Biodegradation of Household Waste Using Micro-Organisms For Production of Important Products

Anita M Chandak¹ and Rupali Balpande²

¹D.R.B. Sindhu Mahavidyalya Nagpur, Maharashtra India

²Kamla Nehru Mahavidyalaya, Nagpur, Maharashtra India Corresponding author email: chandakmb@gmail.com

ABSTRACT

Household waste generated in towns has emphasized on the area for research due to increasing concern about pollution ecosystem and human health. Everyday a huge quantity of waste materials generated from houses. Various type of waste causing adverse effect on living organisms and environment that giving rise to human as well as animal diseases, spoilage in air and soil ecosystem lead to total imbalance in natural ecology. Aerobic biodegradation of organic matter by microbes (aerobes) is most rapid and fast degradation process. No pungent gas produced. Large disposable waste generated. The present study showed vegetable waste was rich source of enzymes, carbohydrates, fats, moisture and phytochemicals and best substrate for Microbial Biodegradation purpose. Analysis of individual enzymatic activity of mixed microbial species on household waste and production of industrially important products.

KEY WORDS: BIODEGRADATION, CELLULASE, AMYLASE, MICROBIAL CONSORTIA.

INTRODUCTION

Biodegradation is the process by which organic substances are broken down into smaller compounds by the enzymes produced by living microbial organisms. The microbial organisms transform the substance through metabolic or enzymatic processes. Biodegradation processes vary greatly, but frequently the final product of the degradation is carbon-dioxide or methane. Organic material can be degraded aerobically with oxygen or anaerobically without oxygen. The microbial population of soil possessed mainly bacteria, fungi, actinomycetes, algae and protozoa. Among these groups, bacteria are most prominent and abundant group. Bacteria use waste for their own metabolism and finally they produce hazardous toxic chemicals into non-toxic or less toxic substances which are important for soil health, plant growing and over all to keep well balance of natural ecosystem.

The genera *Bacillus*, *Corynebacterium*, *Staphylococcus*, *Streptococcus*, *Shigella*, *Alcaligenes*, *Acinetobacter*,

Escherichia, *klebsiella* and *Enterobacter* were the best hydrocarbon degrading bacteria. Mixed microbial communities have the most powerful biodegradative potential because the genetic information of more than one organism is necessary to degrade the complex mixture of organic compounds present in contaminated areas (Bird et al., 1954; Bruckner 2016).

Fungi are an important part of degrading micro biota because like bacteria they metabolized dissolved organic matter, they are principle organisms responsible for the decomposition of carbon in the biosphere. But fungi unlike bacteria can grow in low moisture areas and low pH. Solutions which aids them in the breakdown of organic matter. Filamentous fungi participating in aliphatic hydrocarbons degradation are *Cladosporium* and *Aspergillus* whereas a fungus belongs to penicillin; *Fusarium* and *Aspergillus* can take part in aromatic hydrocarbon decomposition (Chapman et al., 1999).

MATERIAL AND METHODS

1) Sterilization of All glassware's and culture media were sterilised in an autoclave for 15 minutes.

2) Preparation of Sieved Sample of Vegetables waste, then determine the physical parameters of sieved sample of vegetable waste such as colour, weight, pH, temperature, and moisture and water absorption capacity.

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3) Isolation of Bacteria: The Bacteria were isolated using standard serial dilution of soil and sewage water sample and characterised using the specificity of the substrate and gram nature of the organism. They are maintained on Nutrient agar slant at 4°C for further use.

4) Isolation and collection of fungi: *Aspergillus niger* was isolated from onion skin on Potato dextrose agar. The plate were incubated for 5-7 days at 28°C. Black colonies of *Aspergillus niger* was maintained on potato dextrose agar at 4°C. The collected pure culture of *Trichoderma viride*, *Bacillus subtilis* were maintained on selective media and subculture on Sabouraud dextrose agar slant individually at 4°C (Gautam et al., 2012).

5) Screening of Amylase Activity (Starch Hydrolysis Test: In this test, starch agar is inoculated with bacteria and fungi. After incubation at an appropriate temperature, iodine is added to the surface of the agar. Iodine turns blue-black in the presence of starch. Absence of the blue-black colour indicates that starch is no longer present in the medium (Gautam et al., 2010).

