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Development of microbial consortium for degradation of organic kitchen waste

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Abstract

Increasing population and urbanization has led to an exponential increase in organic waste. This waste if not treated properly may lead to pollution and health hazards. So, a study was conducted to develop an efficient bacterial consortium for the degradation of organic kitchen waste. Different bacterial strains were obtained from MTCC, initially grown on specific media and were screened for their enzymatic activities. Eight bacterial strains *viz. Bacillus subtilus* (MTCC 441), *B. amyloliquefaciens* (MTCC 7913), *B. megaterium* (MTCC 8510), *Lactobacillus delbrueckii* (MTCC 2365), *L. plantarum* (MTCC 1407), *L. rhamnosus* (MTCC 1423), *Pseudomonas aeruginosa* (MTCC 4673) and *Staphylococcus aureus* (MTCC 740) were tested for the production of lipase, amylase, protease and cellulase. All the bacterial strains exhibited enzyme activities except *B. megaterium*. So, combinations of these strains were used to make consortia on basis of pathogenicity. Two different microbial consortiums were prepared with Consortia 1 comprising *B. subtilis, L. plantarum, L. rhamnosus, L. delbrueckii, B. amyloliquefaciens* and Consortia 2 comprising *P. aeruginosa, S. aureus, L. delbrueckii, L. rhamnosus, B. subtilus*. Consortium 1 displayed the highest amylase and protease activity as compared to Consortium 2, and therefore selected for monitoring the composting parameters of the organic kitchen wastes. The microbial consortium showed significant outcome for composting parameters such as moisture content (65 – 58 %), temperature (25 – 29 ºC), pH (5.5 – 5.7), organic carbon (41 – 36 %) and decrease in mass (25 %) from 25 to 35 d of composting organic kitchen waste. The C/N ratio was calculated at 38/1 which falls within the optimum composting period. Developing a microbial consortium significantly reduces the degradation time and thereby the composting process. This microbial consortium is environmentally safe and can therefore, be used to effectively improve the management of organic waste at both the community and industrial level.

Introduction

The accelerated industrialization and urbanization in developing countries generate billion tons of domestic waste annually (Singh *et al.* 2011). This waste is not treated properly due to poor state of collection and transportation. Also, the domestic

waste collected from various homes is not properly sorted (Meng *et al.* 2021). This has led to various health issues due to its improper disposal in developing countries like China and India among others (Li *et al.* 2020). In India, 68.8 million tons of municipal solid waste is generated per year at a per capita waste generation rate of 500 grams/person/day (Gupta and Arora 2016). Kashyap *et al.* (2003) stated that about 135.5 million tons/year of municipal solid waste generated in India consists of 30 – 40 % of food waste. The waste produced in India consists of about 50 % organic content as compared to 30 % in the developed countries (Joshi and Ahmed 2016). Carbohydrates, lipids, proteins, and traces of inorganic compounds are the chief chemical components of food waste. According to the available data, Asia alone is estimated to have spent about 25 billion US dollars per annum on solid waste management in 1990 and is projected to rise up to 50 billion US dollars per annum by 2025 (Hoornweg and Thomas 1999). While effective disposal methods such as gasification, landfills etc. are very efficient, these methods tend to have negative impact on the surrounding environment posing risks to public health. The biological handling of such wastes is highlighted to be the best cost-effective method with less adverse impact on the environment (Coker *et al.* 2006).

