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Augmentation of kitchen waste by resident bio-degrading bacteria

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Abstract
Kitchen waste, composed of organic materials such as carbohydrates, amino acids, peptides, and fatty acids, provides an ideal environment for bacterial growth. The enzymatic secretion of microorganisms plays a crucial role in biodegradation, making it a powerful solution for waste management. A bacterial consortium, including enzymes such as amylase, lipase, protease, cellulose, and lactose-fermenting bacteria, is essential for the decomposition of kitchen waste. In this study, twenty bacterial isolates were obtained from kitchen waste collected from Urja girls' hostel at SKUAST-Jammu. The biodegrading capabilities of these isolates were rigorously tested using specific media and screening methods, leading to the selection of nine potential isolates for further analysis. Morphological, cultural, and biochemical identification of these potential isolates was conducted according to Bergey's Manual of Determinative Bacteriology. The compatibility of the potential isolates was assessed using the cross-streak method, and a robust consortium was created by inoculating compatible isolates in nutrient broth. The study rigorously assessed the impact of different pH levels and temperatures on the degradation of 500 g of kitchen waste, with the highest weight reduction (75.60%) observed at 45 °C and pH 7.5. Furthermore, the study investigated the influence of the consortium on pre-treated kitchen waste, with acid pre-treatment resulting in a significant degradation of 21.00% compared to the control. This study conclusively demonstrates that a consortium of resident potential biodegrading bacteria significantly enhances the biodegradation process of kitchen waste.

Keywords: Kitchen waste, biodegradation, biodegrading bacteria, microbial consortium, pre-treatments, consortium

Introduction
Biodegradation is defined as the biologically catalyzed reduction in complexity of chemical compounds (Alexander M *et al.*1994) [1]. Indeed, biodegradation is the process by which organic substances are broken down into smaller compounds by living microbial organisms (Marinescu *et al.* 2009) [14]. Kitchen waste refers to the non-edible parts of food that are discarded during handling, processing, or after consumption. This includes unavoidable food waste from food preparation and refrigerators, as well as waste from households, commercial establishments, institutions, and other sources such as food factories, cafeterias, canteens, or lunchrooms (Iacovidou *et al.* 2012) [10]. Kitchen waste comprises organic materials such as carbohydrates, amino acids, peptides, fatty acids, and their esters, as well as processed foods and paper. These components provide bacteria with the nutrients they need to thrive (Namita *et al.*2012) [17]; (Tsai *et al.* 2007) [24].

In India, a significant portion (70 to 80%) of urban solid waste is residential waste, which is rich in organic matter. On average, a typical family generates 0.5 to 0.75 kg of kitchen waste every day (Tweib *et al.* 2014) [25]. Traditional methods like incineration are not suitable for handling kitchen waste due to its high moisture content. Climate change and global warming issues are expected to reach alarming levels (Broer and Titheridge 2010) [5]. To avoid significant climate change and global warming, recent estimates suggest that a 60-80% reduction in greenhouse gas emissions (CO2, CH4) over 1990 levels is necessary by 2050.

During the degradation of kitchen waste, large amounts of pollutants and foul odours such as NH3 and H2S are produced. Ammonia (NH3) has a strong and intense odour and can cause severe burns to the skin, eyes, and respiratory tract. Hydrogen sulfide (H2S) is an acidic,

flammable gas with a rotten egg odour that is extremely harmful to humans. These gases pollute both the water and the air.

The discharge of toxins into the environment is one of the main causes of biosphere degradation. Inappropriate kitchen waste management and littering can have a significant impact on public health by attracting disease-carrying insects. Improper disposal of waste can also harm the health of municipal workers and rag pickers. Microorganisms, particularly aerobic bacteria, biodegrade organic molecules into simpler substances such as carbon dioxide, water, and ammonia. Microbial enzymes play a crucial role in the breakdown of kitchen waste (Sarkar *et al*. 2011) [21].

Composting, specifically aerobic composting, is widely recognized as a microbially-driven process that is environmentally sound for managing large amounts of domestic organic waste. Effective microorganisms can convert food waste into valuable outputs such as plant nutrients, which help to support soil productivity and maintain nutrient flow between different systems. Various studies have shown that effective microorganisms play a significant role in bioremediation, composting, agriculture, and waste management (Khaliq Abbasi and Hussain 2006) [12].

