# Isolation and functional characterization of house hold waste degrading bacteria

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Waste is anything useless or unwanted, that has no sufficient value or of negligible uses (Harold, 2002). It may include any solid, liquid, or gaseous material. The major waste from households is 'solid waste'. Solid waste may include any solid or semi-solid material that is being discarded from our houses daily. Every day, a huge quantity of waste is generated in all the developed and developing countries (Moqsud, 2003). In rapidly growing cities of the developing world, urban solid waste management is currently regarded as one of the most immediate problems. India is rich in agricultural resources, accounts for 50 million tons of vegetable waste, which is about 30 % of its total production (Verma et al., 2011). Food waste is unwanted raw or cooked food is thrown away during or after food preparation that is no longer fit for consumption or desirable (Jean et al, 2009).

Unscientific disposal can harm all components of the environment and human health (Rathi, 2006; Gupta et al., 1998). As a result, human and animal diseases occur, the air and soil environment are spoiled and the entire natural ecosystem balance is disturbed. Microbes too get a favorable environment for their growth as the household trash contains all types of organic and inorganic matter, both aerobic and anaerobic conditions, moisture and humidity, and all other optimum conditions required for their survival. Thus, accumulated refuse and trash becomes a perfect place for a variety of microbes to grow. Pathogenic microorganisms and harmful chemicals in the solid waste can be contaminated into the environment when the waste is not properly managed (Wai-Ogosu, 2004; Ogbonna and Igbenijie, 2006). Waste can contaminate surface water, groundwater, soil, and air which causes more problems for humans, other species, and ecosystems (Obire et al., 2002).

Due to all such reasons, organic waste needs to be degraded and decomposed. Management and removal of such pollutants are facing a crucial state, due to the unavailability of suitable strategies for treatment and waste disposal. Although organic matter can also be decomposed under anaerobic conditions, the degradation is slow and less efficient and produces less heat and more undesirable products, including C, H, and NO, which are greenhouse gases contributing to global warming (Hao et al., 2001). In comparison, aerobic degradation is accepted as an eco-friendly option for handling food waste, because the predominant aerobic environment can help mitigate methane generation. Thus, degradation is presently gaining more and more attention in treating food waste (Droffner and Brinton, 1995; Elwell et al., 1996). Biological methods of decomposition of solid waste and liquid waste are known. They are regarded, to a high extent, as an effective, relatively cheap, and most environmentally friendly way for utilizing most of the organic waste, in which organic fraction is usually represented by proteins, fats, and carbohydrates, However, one of the biggest problems in this kind of process, is a proper choice of micro-organisms, in terms of both quantity and quality, which determines the utilization effects of the bio preparation as well as the speed of the selected microbes but the biodegradation of most proteins, fats, and carbohydrates in organic waste, originating from for example households seems to be a possible process. The complete biodegradation of organic waste cannot be assumed, as it may contain compounds that cannot be decomposed, i.e.: scleroproteins or hemicellulose, (Bujak and Targoński 1998). Organic manure obtained in this process is being used in agriculture if its chemical composition (the content of heavy metals) and health and sanitary properties are unquestioned. The objective of the study was the evaluation of organic waste biodegradation with bacterial inoculation containing selected proteolytic, lipolytic, and cellulolytic bacteria strains. Reduction of organic waste content may help to a large extent in reducing pollution and various diseases.

## Materials and Methods

### Soil sample collection

Soil samples were collected from various locations in Kanpur, Uttar Pradesh, India, where garbage is dumped.

### Bacterial Strains

Cultures were isolated from soil samples onto nutrient agar plates, by serial dilution method and then incubated overnight at 25°C. After that colonies were differentiated based on their colony morphology. Confirmation of pure cultures was done through Gram's staining of cultures. All three selected cultures were identified based on colony morphology, cellular morphology, and biochemical characteristics by using Bergey's Manual of Systematic Bacteriology (Ninth Edition). After obtaining pure cultures, strains were preserved on agar slants and in glycerol stocks.

**Enzymatic activities**

Screening of all the strains isolated from each sample was done for enzymatic activities namely, cellulase, protease, lipase, and amylase.