Biological treatment of such waste is also referred to as composting. In composting, microorganisms such as bacteria and fungi break down complex organic compounds into simpler stabilized organic matter called compost under aerobic conditions and release carbon dioxide, water, and minerals. The process destroys pathogens and weeds because of the heat release (Tweib *et al.* 2011), converts nitrogen from unstable ammonia to stable organic forms, reduces the volume of waste and improves the physico-chemical properties of the waste. It also makes waste easier to handle and transport, and often allows for higher application rates because of the more stable slow release of N in compost (Fauziah *et al.* 2009). In normal systems, composting has the potential to protect the soil against erosion, reduce soil compatibility, enhance soil water retention and decrease soil acidity, enhance biological and soil biochemical functions, and therefore create an all-encompassing soil ecological symmetry (Cao *et al.* 2021). In these systems, the organic degradation of compost is supported by several coexisting organic microorganisms, and the overall performance of this process is the combined effects of the activity of individual microorganisms with its biochemical functions, which in turn create an all-encompassing soil ecological symmetry (Pfotzer and Schuler 1997; Nevens *et al.* 2003). Synergistic associations are also established among other non-cellulolytic microbial species, and from this association are relied upon to expand the substrate degradation rates (Lynd *et al.* 2002). The synergetic association promotes the decomposition process of kitchen waste, which in turn paves the way for efficient degradation of kitchen waste (Ke *et al.* 2021). Rather than utilizing single strains, utilizing microbial consortia, which is an assortment of microorganisms, for the biodegradation of organic waste enables one to exploit the microbial collaborations and making ideal utilization of their intricate regulatory systems (Wongwilaiwalin *et al.* 2010). The bacterial cultures used therefore must be compatible to make a successful microbial consortium, where it can concomitantly produce all the enzymes required for the degradation of kitchen wastes. This study was therefore undertaken to evaluate and develop a microbial consortium that can concomitantly degrade different kitchen wastes under natural conditions and to optimize the degradation conditions.

Experimental

Procurement of culture and maintenance of culture

Bacterial strains *viz. Bacillus subtilus* (MTCC 441), *Bacillus megaterium* (MTCC 8510), *Bacillus amyloliquefaciens* (MTCC 7913), *Lactobacillus delbrueckii* (MTCC 2365), *Lactobacillus plantarum* (MTCC 1407), *Lactobacillus rhamnosus* (MTCC 1423), *Pseudomonas aeruginosa* (MTCC 4673), *Staphylococcus aureus* (MTCC 740) were obtained from School of Bioengineering and Bioscience, Lovely Professional University, Punjab. The bacterial strains of these microorganisms were inoculated in nutrient broth and incubated at 37 ^{o}C for 24 h. The revived

cultures were maintained on nutrient agar plates and slants and stored at 4 ^{o}C for further use.

Preliminary screening for metabolic characteristics

The selected strains were individually inoculated by streaking on selective media such as starch (2 % starch), skim milk, CMC (Congo red) supplemented agar and nutrient agar plates with 1 % tributyrin to isolate amylase, protease, cellulose, and lipase producers, respectively. The inoculated plates were then incubated overnight at 37 ^{o}C 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.

Preparation of bacterial consortium

Overnight grown bacterial strains were inoculated in 20 mL of nutrient broth to prepare the microbial consortia. Two consortia was prepared – Consortium 1 containing non-pathogenic microorganism (*B. subtilus, B. amyloliquefaciens, Lactobacillus delbrueckii, L. plantarum, L. rhamnosus*) and Consortium 2 containing pathogenic as well as non-pathogenic microorganisms (*B. subtilus, B. amyloliquefaciens, L. delbrueckii, L. plantarum, P. aeruginosa, S. aureus*). The bacterial strains were inoculated on nutrient agar plates and cultivated overnight.

Enzymatic assays of selected bacterial cultures and consortium

The microbial consortia and the selected bacterial cultures were inoculated for 24 h at 37 ^{o}C, and centrifuged at 10,000 rpm for 10 min, respectively, and the supernatants were used for protease and amylase assays.

Protease assay

Protease assay was done according to the method of Sarkar *et al.* (2011). To 1 mL of extract, 1 mL of 1 % casein (substrate) was added and incubated at 60 ^{o}C for 10 min. To this 0.5 mL of trichloroacetic acid (20 % w/v) was added to stop the reaction. The mixture was allowed to stand for 30 min at RT and then filtered. One mL of the filtrate was then mixed with 5 mL of 0.5M Na_2CO_3 solution. For development of color, 0.5 mL of Folin-Ciocalteau reagent was added and kept in dark for 15 min. Absorbance was taken at 660 nm against tyrosine as standard. One unit of protease activity is calculated as the amount of enzyme required to liberate 1 g tyrosine per millilitre in 1 min.