The biological treatment of kitchen waste seems to be the most cost-effective and environmentally friendly approach. Therefore, there is a huge potential for microorganisms to be used for their beneficial applications (Vargas-Garcia *et al*. 2007) [26]. This study aims to enhance the population of resident biodegrading bacteria to efficiently decompose kitchen waste. The study is designed with the objectives of isolating, screening, and characterizing bio-degrading bacteria from kitchen waste, as well as optimizing different physico-chemical conditions to enhance the bio-degradation process.

Materials and Methods

Collection of Kitchen waste sample: The Sample was collected from the mess of Urja girl's hostel, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu. The sample was brought to the laboratory, Division of Microbiology in sealed plastic bags to avoid contamination and stored for further processing (Vaseekaran *et al*. 2010) [27].

Isolation of bio-degrading bacteria: The serial dilution and spread plate approach were used to isolate bacteria from mixed kitchen wastes. One gram of kitchen waste was serially diluted in sterilised distilled water to achieve concentrations of 10^{-1} to 10^{-9}in culture tubes. Dilution 10^{-7} was put aseptically onto nutrient agar in a volume of 0.1 ml. After observing mixed growth each different colony was purified on different agar plates which was also be incubated for 24 hours at 37 °C (Anwar *et al*. 2017) [4].

Screening of potential bio-degrading bacteria

The metabolic ability of the isolates to degrade food waste was determined by observing individual isolates on different media viz. 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. Degradation activity was indicated by the appearance of clearance zones around the colonies. After flooding the plates with iodine, the zone of clearing was detected for starch agar. The plates were stained with Congo red dye as an indicator and washed with 1M NaCl after incubation. The formation of a yellow halo against a red background indicated positive cellulase activity (Anwar *et al.* 2017) [4].

Identification of potential bacterial isolates

On the basis of morphology, Gram staining, and biochemical assays, potential bacterial isolates were identified.

Compatibility test of potential isolates for consortium formation

On nutrient agar media, each potential isolate was streaked vertically and horizontally. Perti plates were incubated at 37 °C for 48 hours to see if any lysis occurred between the two isolates. If there is no lysis or inhibition zone, isolates were compatible; if there is a lysis or inhibition zone, isolates were not compatible. (Denaya *et al*. 2021) [7].

Waste treatment

7kgs of kitchen waste was collected from Urja girl's hostel. 5 kgs of brown material was added in the kitchen waste and mixed well. Kitchen waste was chopped in fine pieces so that microbial enzymes could work effectively.

Standardization of pH and temperature

In several studies with varied temperature ranges, 500 grams of kitchen waste were placed in three heaps which was introduced to different pH levels (5.7, 6.5, and 7.5) and incubated at different temperature ranges (37 °C, 40 °C and 45 °C). The garbage from the kitchen was put to the test. On the other hand, due to these conditions, degradation was detected on the based on physical appearance, texture, color, pH, temperature, EC, C: N, odour. 5ml consortium solution was mixed with pre-treated waste before optimization of pH and temperature. Kitchen waste reduction was measured up to 21 days (Ma *et al*. 2011) [35].

Pre-treatment of kitchen waste

Kitchen waste was pre-treated by following methods:

Acid pre-treatment*:*500 g of raw mixed kitchen waste was acidified with HCl (10 N) at room temperature until pH 2 was reached. After 24 hours of contact time, reduction value was observed. The pre-treated kitchen waste was continuously blended. At several locations across the pretreated kitchen waste, HCl and pH were monitored (Ma *et al*. 2011) [35].

Freeze-thaw pre-treatment*:* In an ultra-low temperature freezer, 500 g of raw mixed kitchen waste was frozen to -4 °C. The frozen kitchen waste was thawed in a thermal oven at 55± 2 °C for 30 minutes after being frozen for 24 hours (Ma *et al*. 2011) [35].

Effect of consortium in pre-treated kitchen waste*:* Efficacy of consortium on pre-treated kitchen waste was evaluated by addition of 5 ml of microbial consortium into it and incubated at 45 °C temperature. Weight reduction percentage was measured up to 14th day (Anwar *et al*. 2017).