**Cellulase test**

Single colonies were inoculated onto Mandels and Reese medium (Mandels and Reese,1957) and incubated at 28°C for 48 h. All the plates were stained with 1% (w/v) Congo-red solution for 15 min and decolorized with 1 M NaCl for 15 min ( Teather anood, 1982). The degradation zones were visible around the colony, showing that the strains could hydrolyze carboxymethylcellulose.

**Protease test**

Spot inoculation on Gelatin agar medium with Congo Red and incubated for 48 hrs at 28°C. A black zone in the middle of the colony was obtained in protease-producing colonies. The colony obtained was found to be protease-producing and was sub-cultured in Gelatin yeast extract glucose broth.

**Lipase test**

Inoculation of samples on tributyrin agar plates and incubated at 28°C for 48 hrs. Colonies showing clear zones around them were picked out and purified on tributyrin agar plates.

**Amylase test**

Inoculated the culture on Starch agar media and incubated for 48 hrs at 28◦ C then iodine solution was poured. Colonies forming clear zones were amylase positive

Colonies having all the mentioned enzymatic activities were selected and identified.

**Biodegradation of selected waste**

Biodegradation of wastes was performed on potato. The 3 selected strains were inoculated separately and in consortia to the above-mentioned material. And then test in equal intervals (0,15,30,45,60,75 days) for following parameters.

**Estimation of weight**

The weight of each sample was taken at regular intervals (0,15,30,45,60,75 days) by a weighing machine.

**Estimation of protein**

Estimation of protein was done by Lowry's method

**Estimation of sugar**

Estimation of sugar was done by the phenol sulphuric acid method. Take 2 ml of the standard solution or appropriately diluted unknown sugar sample solution, 1 mL of phenol reagent was added, followed by the rapid addition of 5 mL of concentrated sulphuric acid, using a glass pipette. After 30 minutes the yellowish-orange colour developed against a reagent blank at 490 nm was read in a photometer and the absorbance was recorded. A calibration curve was constructed, by plotting the sugar concentration of the sugar in the sample was computed in the sample from the calibration curve. While calculating the sugar concentration in the unknown sample, the dilution factor was taken into account.

## Results and Discussion

### Bacterial Strains

33 different bacterial strains were isolated and purified (Table 1), among which the majority of the strains i.e., 22 were Bacillus while others were cocci. 9 strains show fluorescence (Table 1). A similar study was done by Kaining Zhao et al., (2016). He isolated 104 strains, based on morphological and molecular identification of which majority (63) were bacterial strains and genus *Bacillus* exhibited the greatest number of species and strains. Members of this genus form endospores tolerant to high temperature, corrosion, and detrimental environmental

parameters. They can grow and reproduce even 80°C and are important microorganisms in the composting process.

**Table-1: No. of isolates from each sample**

| SOIL SAMPLE | NO. OF ISOLATES | ISOLATE NAME |
|---|---|---|
| Shashtri chowk | **7** | 1A,1B,1C,1D,1E,1F,1G,1H |
| Polytechnique | **17** | 2A,2B,2C,2D,2E,2F,2G,2H,2I,2J,2K, 2L,2M,2N,2O,2P,2Q |
| Geeta nagar | **9** | 3A,3B,3C,3D,3E,3F,3G,3H,3I |

**Enzymatic activities**

Results of enzymatic activities (cellulase, protease, lipase, and amylase) of all the isolated strains are shown in Table no. 2.

**Table 2: Enzymatic activities of all the isolated strains (diameters in cm)**

| STRAIN | CELLULASE | PROTEASE | LIPASE | AMYLASE |
|---|---|---|---|---|
| 1A | **0.02** | **0.4** | - | **0.3** |
| 1B | - | - | - | **0.3** |
| 1C | - | - | - | **0.2** |
| 1D | - | - | - | **0.1** |
| 1E | **0.1** | **1.1** | - | **0.5** |
| 1F | **0.3** | - | - | - |
| 1G | **0.1** | **0.6** | - | - |
| 1H | **0.9** | **0.8** | **0.5** | **1.1** |
| 2A | **0.5** | **0.8** | - | **0.1** |
| 2B | **0.4** | - | - | **0.5** |
| 2C | - | **0.5** | **0.8** | **0.1** |
| 2D | - | - | - | - |
| 2E | **0.2** | **0.1** | - | - |
| 2F | - | - | - | **0.8** |
| 2G | **0.5** | **1.2** | **0.1** | **0.1** |
| 2H | **0.1** | **0.5** | - | **1.1** |
| 2I | - | **0.1** | - | - |
| 2J | - | - | - | **0.1** |
| 2K | **0.1** | - | **0.7** | **0.5** |
| 2L | - | **0.1** | - | - |
| 2M | - | - | - | **0.3** |
| 2N | **0.1** | - | - | **0.4** |
| 2O | - | **0.8** | - | - |
| 2P | **0.8** | **1.0** | **0.9** | **0.7** |
| 2Q | **1.2** | **0.8** | **1.1** | **1.0** |
| 3A | - | **0.6** | - | **0.8** |
| 3B | - | - | - | **0.1** |
| 3C | - | - | - | - |
| 3D | **0.5** | **0.2** | - | - |
| 3E | **0.1** | **0.5** | - | **0.5** |
| 3F | **0.9** | - | - | **0.8** |
| 3G | **0.1** | - | - | **0.5** |
| 3H | **0.2** | - | - | **1.4** |
| 3I | - | **0.5** | **0.1** | **0.4** |