6) Screening of Cellulase Activity: Cellulase-producing bacteria and fungi were inoculated by the streak plate method using Carboxy methyl cellulose agar media. The plates were incubated at 37 degree centigrade for 24 hours for bacteria and 3-4 days for fungi at 37 °C. The plates were flooded with 0.1% of Congo red solution for 15min and washed with 1M NaCl, and the zone of hydrolysis was recorded. The zone of hydrolysis shows positive test (Indumathi 2017).

7) Preparation of microbial consortia: Take three test tubes containing 5ml nutrient broth and inoculate loopful of pure culture of selected microbes separately and incubate for 37degree centigrade for 24 hours. Transfer 3 ml (1-1-1) of 24 hour grown nutrient broth in 27 ml freshly prepared broth that is equal to 30 ml. Again incubate the 30ml broth for 24 hr and store for further use (Ingale et al., 2014).

8) Preparation of experimental test samples (N, BC, ABC, FC, AFC) and enzymatic activity assayed calorimetrically.

9) Estimation of Amylase activity :(DNSA 3,5Dinitro-salicylic acid method).

Procedure:

- 1% starch solution was prepared for performing the test.
- 1ml of 1% starch solution and different dilutions of extract of enzymes along with 1ml of citrate phosphate buffer was incubated for 30mins at 50 °C
- 2 ml of DNS solution was added to stop the reaction.
- Tubes were kept for boiling in water bath for 10 minutes.
- Absorbance was recorded at 540nm against glucose standard.

The amount of enzyme, which releases 1 micromole of reducing sugar as glucose per minute is called as one unit of enzyme activity.

10) Estimation of Cellulase activity:

Procedure:

- 1% of carboxy methyl cellulose was prepared
- 0.45 ml of the above was taken as a substrate and incubated with 0.05 ml of enzyme extract
- 0.05 ml of Citrate Phosphate buffer was added to it and incubated at 55 C for 15 minutes.
- 0.5 ml DNS was added to stop the reaction and kept in boiling water bath for 5 min
- Absorbance was recorded at 540nm against standard glucose.

11) Assay of total protein by Folin-Lowry method

Table 1. Determination of Physical Parameters of Test Samples

Test	SampleRate Of Degradation In gm/ Moisture Content In gm/day			
	7 th	14 th	21 st	28 th
1) N	80/3.2 gm	60/ 5.6 gm	28/6.7 gm	18/7 gm
2) BC	96/3.8 gm	58/ 6.1 gm	41/4.5 gm	32/ 3.3 gm
3)ABC	100/4 gm	93/ 3.7 gm	53/5.8 gm	47/ 4 gm
4) FC	93/3.7 gm	85/ 3.4 gm	72/2.8 gm	41/ 1.6 gm
5)AFC	100/4 gm	86/ 3.4 gm	66/ 2.6gm	46/ 1.8 gm

Procedure: Into a series of test tubes pipette out 0.2 ml to 1ml working standard solution (concentration between 20 to 100 ug . Makeup unknown sample solution to 50ml distilled water and pipette out 0.5 ml into a test tube. Add NaOH to all tubes to make up volume uniformly to 1ml. Pipette out 1ml NaOH into a separate tube to

serve as blank. To all tubes add 5ml ml alkaline copper reagent. Mix well and incubate at room temperature for 10 minutes. Into all the tubes pipette out 0.5 ml of Folin's - Ciocalteau Reagent, mix well reaction contents and incubate at temperature 37 °C of for 30 minutes Measure absorbance of color blue formed calorimetrically

at 750 nm. The intensity of color is directly proportional to amount of protein present in sample. Draw a standard calibration curve by plotting the concentration of protein on X - axis and the absorbance on Y-axis. Calculate the concentration of unknown protein from the graph.

Figure: Rate Of Degradation Within 28 Days Of Incubation Period

12) Assay of Free Reducing Sugars by 3, 5 -Dinitrosalicylic Acid Method. Same as amylase activity assay.

13) Estimation of Total an Antioxidant Capacity by Phosphomolybdenum Method (Janarthanan et al., 2014).