Amylase assay

Amylase activity was measured according to Sarkar *et al.* (2011). To 1 mL of extract, 1 mL of 1 % starch (substrate) and 1 mL of citrate-phosphate buffer (pH 6.0) was added and incubated at 50 °C for 30 min. The reaction was ceased by adding 2 mL of 3,5-dinitrosalicylic acid (DNSA) reagent and kept in boiling water bath for 10 min. Absorbance was read at 540 nm against glucose as the standard. One unit of amylase activity is defined as the amount of enzyme which releases 1 µM of reducing sugar as glucose per minute, under the assay conditions ($U.mL^{-1}$).

Evaluation of kitchen waste degradation

The consortium capable of concomitantly producing both proteases and amylases was selected for laboratory trials. Three kilograms each of small heaps of kitchen wastes was collected from different canteens located in the campus of Lovely Professional University (Phagwara, Punjab, India). Each heap was inoculated with 5 % of selected consortium by evenly mixing the inoculum with the wastes, and observations for changes in pH, temperature, weight loss, organic carbon, and moisture content were taken at 1, 10, 20 and 35 d, respectively.

pH, temperature, and moisture content

The pH of the organic waste of both the control and consortia treated organic waste was checked at 1, 10, 20, and 35 d of composting, respectively. Temperature was also noted for the same using a thermometer at 1, 10, 20 and 35 d of composting, respectively. Similarly, for moisture content 10 g of each sample was weighed and dried for 24 h in the oven at 100 – 110 ^{o}C at 1, 10, 20, and 35 d of

composting, respectively, and moisture content was determined by calculating the weight difference.

Organic carbon

Organic carbon was estimated by rapid titration method of Walkley and Black (1934). To 1 g of oven-dried waste, 20 mL of 0.1 N $K_2Cr_2O_7$ was added. To this, 20 mL of concentrated H_2SO_4 was added and homogenized by gentle swirling and allowed to stand for 30 min. After completion of the reaction, 250 mL distilled water and 10 mL of 85 % orthophosphoric acid (H_3PO_4) was added with 1 mL of diphenylamine indicator. The mixture was back titrated against 0.5 N Ferrous ammonium sulphate with diphenylamine indicator, and the developing reddish brown color was taken as the endpoint. A blank is run without the sample and the final value was derived as per the calculation of Khan (2016).

Carbon, Hydrogen, Nitrogen and Sulphur (CHNS) analysis

CHNS content in the composting kitchen waste inoculated with selected Consortium 1 was analysed with CHNS-O Elemental Analyser (Thermo Scientific™ FlashSmart™, Waltham, USA).

Statistical analysis

The assays and experiments were carried out in triplicates, and mean values and standard error were calculated using SPSS 21.0 software for Windows.

Results and Discussion

Preliminary screening for metabolic characteristics of bacterial strains

Seven bacterial cultures were selected which produced the desired enzymes required to degrade organic kitchen wastes. The strains of *S. aureus*, *B. subtilus*, *P. aeruginosa*, *L. plantarum*, *L. delbrueckii*, *L. rhamnosus*, and *B. amyloliquefaciens* showing the hydrolysis zones are presented in Fig. 1 and Table 1. All the strains showed positive results for enzyme production except for *B. megaterium* which did not exhibit lipase and cellulase activities.

Table 1. Enzyme production by different bacterial strains.

Microorganisms	Amylase	Lipase	Protease	Cellulase
B. subtilis	+	+	+	+
B.megaterium	+	-	+	-
P. aeruginosa	+	+	+	+
S. aureus	+	+	+	+
L. plantarum	+	+	+	+
L. rhamnosus	+	+	+	+
L.delbrueckii	+	+	+	+
B.amyloliquefaciens	+	+	+	+

+ indicates enzyme activity and - indicates no enzyme activity.