Weight reduction analysis

Weight reduction of kitchen waste due to bio-degradation was measured by weighing balance. Fresh weight was

noticed on day 1 prior to any pre-treatment and after 7, 14, 21 days of treatment the weight was measured.
Weight loss was measured by the formula:

Dry weight calculation

- Weight loss (%) = $W\text{-}W_1/W*100$
- W= Initial weight
- W_1=Final weight

Results, Discussion and Conclusion

Bacterial isolates were isolated from the sample collected from Urja girl's hostel, SKUAST-Jammu and isolation was carried out by using serial dilution method. Total twenty resident bio-degrading bacteria were isolated from collected sample. Nine potential biodegrading bacteria showed positive result on specific media viz. starch agar, skimmed milk agar, MacConkey agar, and Congo red agar as clear hydrolysis zones were formed around potential bacterial isolates.

The effective potential biodegrading bacteria were identified on the basis of morphology, Gram's staining and biochemical characterization. Table 1 represents the Biochemical identification of potential isolates. All the potential isolates were grown on nutrient agar medium (NA) and incubated at 28±2 °C to study various morphological characters. The growth in potential isolates initiated after 24 hours of incubation. Isolate I_1 was yellowish in colour and slimy in nature. I_2, I_3, I_4, I_5 and I_6 were creamish in colour and mucoid whereas I_7 and I_8 were creamish and slimy and $I_9$was whitish in colour and showed patchy growth. Gram's staining showed that the isolatesI_1, I_3, I_9 were Gram positive and rod shaped and rest of the isolates were Gram positive and rod shaped.

Table 1: (Biochemical identification of potential isolates)

S.NO.	Isolates	Oxidase Test	Catalase Test	Indole test	Mr test	Vp test	Citrate utilization test	TSI test
1	I_1	-VE	+VE	-VE	+VE	+VE	+VE	-VE
2	I_2	+VE	+VE	-VE	+VE	+VE	+VE	+VE
3	I_3	+VE	+VE	+VE	+VE	+VE	+VE	+VE
4	I_4	+VE	+VE	-VE	-VE	+VE	+VE	+VE
5	I_5	+VE	+VE	+VE	+VE	+VE	+VE	+VE
6	I_6	+VE	+VE	+VE	+VE	-VE	+VE	+VE
7	I_7	+VE	+VE	+VE	+VE	-VE	+VE	+VE
8	I_8	+VE	+VE	-VE	+VE	+VE	+VE	-VE
9	I_9	+VE	+VE	+VE	-VE	+VE	+VE	-VE

The formation of a consortium was carried out to assess the compatibility of the potential isolates using the modified cross-streak method. All the potential isolates supported each other's growth, so a microbial consortium of potential isolates was created to improve the bio-degrading process.

The activity of all the isolates present in the consortium was rechecked by streaking on specific media. Initially, the physico-chemical properties of the organic waste were evaluated (refer to Table 2)