The majority of species were able to degrade cellulose, protein, and starch while a limited number of strains degraded lipids. The diameters (d) of the clearance zones around the colonies were measured to assess the degradative activity of the strains. Degradation of cellulose present in the medium (microcrystalline cellulose as only C- source) was easily detected, indicator

Congo red dye was added which formed a transparent clearance zone around the colony in the area where cellulose was decomposed by the bacteria. Nineteen bacterial strains had clearance zones, strain 1H (d=09mm), 2P (d=08mm), 2Q (d=12mm) and 3F (d=11mm) show maximum diameters of clearance zones. A similar study was performed by Yan-ling-Liang in 2014. He isolated a total of 245 cellulose-degrading aerobic bacterial strains from different natural reserves in the subtropical region of China, which were cultured in an agar medium containing sugarcane bagasse pulp as the sole carbon source. Out of these strains, 22 isolates showed hydrolyzing zones on agar plates containing CMC-Na after Congo-red staining. Based on the enzymatic activities of all the strains, 3 strains were selected. Nair J, Okamitsu (2010) also performed a similar study. He isolated *Bacillus subtilis* as it showed the greatest cellulase activity and used it to degrade kitchen waste by its high-temperature tolerance. Our results also indicated that 18 bacterial strains formed a black zone in the center of the colony, showing degradation of the protein. The minimum diameter of the black zone was d=0.2cm (2E) and the maximum diameter was d=1.2 cm (2Q). Kaining Xhao et al (2016) performed a similar study and found that all their 104 isolated strains were capable of degrading protein. Some studies also showed that efficient protein degradation mainly contributed to nitrogen circulation in compost systems and promoted compost fermentation (Lim et al, 2014). Only 7 strains were isolated that formed clearance zone, showing their ability to degrade lipids. The maximum clearance zone was observed in 2Q (d=11 mm). The major factor for the expression of lipase activity has always been carbon, since lipases are inducible enzymes and are thus generally produced in the presence of a lipid source such as oil or any other inducer, such as triacylglycerols, fatty acids, hydrolyzable esters, tweens, bile salts and glycerol ( Mobarak-Qamsari et al.,2011).

Iodine was added to the starch medium to identify amylolytic strains. Carbohydrates are required for cell growth and metabolism. Microorganisms were inoculated into starch media and transformed starch into carbohydrates of low molecular mass. Twenty-six bacterial strains had high starch degradative activity. The maximum degradative activity was shown by 1H (d=11 mm). Xhao, 2016 isolated 104 strains out of which 5 had amylase activity, the top three were A3 (*Paenibacillus polymyxa*, d = 12 mm), J2 (*Bacillus cereus*, d = 16 mm), and D6 (*B. amyloliquefaciens*, d = 23 mm).

Strains 1H, 2P and 2Q gave positive results for all the enzymes tested. (Table 3). Hence, they were selected for further functional analysis of their degradative activity of household waste.

**Table 3: clearance zone of selected strains**

| Strains | Cellulase | Protease | Lipase | Amylase |
|---|---|---|---|---|
| **1H** | 0.9 cm | 0.8 cm | 0.5 cm | 1.1 cm |
| **2P** | 0.8 cm | 1.2 cm | 0.9 cm | 0.7cm |
| **2Q** | 1.2 cm | 0.8 cm | 1.1 cm | 1.0 cm |

**Biochemical characterization**

Biochemical analysis of strains indicated that strain 2P tends to be *Pseudomonas*, strain 2Q could be *Paenibacillus*, and strain 1H could be Pseudomonas.