Enzymatic assays of selected bacterial cultures and consortium

Consortium 1 exhibited higher amylase activity (17.66 $U.mL^{-1}$) as compared to Consortium 2 and individual bacterial strains which are statistically different from each other ($P < 0.05$) (Fig. 2A). Bacterial strains in Consortium 1 especially *B. subtilis*, *B. amyloliquefaciens*, and *L. plantarum* show appreciable levels of amylase activities; their cumulative action in Consortium 1 positively and significantly increased the level of starch degradation in the kitchen waste. Studies have shown that the genus *Bacillus* yields a large variety of extracellular enzymes of which amylases and proteases are of major industrial importance.

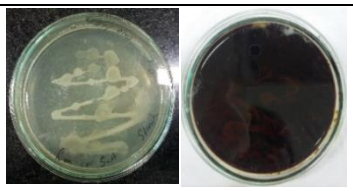
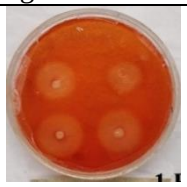
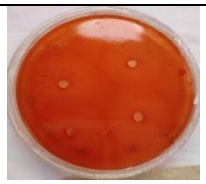
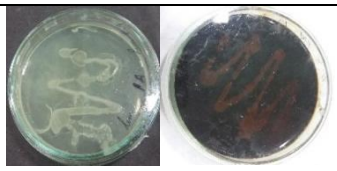
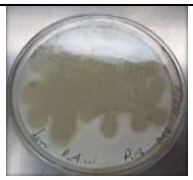
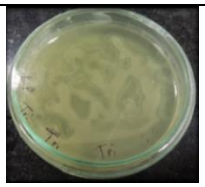
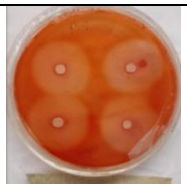
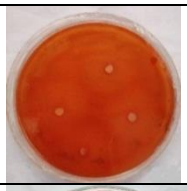
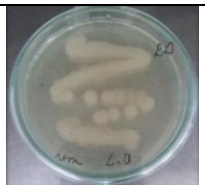
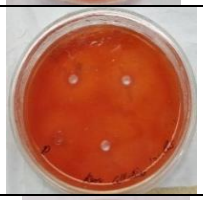
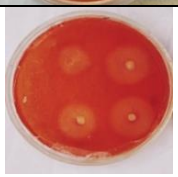
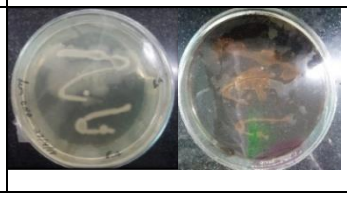
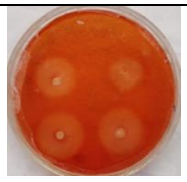
	Amylase activity on 1 % starch agar	Protease activity on skim milk agar	Lipase activity on nutrient agar with 1 % tributyrin	Cellulase activity on CMC (congo red) supplemented agar
Staphylococcus aureus				
Bacillus subtilus				
Pseudomonas aeruginosa				
Lactobacillus plantarum				
Lactobacillus delbrueckii				
Lactobacillus rhamnosus				
Bacillus amyloliquefaciens				

Fig. 1. Bacterial strains showing zones of hydrolysis on plates of starch agar, skim milk agar, nutrient agar of 1 % tributyrin, and CMC (Congo red) agar.

Mesophile *Bacillus* sp. has showed to offer highly alkaline and thermostable α-amylases (Saxena *et al.* 2007; Naidu *et al.* 2013; Awasthi *et al.* 2018). Similarly, for protease assay while both consortia exhibited considerable protease activities, Consortium 1 produced a higher activity at 20.71 $U.mL^{-1}$ (Fig. 2B) that was significantly higher from Consortium 2 and individual bacterial strains ($P < 0.05$). Among individual bacterial strains *L. delbrueckii*, displayed the highest protease activity.