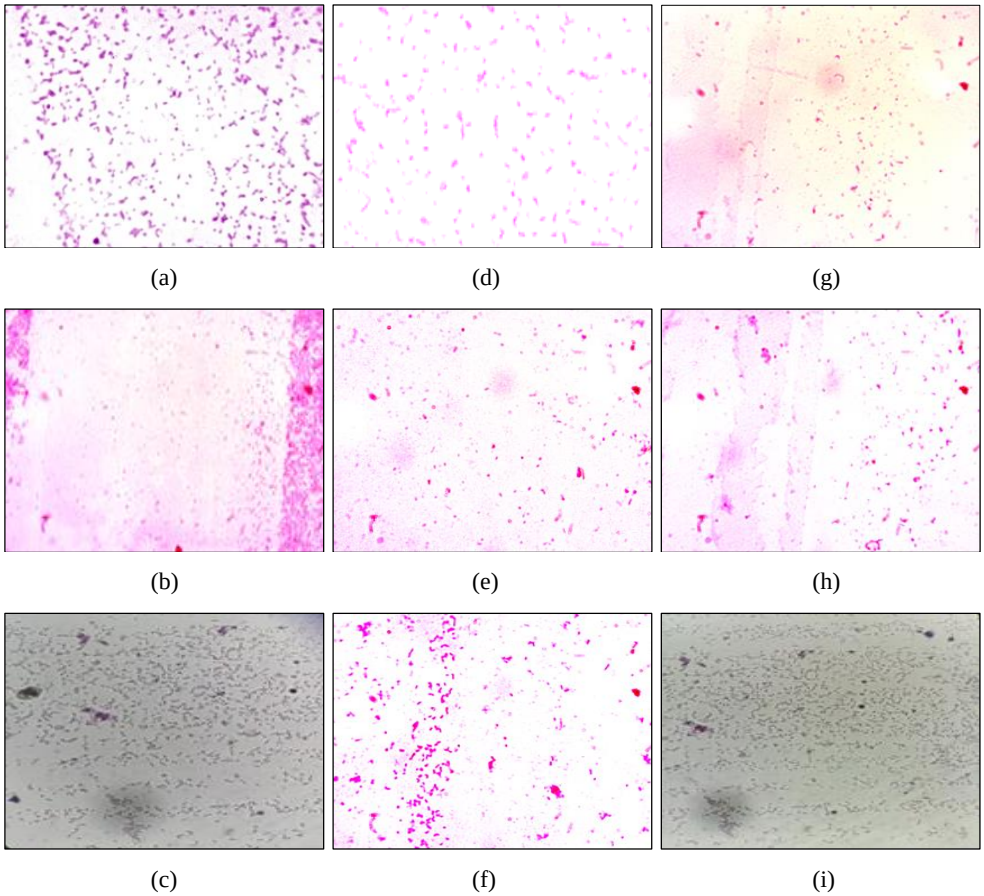

Fig 1: Microscopic characteristics of potential isolates (a)I1 (b)I2 (c)I3 (d)I4 (e)I5 (f)I6 (g)I7 (h)I8 (i)I9

Table 2: Physiochemical properties of organic waste

S. no.	Physiochemical Properties	Organic waste
1	moisture (%)	55.20
2	pH	4.10
3	Temperature	31.00
4	C: N	28:1
5	Color	Brown
6	Odor	Rancid
7	EC (µs)	3.15

The standardization of different parameters of kitchen waste was done by adjusting different pH ranges (5.7, 6.5 and 7.5) at respective temperatures (37 °C, 40 °C and 45 °C) in different trials (Table 3). The max reduction was observed on 21st day in all trials, but the temperature at 45 °C with different pH (5.7, 6.5 and 7.5) showed best results among all (70.60%, 68.00% and 75.60%) respectively.

Table 3: Weight reduction (%) of Kitchen waste at different temperature

pH	Weight reduction (%)								
	37 °C			40 °C			45 °C		
	Days								
	7th	14th	21st	7th	14th	21st	7th	14th	21st
5.7	16.00	35.20	48.00	6.60	26.92	50.00	30.00	51.40	70.60
6.5	10.00	24.40	44.00	2.20	25.42	54.00	32.00	45.98	68.00
7.5	20.08	39.80	49.00	18.00	30.76	58.00	34.60	55.00	75.60

Figure 1, 2 & 3 showed weight reduction percentage of kitchen waste at 37 °C, 40 °C and 45 °C respectively.

