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Development of Microbial Inoculum to Enhance the Degradation of Organic Waste

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Abstract:
Organic waste has become a critical issue with rising population, urbanization and economic activities in Sri Lanka. There are number of methods that are used for organic waste management majoring organic composting. However, the main problem of this process is that it needs much time to degrade naturally. Therefore, this study is focused on the development of microbial consortium to enhance the degradation of organic waste. This study found out the effective method to reduce the time taken for degradation of 5kg of organic waste within 30 days by using Pseudomonas sp., Bacillus sp. and Aspergillus sp. which are isolated from the Kaduwela garbage site, Sri Lanka. These microorganisms were selected based on their extracellular enzyme activity by an enzyme assay and identified by using biochemical and morphological identification methods. There were two types of treatments that were used as individual and combination of the above-mentioned microorganisms in three different concentrations such as Mcfarland 1, 3 and 5 of bacterial isolates and spore suspension of Aspergillus sp. which equals to $3x10^8$spores /ml, $9x10^8$spores/ml and $1.2x10^9$spores/ml. Each of these concentrations was applied individually and 3 combined concentrations as low (T1), moderate (T2) and high (T3) of above mentioned microorganisms. The efficiency of degradation was analyzed by comparing C/N ratio, mean of degradation using One-way-Analysis of Variance (ANOVA) and degradation percentage. According to the results, the highest concentration of combined consortium (T3), showed the lowest C/N ratio as 15:1 and the highest mean of degradation as 66.67%. With regard to the use of One-way ANOVA for combination, the mean value of control and T1 (P = 0.004), control and T2 (P = 0.001), control and T3 (P = 0.000) results obtained revealed that there were significantly different, confirming that highest concentration of combination of these three microorganisms was the best consortium to degrade organic waste efficiently.

Keywords*: Organic waste, microbial consortium, gravimetric analysis, C/N ratio*

1. Introduction

Municipal solid waste (MSW) comprises household waste, construction and demolition debris, sanitary residue and waste from the street. It is revealed that with increasing urbanization, change in lifestyles and un-thoughtful habits of people showing that there is marked increase in the amount of municipal solid waste generated. There are a number of strategies available for the treatment and management of waste, including minimizing the rate of production, reusing and recycling some of the materials, energy recovery, correct disposal methods and composting. With unplanned expansion in urbanization, pollution has created a negative impact on land, in air and on open water bodies[12]. The waste is thrown on the roadsides and then collected by the workers of the municipalities is ultimately put into low lands popularly known as cheaper ways of landfills[13]. This leads to loss of potentially valuable materials that can be processed as fertilizer and fuel[1]. The bulk of organic manure comprises mainly carbohydrates, proteins, volatile acids and fatty acids which are easily biodegradable[13]. The biological treatment of solid waste appears costly despite its negative impact on environment seems to be less harmless and it introduces a sustainable process[7]. This process of microbial treatment of waste is known as microbial composting for organic matter. Microbial composting is an autonomous, aerobic solid phase biodegradative process of organic materials under controlled conditions[13]. The shortening of composting period with considerable reduction in the C/N ratio is one of the options for making the composting more productive[11]. Another difficulty in the composting technology is the assessment of maturity

in compost. Many parameters to evaluate the maturity of compost from food wastes or city refuse, such as the change of physico-chemical properties[9]calorimetric and spectroscopic methods[3] and enzymatic activity[15]have been identified. The water soluble organic-C/total organic nitrogen ratio is considered to be a suitable parameter for assessing compost maturity[6].

The enzymes released by the microorganisms during composting effectuating the breakdown of several organic compounds characterized by a complex structure, finally leading to the solubilisation of simple water soluble compounds[2]. Various identified hydrolytic enzymes having the ability to control the rate of various substrates are degraded. Important enzymes involved in the composting process include: cellulases, which depolymerise cellulose, B-glucosidases which hydrolyse glucosides, and urease involved in N-mineralization, phosphatases and arylsulphatase that remove phosphate and sulphate groups from organic compounds[10].The ccharacterizing and quantifying enzymatic activities during composting could reflect the dynamics of the composting process in terms of the decomposition of organic matter and nitrogen transformations, and may provide information about the maturity of composted products[14]. The matured compost product can be applied into lands without any adverse environmental effect due to destruction of pathogenic and thermophilic microorganisms within maturation stage.

In view of the above, present study aims to develop a microbial consortium to enhance the degradation of organic waste within 30 days by facilitating microbial dynamics in the composting process.

2. Material and Methodology

2.1. Sample Collection

Municipal solid waste and soil from three different stages of composting were collected from Kaduwela Municipal Council, Kaduwela and Homagama home garden compost bins. Waste materials were brought to the laboratory in safe containers. After initial screening, the waste materials were air-dried for isolation of abundant microorganisms in the three different composting stages.

2.2. Isolation of Microorganisms

Abundant microorganisms in organic waste were selected by preparing dilution series and plate count. Isolated microorganisms were observed in all the spread plates and abundant microorganisms were selected by observing the highest dilutions among other dilutions. Four way streak and quadrant method was used to separate the bacterial cells and to grow them into isolated colonies. These streak methods were repeated until the pure culture was obtained.

2.3. Extracellular Enzyme Activity of Selected Microorganisms

The enzymes involved mainly serving a hydrolytic function as extracellular or exo-enzymes. Therefore the degrading ability of selected microorganisms was tested using starch hydrolysis test, casein hydrolysis test, lipid hydrolysis test and cellulose hydrolysis test respectively.

2.4. Selection of Efficient Microorganisms for Organic Waste Degradation

Two bacterial isolates and one abundant fungal species were selected based on their ability to produce hydrolyzing enzymes and extracellular enzyme activity.