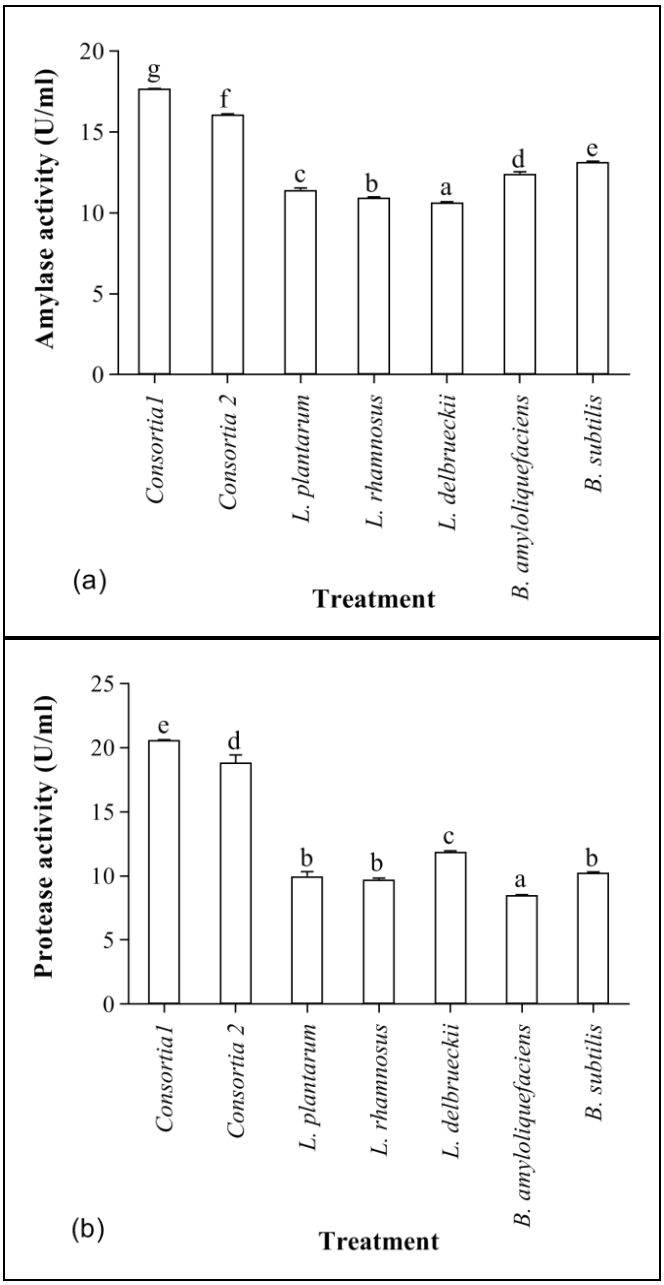


Fig. 2. Enzyme activities of consortia and specific microbes (**a**) Amylase, (**b**) Protease. Data presented are the mean ± standard error of three replicates. Means with different superscript letters are significant by Tukey's post hoc test ($P < 0.05$).

Studies by Kliche *et al.* (2017) observed that the *Lactobacillus* genus, specifically *L. delbrueckii* and *L. helveticus*, produce proteases that can hydrolyze α- and ß-casein, while its amylase activity was comparatively the least. Also, bacterial strains showing higher protein degrading activity include *B. subtilis* and *L. plantarum*. These bacteria also indicated higher amylase activities (Lim *et al.* 2019; Chen *et al.* 2020). Given that Consortium 1 presented enhanced concomitant activities of both amylase and protease in comparison to Consortium 2 and the individual bacterial strains, respectively, Consortium 1, which consist of non-pathogenic bacterial species, was thus taken for further evaluation on degradation of kitchen waste.

Evaluation of degradation of kitchen waste – pH

Generally, during composting period, pH is affected due to changes in the chemical composition of the waste matter. Initially, pH falls below neutral due to the formation of organic compounds by the microbes and the formation of ammonium (Palaniveloo *et al.* 2020). Studies have shown that from the 2nd week of composting the pH values increased because of the elimination of carbon dioxide and the decomposition of proteins in the waste (Saad *et al.* 2013). The initial pH value of both control and consortium-treated waste was 4.0. The pH of consortium-treated kitchen waste increased to 5.3 and 5.7 on day 20 and day 35, respectively, whereas the pH Value in control increased slightly to 4.7 and 5.0 on day 20 and day 35, respectively. Compost microorganisms are reported to operate best under neutral to acidic conditions, with pH in the range of 5.5 to 8.0 (Mehta *et al.* 2014; Palaniveloo *et al.* 2020).