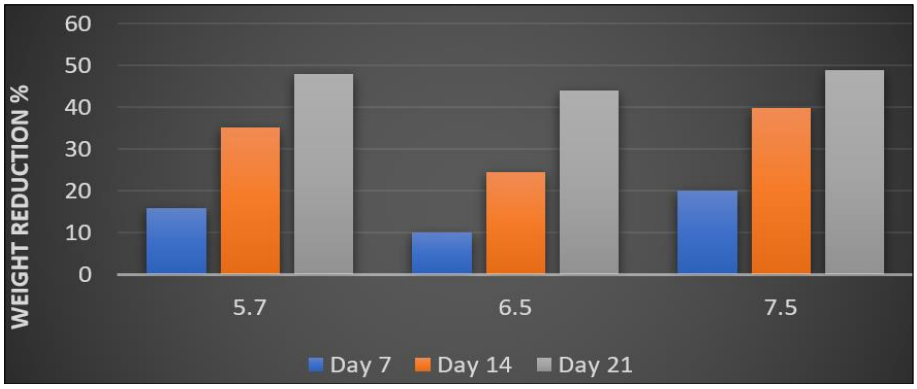

Fig 2: Weight reduction (%) of kitchen waste at 37 °C

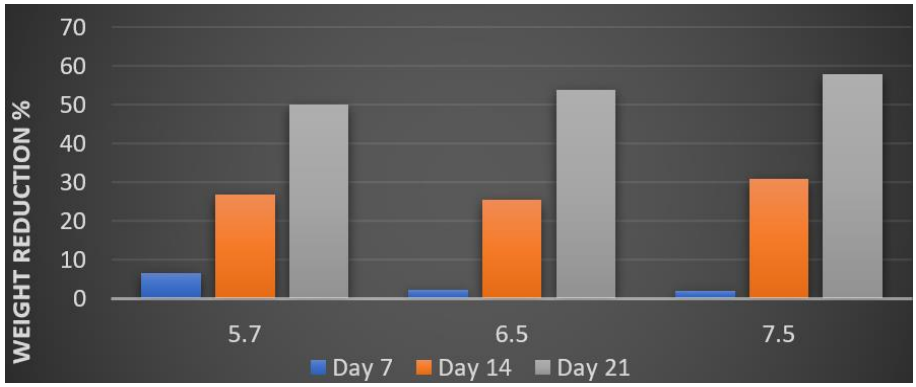

Fig 3: Weight reduction% of kitchen waste at 40 °C

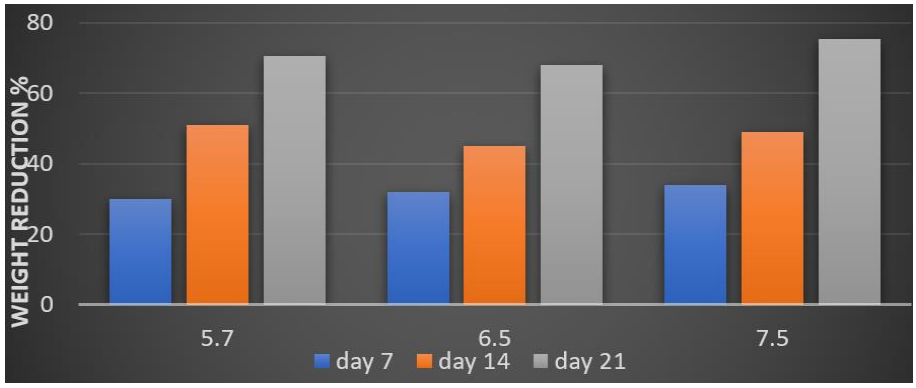

Fig 4: Weight reduction% of kitchen waste at 45 °C

Physio-chemical properties of mature organic waste compost on 21st day of the composting process is mentioned in table 4.

Table 4: Physiochemical properties of kitchen waste compost

S. No.	Physiochemical properties	Kitchen waste compost
1	Moisture (%)	23.00
2	C: N	23:1
3	EC (µS)	4.10
4	Color	Dark Brown
5	Odor	Earthy
6	Texture	Smooth

Efficacy of consortium on pre-treated kitchen waste was done by acid and freeze- thaw treatment and the effect of consortium on pre-treated kitchen waste was done by adding 5ml of consortium into it. The pre-treated kitchen waste showed significant reduction over control but among all the treatments the pre-treated kitchen waste with consortium showed maximum reduction and between them the acid pre-treated kitchen waste (21.00%) with consortium significantly degraded the kitchen waste (Table 5).

Table 5: Bio-degradation of pre-treated kitchen waste

Treatments (500 g)	24 Hours (%)	7 Days (%)	14 Days (%)
Control	0.00	2.10	2.20
Acid pre-treatment	4.20	8.00	5.10
Freeze-thaw treatment	1.20	3.00	1.50
Acid pre-treatment + consortium	3.00	13.38	21.00
Freeze thaw + consortium	2.00	8.82	15.20

Figures 5 & 6 represents comparison of different pre-treatment in weight reduction after 24 hours without consortium and Representing weight reduction of two pre-treated waste samples by microbial consortium after 7 days respectively.