**Table 4: Biochemical characterization of screened strains**

| BIOCHEMICAL TEST | 2P | 2Q | 1H |
|---|---|---|---|
| **GRAM STAINING** | - | + | - |
| **UREASE** | + | + | + |
| **CATALASE** | + | + | + |
| **OXIDASE** | + | - | + |
| **SIMON CITRATE** | + | + | + |
| **MR** | - | + | - |
| **VP** | - | + | - |

| CARBOHYDRATE FERMENTATION | ACID | GAS | ACID | GAS | ACID | GAS |
|---|---|---|---|---|---|---|
| **GLUCOSE** | + | + | + | + | + | + |
| **LACTOSE** | - | - | - | - | - | - |
| **MALTOSE** | + | + | + | + | + | + |
| **SUCROSE** | + | + | + | + | + | + |
| **FRUCTOSE** | + | + | + | + | + | + |
| **RAFFINOSE** | + | + | + | + | + | + |

**Biodegradation of waste**

Functional analysis of selected strains was performed on selected samples. Samples were selected based on the biomolecules present in them.

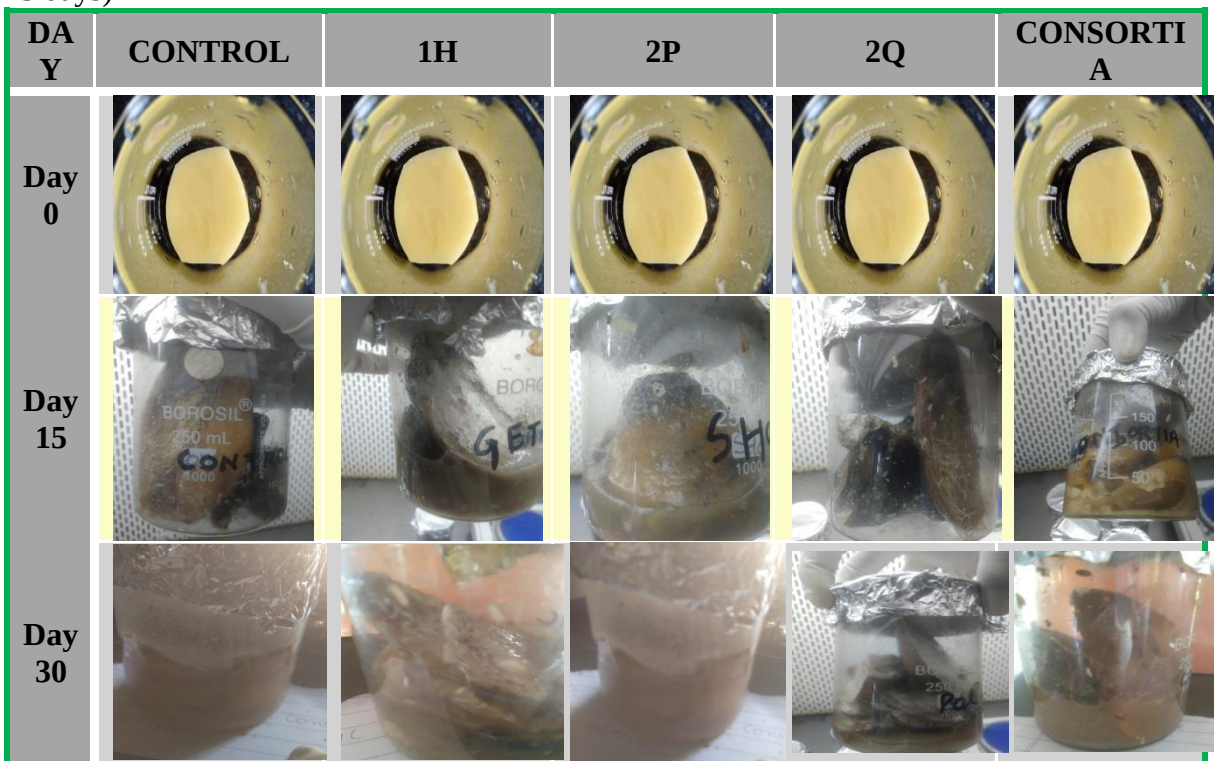
**MORPHOLOGICAL CHANGES IN POTATO**

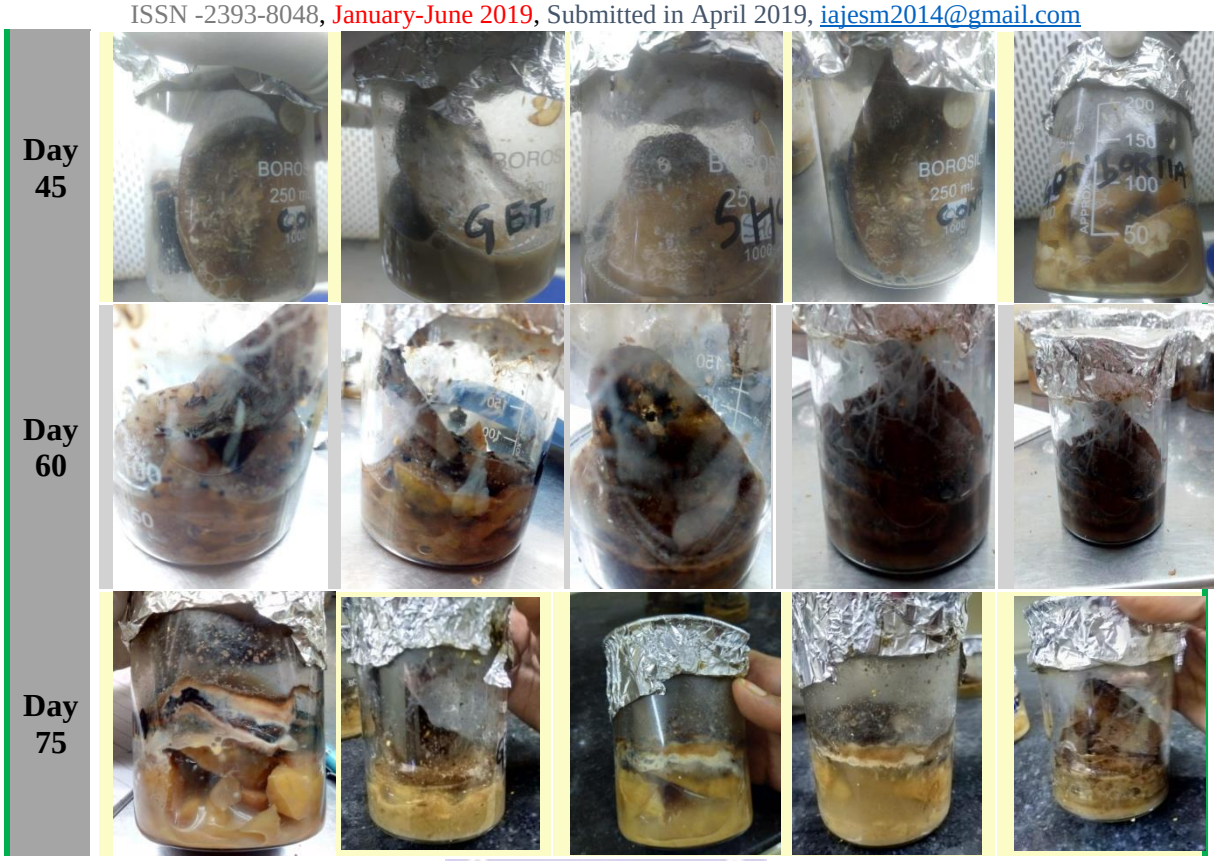
Based on our study on household waste degradation, several morphological changes were shown due to degradative activity of our selected bacterial strains and their consortia. The color of the potato changed to dark brown. A large amount of potato pulp was degraded and it turned to a semi-solid paste-like form. Maximum semisolid pulp was observed in potato containing strain 2Q while the minimum was observed in control (Figure 1). The potato peel thinned and remained intact while the pulp being semi-solid came out of the potato in the broth. No pores were created in the peel of the potato. It could be concluded here that due to the greater amylase activity of strain 2Q, it degraded starch in the potato in less time.