Temperature

During composting, the change in temperature has a major influence on the efficiency of the composting process. It is an important parameter to monitor composting efficiency, because it affects not only the biological reaction rates and the dynamic population of microbes, but also the physico-chemical characteristics of composts (Chang *et al.* 2019). From an initial temperature of 20 ºC in both consortium-treated kitchen waste and in control was observed, where a linear increase is observed up to day 20. Control sample registered temperature increase up to 25 ºC while Consortia 1 exhibited a higher temperature of 29 ºC. A gradual decreasing trend towards the end of the composting process at day 35 was noted. Similar temperature variations were observed in various studies of compost of plant residues, pineapple leaves (PL) wastes, mixed vegetables and fruit waste with

ambient temperature 24 to 30 ºC; 25 to 32.5 ºC and 35 to 45 ºC (Gautam *et al.* 2010; Cheng *et al.* 2013; Abu-Zahra *et al.* 2014). More linear changes in temperature were observed in Consortia 1 as compared to control.

Moisture content

The moisture content of the consortium-inoculated wastes demonstrated a gradual decline from an initial 74.3 % to 58 % at day 35; in control only slight decrease in the moisture content was observed with increase in composting time and 68 % was recorded at day 35 Fig. 3C. According to Jain *et al.* (2019), moisture content has a central influence on the biodegradation of organic matter where it affects the microbial activity, as well as the physical structure, in the composting process. Proper moisture content in the composting process is one of the key factors for the success of composting (Makan *et al.* 2013). Many researchers have investigated the range of optimum moisture content for composting process to be within 50 % and 70 % (Iqbal *et al.* 2015; Wu *et al.* 2015). Thus, the reduction in moisture content was significant in the kitchen waste treated Consortium 1 as compared to control.

Weight loss

Weight loss is another factor for determining the process of composting (Pan *et al.* 2012). From the data represented in Fig. 3D during the laboratory trail the weight of the kitchen waste with Consortia 1 reduced from 447 g to about 333 g as compared to control reduced from 470 g to about 400 g. Weight loss of 25 % was observed (Tiquia *et al.* 2002). Microbial metabolic activity was the primary reason for kitchen waste mass loss. Microbes decomposed organic components into micro-molecular organics such as glucose and produced and emitted large amounts of CO_2 (Zhang *et al.* 2013; Yeh *et al.* 2020). A drastic change in weight loss was observed when kitchen waste was inoculated with microbial Consortia1 as compared to control.