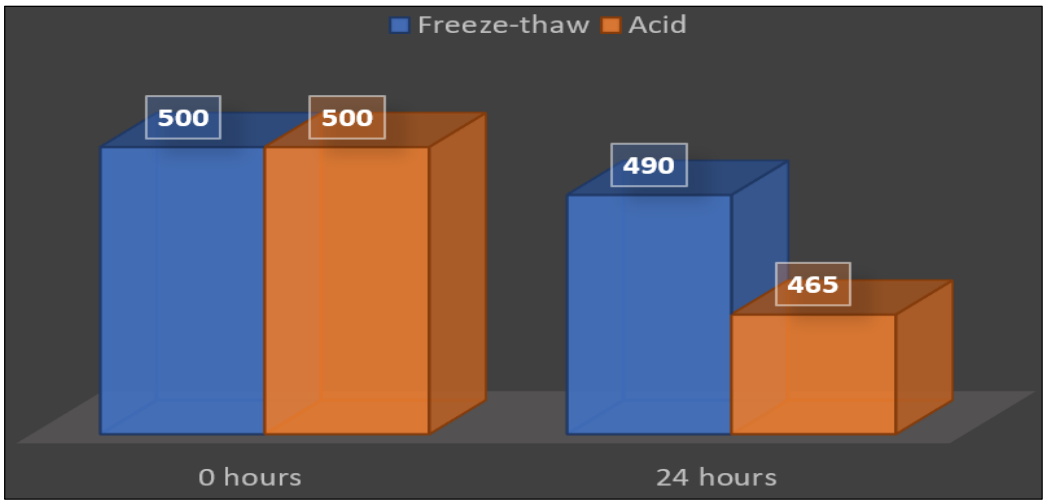

Fig 5: Comparison of different pre-treatment in weight reduction after 24 hours without consortium.

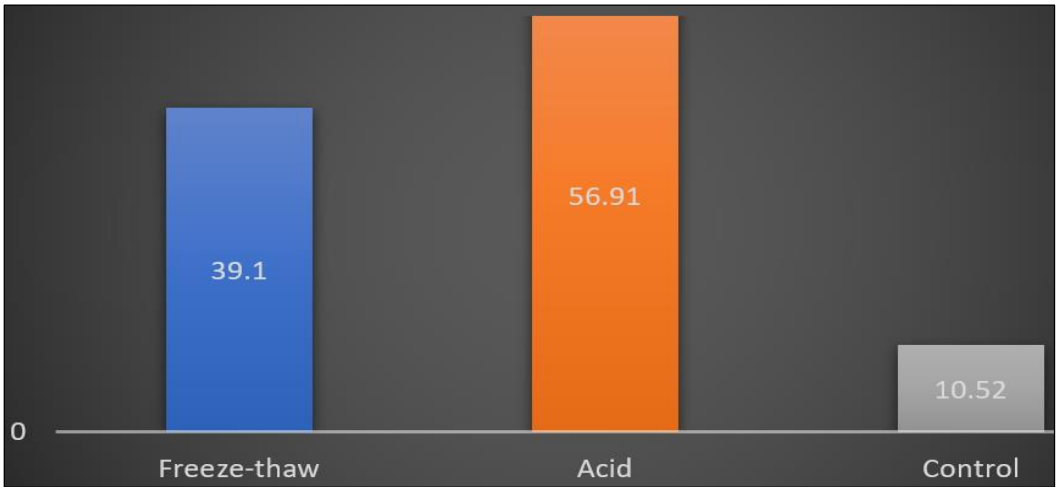

Fig 6: Representing weight reduction of two pre-treated waste samples by microbial consortium after 7 days. (Weight reduction is calculated in grams).

(Vaseekaran *et al.*, 2010) [27] described the method of collecting samples in containers under sterile circumstances from contaminated decaying materials of kitchen waste. Similarly, the sample was collected as kitchen waste from Urja girl's hostel, SKUAST-Jammu. The isolation of bacterial isolates from organic waste has been reported by (Maraveas 2020) [15].

The screening was based on the size of the clear zones formed by isolated biodegrading bacteria on various media, including starch agar, skimmed milk agar, McConkey agar, and cellulose-containing agar. Bacterial isolates were grown on starch agar and then flooded with iodine solution; positive isolates displayed below around gram iodine. Blue-black colouring on agar with 1% starch. The manufacture of

amylase yielded a positive effect. (Mazzucotelli *et al*. 2013) [16] observations are comparable to ours. Skim milk agar was used to isolate protease-producing bacterial colonies. Our findings are similar to those of (Anwar *et al*. 2017), who studied protease-producing bacteria and found similar outcomes. Protein degradation in skim milk agar as measured by protein hydrolytic zones. Cellulase-producing bacteria were isolated using carboxy-methyl cellulose agar. The isolates were grown in Congo red agar media, a modified media for fast cellulose hydrolysis screening developed by (Mazzucotelli *et al*., 2013) [16].