Sarian (2012) presented the characteristics of *Mycobacterium aurum* with the ability to degrade granular potato starch. Other studies have reported enzyme systems that degraded the granules by peeling off layers from the surface, without apparent erosion of their internal regions (no pores detected) (Taniguchi et al. 1982). Potato is generally less susceptible to enzymatic degradation than other starches. It has been proposed that larger granules are less easily degraded than smaller granules of starch (Heitmann et al., 1997; Kong et al., 2003; Tester et al., 2004).

**Fig. 1: Morphological changes in potato**

Morphological changes due to bacteria on potato at different time intervals (0, 15, 30, 45, 60, 75 days)






**ESTIMATION OF WEIGHT OF POTATO AT REGULAR INTERVALS**

In this study, weight of samples was measured at regular intervals for 90 days. Weight of potato reduced as its degradation proceeded. Each of the strain reduced the weight of potato but maximum reduction was observed in the consortia of strains. Control also shows some reduction in weight of potato due to natural environment but its rate is too slow as compared to the consortia. Consortia contained all the three strains with high amylase and cellulose activity which degraded the starch and other sugars present in it. Efficient degradation of potato by consortia indicates that amylase and cellulase activity of all the three enhanced when they form a multicellular organization.

Some studies showed that dried potato waste solids had a wide range of N percentages that influences its decomposition (Smith, 1996). Similarly, isolated amylase producing bacteria from a soil of potato dump sites (Vaidya et al., 2015).

Table no. 6 show that only 25.80% of potato weight reduction was in control while 48.09% of potato weight reduction in consortia of all the three bacterial strains.

Other bacterial strain also reduced potato, 36.04% by 1H, 42.60% by 2P and 28.82% by 2Q.

**Table 5 : Estimation of protein and total sugar content of potato at regular intervals**

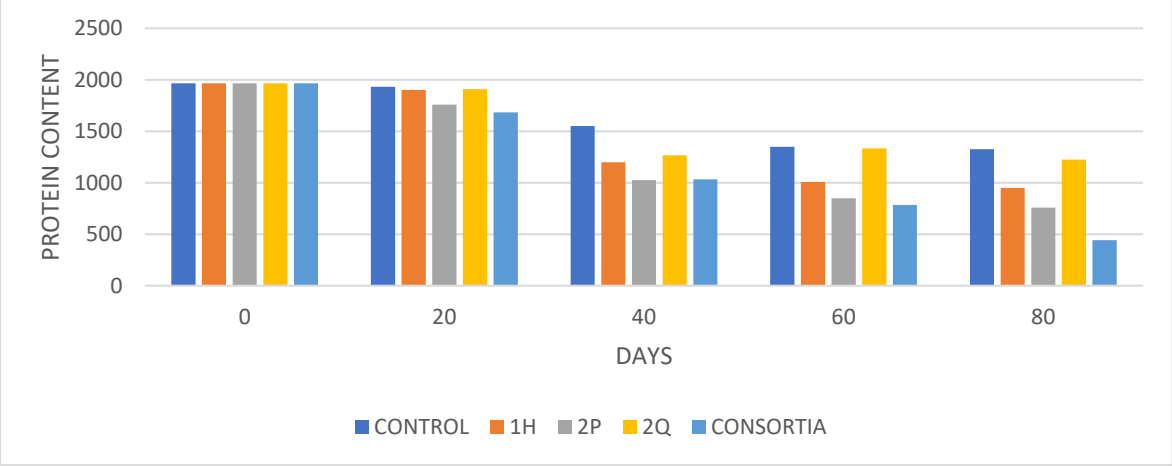
| Day | Strain | Total sugar content (in µg/ml) | Protein content (in µg/ml) |
|---|---|---|---|
| Day 0 | Control | 210 | 1966 |
| | 1H | 210 | 1966 |
| | 2P | 210 | 1966 |
| | 2Q | 210 | 1966 |
| | Consortia | 210 | 1966 |
| Day 20 | Control | 190 | 1933 |
| | 1H | 180 | 1900 |
| | 2P | 180 | 1758 |

| | | | |
|---|---|---|---|
| | 2Q | 200 | 1908 |
| | Consortia | 150 | 1683 |
| Day 40 | Control | 170 | 1550 |
| | 1H | 150 | 1200 |
| | 2P | 120 | 1025 |
| | 2Q | 160 | 1266 |
| | Consortia | 110 | 1033 |
| Day 60 | Control | 110 | 1350 |
| | 1H | 160 | 1008 |
| | 2P | 150 | 850 |
| | 2Q | 130 | 1333 |
| | Consortia | 80 | 783 |
| Day 80 | Control | 140 | 1325 |
| | 1H | 130 | 950 |
| | 2P | 80 | 758 |
| | 2Q | 90 | 1225 |
| | Consortia | 30 | 441 |