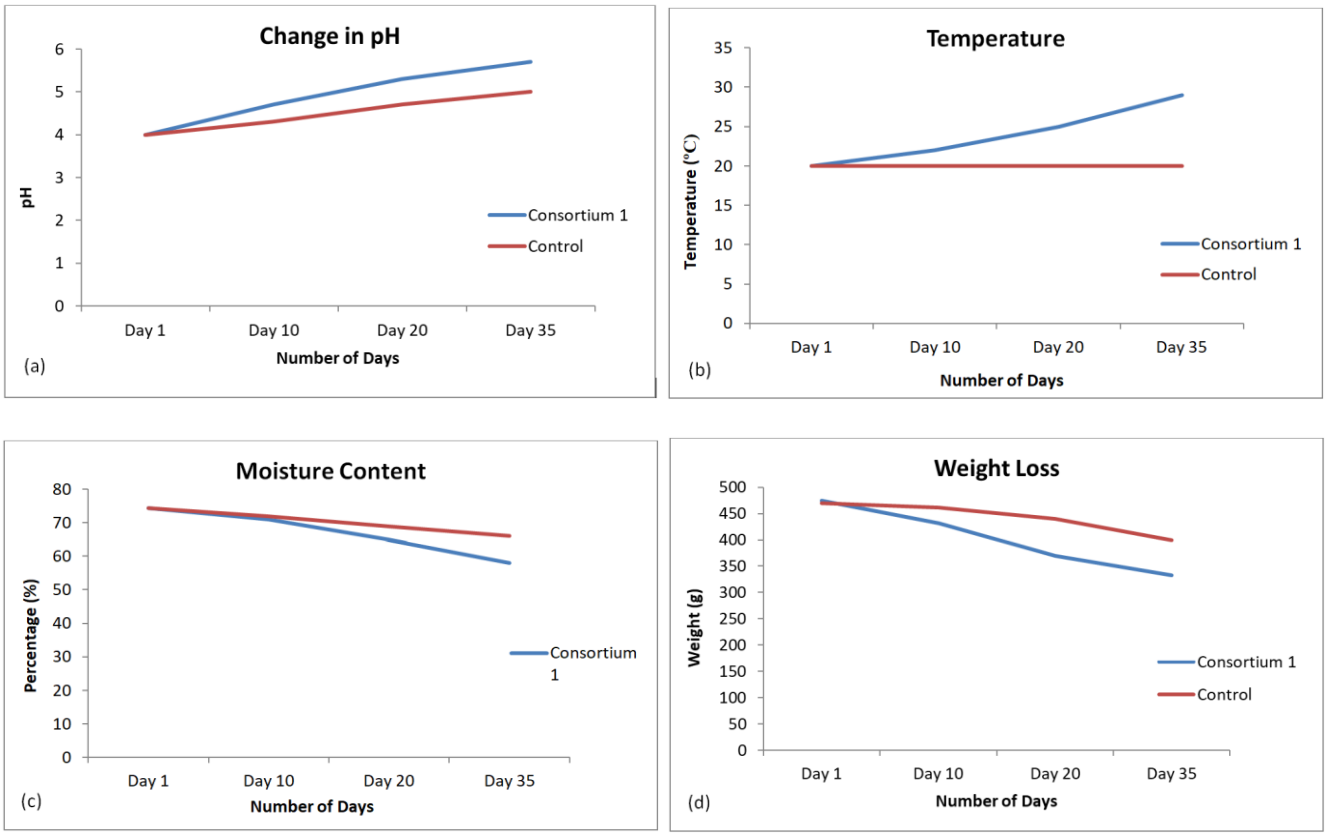


Fig. 3. Various parameter of degradation of kitchen waste and consortium 1: **a** – change in pH; **b** – change in temperature; **c** – moisture content; **d** – weight loss.

Organic carbon

The C : N ratio is one of the most important indicator of the composting process where the occurrence of carbon during composting assists microbes during the decomposition process, while nitrogen helps build microbial cell structures (Saad *et al.* 2013). According to Rastogi *et al.* (2020), the optimum composting process befalls through a range of C : N ratio 30 – 40 : 1. Also, Jain *et al.* (2019) found that C : N ratio lower than 20 can also be effective for composting process. The initial carbon content of kitchen wastes was 73 % on both the control and Consortia 1 treated waste, respectively. The carbon content showed a sharp decrease in Consortia 1 treated waste up to 41 % on the 20th d of degradation and 36 % on 35th d of degradation, in contrast the control showed slight decreased of 67 % on the 20st d of degradation and only 64 % up to 35th d of degradation (Fig. 4) Thus the reduction in organic carbon content was significant in the kitchen waste by Consortium 1 as compared to control.

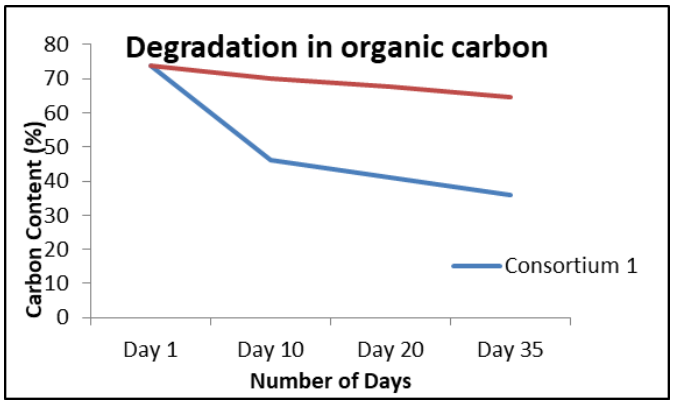


Fig. 4. Organic carbon content of Consortium 1 and control during degradation.