After flooding with congo red as an indicator and 1M NaCl solution to indicate cellulase destruction, a clear zone emerged around the culture. Our findings are consistent with those of (Mazzucotelli *et al*. 2013) [16]. Enteric lactose fermenting bacteria was screened using McConkey agar media by forming a clearance zone around the culture. Our findings are similar to (Anwar *et al*., 2017) [4].

All the potential isolates were morphologically and biochemically identified using biochemical tests. (Al-Dhabaan 2019) [3] evaluated the morphological and biochemical of petroleum hydrocarbon biodegradation bacteria isolated from oil-polluted soil. Oxidase test, Simmon citrate test, methyl red test, V-P test, Indole test, catalase Test, triple sugar iron test and citrate utilization test were all carried out at the same time.

Consortium formation was carried out by using a modified cross-streak method (Velho-Pereira *et al*. 2011) [28]. All the cross-streaked isolates supported each other's growth and hence microbial consortium of isolates was made to enhance the bio-degrading process. Microbial consortium activity was again rechecked on specific media. (Ke *et al*. 2021) [11] studied synergetic degradation of waste oil by constructed bacterial consortium for rapid in-situ reduction of kitchen waste. (Xu *et al*.2021) [31] aimed to relate bacterial dynamics to gaseous emissions during the composting of kitchen and garden wastes. (Poszytek *et al*. 2016) [19] formed a consortium with high cellulosic activity for enhanced biogas production. (Velho-Pereira 2011) [28] evaluated the antimicrobial activities of actinobacteria was carried out by using the cross-streak method. (Sarkar *et al*. 2011) [21] prepared efficient microbial consortia with concomitant enzymatic activity for the effective degradation of organic kitchen waste.

At various temperatures and pH levels, numerous researches have been undertaken to optimise kitchen waste parameters. In the present-day study, the maximum weight loss (%) was observed at 45 °C temperature and pH 7.5 during the degradation of kitchen waste for 21 days. Our efforts are backed up by (Anwar *et al*. 2017) [4] showed various chemical, physical, and biological pre-treatments for organic solid residues, with a focus on those used on the organic fraction of municipal solid waste. (Gao *et al*. 2021) [9] investigated how bacterial dynamics affected the gaseous emission and humification of kitchen and garden wastes during composting with microbial inoculants. (Shen *et al*. 2015) [22] evaluated changes in water states throughout the composting of kitchen waste. In a similar study, (Anwar *et al*. 2017) [4] found that beneficial biodegrading bacteria from the surrounding environment may be engaged in the formation of a microbial consortium that will enhance environmentally favourable bioconversion of solid organic waste.

Before consortia addition, kitchen waste was subjected to acid and freeze-thaw pre-treatments to improve biodegradation. Freezing the material at a low temperature causes cell disruption due to the development of intracellular ice crystals, which damages the cell membrane (Stabnikowa *et al*. 2008) [23]. Acid pre-treatment resulted in higher degradation (5.10%) than freeze-thaw pre-treatment (1.50%) without consortium. The acid lowers the pH of the medium, and excessive amounts of undissociated acids disrupt macromolecules (Cotter *et al*. 2003) [6]. Acid pre-treatment may break down polymers into monomers or oligomers, allowing microorganisms to digest them more quickly (Devlin *et al*., 2010) [8]. After consortia addition, weight reduction was 21.00% by acid treatment and 15.20% by freeze-thaw treatment respectively, and 2.20% in the control condition. (Sarkar *et al*., 2011) [21] found a 55-65% weight reduction in kitchen garbage after 21 days due to microbial consortia.

45 °C temperature and pH 7.5 among all the trials resulted in the highest weight reduction of 75.60%. Kitchen waste was subjected to acid and freeze-thaw pre-treatment and 5 ml of the consortium was added to pre-treated kitchen waste. Pre-treated kitchen waste showed a significant reduction than control but among the treatments, the pre-treated kitchen waste with consortium showed the best result. Consortium mixed acid pre-treatment showed high degradation (21.00%) as compared to freeze-thaw pre-treatment. This study concluded that the consortium of resident potential biodegrading bacteria enhanced the bio-degradation process and this study will contribute towards the bioprocess technology by using these bio-degrading bacteria for the kitchen waste treatment.

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