polymers convert into monomers or oligomers due to acid pre-treatment which increases the digestion rate of microbes [22].

5. CONCLUSION

Present study showed that various microbes are present in the kitchen waste of winter season which leads its decomposition. With colony-forming unit and doing secondary culture of those microbes pure culture of bacteria present in it can be observed. Various biochemical tests can help to know the morphology and nature of microbes. The environment also plays a major role in the weight reduction of kitchen waste was seen by doing heat, cold, and acid pre-treatment.

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