The sample of kitchen waste treated with Consortia1 showed the presence of 38 % carbon content, 1 % nitrogen, and 6 % hydrogen (Fig. 5). The C/N ratio in the current study is calculated at 38. C : N ratio of Consortium 1 at 18 d was 39 : 1.2 which indicates an efficient degradation process. While the optimum composting process occurs in the C/N ratio of 30 to 40, other studies have also reported that C/N ratio lower than 20 can also be effective for composting process (Cao *et al.* 2021).

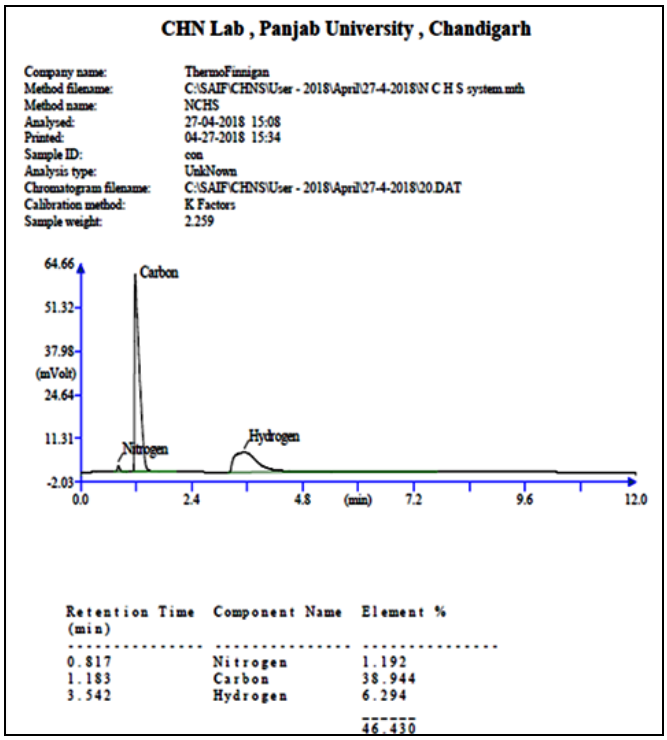


Fig 5. CHNS analysis of Consortia 1.

Conclusion

In this study bacterial strains were selected and screened based on their enzymatic activity to create a microbial consortium to degrade organic kitchen waste. Two bacterial consortia were prepared, one containing non-pathogenic bacterial species (*B. subtilus*, *B. amyloliquefaciens*, *L. plantarum*, *L. rhamnosus*, and *L. delbrueckii*) (Consortia 1) and the other a mixture of pathogenic and non-pathogenic bacterial species *P. aeruginosa*, *S. aureus*, *L. delbrueckii*, *L. rhamnosus*, *B. subtilus* (Consortia 2). Consortia 1 showed better enzymatic production as compared to Consortia 2 and Consortia 1 was thus taken into consideration as the main consortia for degradation of kitchen waste.

Bacillus sp. can interact with each other and form a stable micro-ecology system to improve the degradation rate of organic matters, increasing the maturity of kitchen waste and reducing toxin products of the reaction. The bacterial strains used were non-pathogenic and thus will not be harmful to the environment and showed positive results for enzyme production required to degrade the kitchen waste. Thus, degradation of organic kitchen wastes by the bacterial consortia is extremely significant.

The utilization of microbial consortium created through characteristic choice or change of the execution of these microorganisms in natural kitchen waste degradation for treatment of organic kitchen waste in the near future might be the best option. The utilization of microbial consortium created through characteristic choice or change of the execution of these microorganisms in natural kitchen waste degradation in the near future might be the best option. Consortia of non-pathogenic bacteria are also an efficient and viable option for an environment and health safety method of waste management. This study warrants further metabolic studies of each microorganism working in synchronization for efficiently degrading the kitchen waste. These consortiums can be scaled to industrial level in fermentors for bulk municipal waste management in the near future. The biogas released and the by products can be used as manure and for other purposes.

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Conflict of Interest

The authors declare that they have no conflict of interest in the publication.

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