Procedure: The tubes containing 0.2 ml of extract (100-1000 ug/ml) is mixed with, 1.8 ml of distilled water, 2 ml of phosphomolybdenum reagent solution. Incubate it at 95°C for 90 minutes. The mixture is cooled to room temperature and the absorbance is measured at 695 nm against a reagent blank. The antioxidant capacity is expressed as Ascorbic Acid Equivalent (AAE).

Table 2. Screening Of Enzyme Activity

Enzyme Activity	Substrate	Name Of Microbe s	Observations	Results
Amylase Activity	Starch Agar	Escherichia coli	Clear Zone Of Inhibition Was Not Observed After Addition Of Iodine Solution	Negative
		Bacillus subtilis	Clear Zone Of Inhibition Was Observed After Addition Of Iodine Solution	Positive
		Aspergillus niger	Clear Zone Of Inhibition Was Not Observed After Addition Of Iodine Solution	Negative
		Trichoderma viride	Clear Zone Of Inhibition Was Not Observed After Addition Of Iodine Solution	Negative
Cellulase Activity	Carboxy methyl Cellulose [CMC]	Escherichia coli	Clear Zone Of Inhibition Was Observed After Addition Of Congo Red Dye Solution	Positive
		Bacillus subtilis	Clear Zone Of Inhibition Was Observed After Addition Of Congo Red Dye Solution	Positive
		Aspergillus niger	Clear Zone Of Inhibition Was Observed After Addition Of Congo Red Dye Solution	Positive

Experimental Results

Results and Discussion:

1. Estimation of Amylase activity

2. Estimation of Cellulase activity

Table 3. Standard Glucose concentration curve

Test Tube	Standard Solution Of Glucose (ug/ml)	Optical Density (At 540 Nm)
1	0	0.00
2	200	0.13
3	400	0.26
4	600	0.40
5	800	0.51
6	1000	0.64

Figure: 1: Standard Glucose Concentration Curve

Table 4. Amylase Activity Chart

Test Sample	Rate Of Degradation In gm/ Moisture Content In gm/day			
	7 th	14 th	21 st	28 th
N	0.19	0.21	0.16	0.14
BC	0.10	0.14	0.02	0.01
ABC	0.04	0.07	0.13	0.10
FC	0.09	0.08	0.07	0.12
AFC	0.02	0.04	0.06	0.08

Figure 2: Amylase activity graph

Table 5. Standard Glucose concentration curve

Test Tube	Standard Solution Of Glucose (ug/ml)	Optical Density (At 540 Nm)
1	500	0.01
2	2500	0.015
3	5000	0.03
4	7500	0.045
5	10,000	0.06

RESULTS AND DISCUSSION

Enzymes are large biological molecules responsible for the thousands of metabolic processes that sustain life. They are important product obtained for human needs from several sources are generally used in industries. An enzyme remains a major cost factor for the lignocellulose fuels and chemical industries. Enzymatic screening carried out on specific selective medium of microbes. *Bacillus subtilis* gives clear zone of inhibition after addition of iodine solution on starch agar i.e. it shows positive amylase activity. The positive cellulase activity screened on Carboxymethyl cellulose agar. Clear zone around growth after addition of Congo red dye solution on CMC agar given by *E. coli*, *B. subtilis* and *A. niger*. The experimental sample N shows highest degradation rate at high acidic condition and moisture content after 28th day. A colour changes from light green to Blue green at pH 8.5 and the volume reduces to 18 gm. with 7 gm. moisture content (Krishna et al., 2016).

Figure: 3: Standard Glucose Concentration Curve

3. Estimation of Protein Concentration

4. Estimation of Reducing Sugar Concentration high

5. Antioxidant Activity

Table 6. Cellulase Activity Chart

Test Sample	Rate Of Degradation In gm/ Moisture Content In gm/day			
	7 th	14 th	21 st	28 th
Normal	0.02	0.03	0.04	0.01
BC	0.06	0.05	0.04	0.02
ABC	0.04	0.06	0.03	0.02
FC	0.03	0.02	0.01	0.01
AFC	0.01	0.02	0.03	0.02

Figure 4: Cellulase activity graph

Table 7. Standard Glucose concentration curve

Test Tube	Standard Solution Of Glucose (ug/ml)	Optical Density (At 750 Nm)
1	200	0.13
2	400	0.20
3	600	0.40
4	800	0.53
5	1000	0.70