**EFFECT ON PROTEIN AND TOTAL SUGAR CONTENT ON POTATO**

This study showed that due to protease activity of bacterial strains, protein concentration was reduced in each sample. Maximum reduction was observed in consortia i.e., 77.56% reduction while minimum observed in control i.e., 32.50%. Reduction in protein in strain 1H, 2P and 2Q is 52.67%, 61.44% and 37.69% respectively (Table no. 5, figure no. 2).

**Figure 2: Effect on protein content of potato**




Maximum reduction in carbohydrate was observed in consortia (85.70%) while minimum was observed in control (33.33%). Other strains also reduced carbohydrate content as follows, 1H by 38%, 2P by 61.9% and 2Q by 57.1%.

**Figure 3: Estimation of total sugar content of potato**

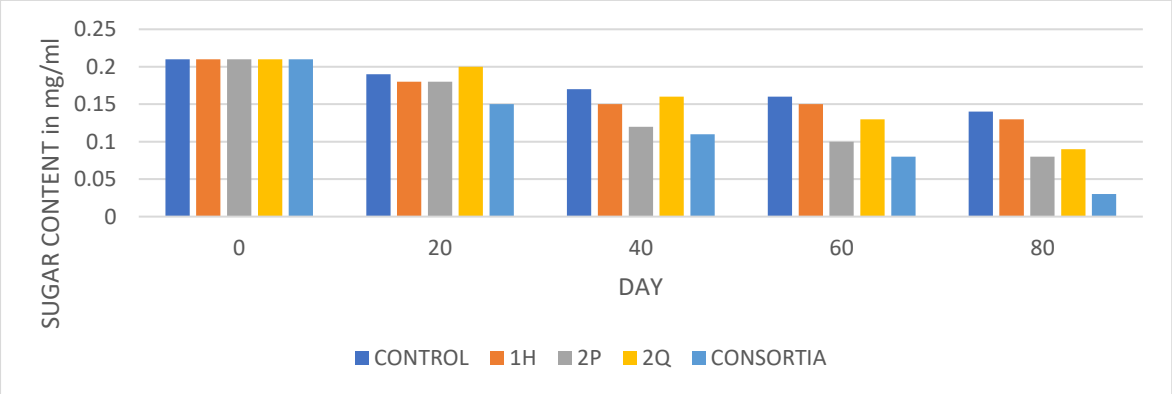



Table 6 suggests that consortia worked best in degradation of potato, we can say that due to strong amylase and cellulase activity of all the selected strains, they exhibited great co-metabolism for reduction in protein, starch and other carbohydrate content, there by leading to reduction in weight.

**Table 6: DEGRADATION OF POTATO**

| STRAIN | WEIGHT | PROTEIN CONTENT | SUGAR CONTENT |
|---|---|---|---|
| **CONTROL** | 25.80% | 32.50% | 33.33% |
| **1H** | 36.04% | 52.67% | 38% |
| **2P** | 42.60% | 61.44% | 61.9% |
| **2Q** | 28.82% | 37.69% | 57.1% |
| **CONSORTIA** | 48.09% | 77.56% | 85.70% |

Table 6 suggests that consortia worked best in degradation of potato, we can say that due to strong amylase and cellulase activity of all the selected strains, they exhibited great co-metabolism for reduction in protein, starch and other carbohydrate content, there by leading to reduction in weight.

## CONCLUSION

Thirty-three different bacterial strains were isolated from various municipal waste soil sample. Three of the dominant strains were selected for the biodegradation on the basis of their enzymatic activities (cellulase protease, lipase, and amylase). Two of them were may be *Pseudomonas* (1H and 2P) and one as *Paenibacillus* (2Q). They were examined to check their ability to degrade common household waste (potato). Morphology, weight, protein content and total sugar content were analyzed. Strain 2Q had the great effect in terms of reduction of all the parameters in the samples. Consortium of all the three strains was also tested for bio degradation and it degraded each sample efficiently, which leads to reduction of household waste to a great extent.

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