Figure 5

Table 8. Cellulase Activity Chart

Test Sample	Rate Of Degradation In gm/ Moisture Content In gm/day			
	7 th	14 th	21 st	28 th
N	0.31	0.17	0.11	0.07
BC	0.01	0.28	0.08	0.05
ABC	0.18	0.17	0.15	0.11
FC	0.01	0.13	0.18	0.26
AFC	0.16	0.08	0.04	0.01

Various assay carried out to measure the amount of enzymes and other nutrients present in selected test samples by taking standard. After incubation period, it was observed that Amylase concentration was increases

after 14th day of incubation. In test sample N, The maximum amylase production was observed on 14th day of incubation which is 330 ug/ ml .BC shows maximum amylase concentration of 220 ug/ml within 14 days of incubation. But in case of FC it was just 200 ug/ml after

21days of incubation. ABC and AFC possess 200 ug/ml after 21th day and 130 ug/ml after 28th day of incubation respectively (Laufenberg et al., 2003).

Figure 6

Table 9. Standard Glucose concentration curve

Test Tube	Standard Solution Of Glucose (ug/ml)	Optical Density (At 520 Nm)
1	200	0.13
2	400	0.20
3	600	0.40
4	800	0.53
5	1000	0.70

The substrate Carboxy methyl cellulose required for cellulase production. The maximum cellulase production observed after 21th day of incubation in N and AFC, which is 6700 ug/ml and 5000 ug/ml respectively. Both BC and FC shows maximum cellulase production is 10000 ug/ml after 7th day. ABC shows substrate utilization is 5000 ug/ml after 21th day of incubation. The maximum protein Concentration observed in N test sample is 460 ug/ml after 7th day of incubation. 420 ug/ml concentration observed in test sample BC after 14th day and 270 ug/ml in test sample ABC after 7th day of incubation. Test sample FC and AFC Shows maximum protein concentration are 390 ug/ml after 28th day and 240 ug/ml after 7th day of incubation period.

Figure 7

Table 10. Cellulase Activity Chart

Test Sample	Rate Of Degradation In gm/ Moisture Content In gm/day			
	7 th	14 th	21 st	28 th
N	0.13	0.07	0.05	0.01
BC	0.17	0.13	0.05	0.04
ABC	0.09	0.04	0.03	0.01
FC	0.13	0.08	0.03	0.02
AFC	0.05	0.03	0.02	0.01

Figure 8

Table 11. Standard Glucose concentration curve

Test Tube	Standard Solution Of Glucose (ug/ml)	Optical Density (At 700 Nm)
1	100	0.10
2	300	0.27
3	500	0.45
4	700	0.70
5	1000	0.90

Figure 9

Table 12. Cellulase Activity Chart

Test Sample	Optical Density at 700 nm [days]			
	7 th	14 th	21 st	28 th
N	0.34	0.53	0.57	0.60
BC	0.64	0.41	0.32	0.31
ABC	0.79	0.83	0.51	0.40
FC	0.52	0.41	0.26	0.24
AFC	0.72	0.33	0.26	0.22

Figure 10

The high reducing sugar concentration observed in test sample N, BC and ABC are 1800 ug/ml, 2350 ug/ml and 1250 ug/ml after 7th day of incubation period. The test sample FC and ABC shows maximum glucose concentration are 1800 ug/ml and 700 ug/ml after 7 days of incubation period. Antioxidant activity of test sample estimated calorimetrically at 695 nm, it shows maximum antioxidant production after 7th day of incubation from BC, FC, AFC are 710 ug/ml, 570 ug/ml, 800 ug/ml respectively. The maximum antioxidant production by Control and ABC shows 660 ug/ml after 28th day and 930 ug/ml after 14th day of incubation. The present study concluded that Microbial conversation of vegetables waste to bio fertilizer is a feasible and potential technology in the future to maintain the natural resources and to reduce the impact on environmental quality. It is a simple biotechnological process of composting in which certain species of bacteria are used

to enhance the process of waste conversion and produce a better end product (Mussatto et al., 2011; Novinsak et al., 2011; Pothiraj & Eyini 2007; Panda et al., 2016; Steger et al., 2017).

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