2.5. Morphological Characterization and Identification of Isolated Microorganisms

Bacterial isolates and fungal isolate from Kaduwela and Homagama sites were stained and observed under the light microscope in order to observe the shape, morphological features and the gram reaction of the bacterial isolates.

2.6. Biochemical Identification for Selected Bacterial Isolates

As a tool in identification of an unknown bacterial species, scientists generally investigate their biochemical characteristics such as ability to ferment various sugars. Usually this is associated with the breakdown of sugars into organic acid and a gas. This can be detected by growing bacteria in a broth culture containing a particular sugar and a pH indicator and by having an inverted Durham tube to trap any gases evolved. Hence, glucose fermentation test, lactose fermentation test and casein hydrolysis test were followed for further identification of selected bacterial isolates.

2.7. Experimental Set Up

Rapid composting experiments were carried out in specially designed composters of 5 Kg capacity and two trials were performed with 9 of replicates from each treatment. The composters were designed in such a way as to provide sufficient air supply to the decomposing material. The bottom of the composter had a 3mm diameter holes to remove the leachate generated during composting. Two kilograms of one day old municipal solid waste and 0.1 kg of saw dust were put into composters with addition of both bacterial and fungal species separately and as a combination in three different concentrations such as Mcfarland 1, 3 and 5 of bacterial isolates (HU2 and HB2) and spore suspension of selected fungal species (Asp) equals to $3x10^8$spores /ml, $9x10^8$spores/ml and $1.2x10^9$spores/ml. Each of these concentrations was applied

individually and the three combined concentrations were designated as low (T1), moderate (T2) and high (T3) of the selected three microorganisms.

2.8. Testing of Organic Waste Degradation

After obtaining the pure cultures of organic waste degrading bacterial and fungal isolates, organic waste degradation efficiency was examined for both the individual isolates as well as for a selected bacterial and fungal combination using the gravimetric analysis. To certify the gravimetric analysis, carbon content, nitrogen content and carbon to nitrogen ratio was calculated as a compost maturity analysis.

Analysis was performed using One-way Analysis of Variance. Samples were drawn from combination experiments and were analyzed for both the physico-chemical parameters. The chemical analyses of the samples were performed on air-dried (29-30 ^{o}C for 4 days) samples. The organic carbon content of the compost was estimated using Walkey and Black method (Smith and Weldon, 1982) and the total nitrogen applying modified Kjeldahl method (Faithfull, N.T., 2002). The microbial (bacteria and fungi) biomass was assessed by counting the colony forming units.

3. Results and Discussion

3.1. Isolation of Microorganisms for Organic Waste Degradation

The number of bacterial species per milliliter in the original compost suspension (initial stage, thermophilic stage and maturation stage) was calculated.

Stage of composting	No. of bacteria c.f.u/ml wet compost	No. of fungal count/ml
Initial stage	1.156x 10^8c.f.u./ml	1.83x 10^3/ml
Thermophilic stage	1.962x 10^8c.f.u./ ml	6.53x 10^3/ml
Maturation stage	1.000x 10^8 c.f.u./ml	6.80x 10^3/ml

Table 1:Changes in the bacterial and fungal density in three stages of composting process

High amount of bacterial population was observed in thermophilic stage and high amount of fungal population was observed in maturation stage apropos of their activity.

Colombo municipal solid waste and the soil from three different regions of composting were collected from each of the site of Kaduwela Municipal Council, Kaduwela and the home garden compost bin. The distribution of microorganisms differs with the different environmental conditions such as moisture, aeration, temperature, etc. and the degree of contamination. Thus sampling was carried out by selecting three different sites. After initial screening, the samples from three layers of composting were air dried and counted the total number of bacterial and fungal isolates according to the three stages (initial, thermophilic and maturation stages) of composting.

The isolated microorganisms were observed in every spread plate and the microorganisms were selected by observing their abundance in the highest dilution. Presence of microorganisms in abundance is indicative of their active involvement in the decaying process of organic waste[13]. Then the bacterial isolates were selected based on the abundance.

These selected bacterial species were further studied to elucidate their ability to degrade high molecular weight macromolecules such as polysaccharides, lipids and proteins.

3.2. Determination of Extracellular Enzyme Activities of Isolated Microorganisms

Isolated abundant microorganisms were inoculated as a streak on skim milk agar, Tributyrin agar, starch agar and Cellulose Congo Red agar media to determine the degradation efficiency for casein, lipids, starch and cellulose respectively.

Bacterial isolate code	Proteases	Carbohydrases	Lipases	Cellulases
BK1	-	++	-	+
BK2	-	+++	+	++
BK3	-	++	+	+
BK4	+++	+	+	+
HU1	++	+++	++	+
HU2	++	+++	+	+++
HM3	+++	-	-	+++
HM4	+++	+	-	+++
HB1	+++	++	-	++
HB2	+++	+++	-	+++

Table 2: Summary of the results obtained from extracellular enzyme activity of bacterial isolates

Distance from the colony to the outer boundary of clear zone (-) not detected, (+) 0.5 cm, (++) 1.0 cm, (+++) 2.0 cm.

Based on the results of 3.2, HU2 and HB2 bacterial isolates were selected for further analysis of organic waste degradation

Since, these high molecular weight macromolecules are the main constituents of organic waste, theses polysaccharides, lipids and proteins are digested into low molecular weight molecules by microbes before they are transported into the bacterial cell and subsequently metabolized. Microbes secrete several hydrolytic extracellular or exoenzymes into the medium in order to digest these macromolecules during degradation of organic waste[9]. Biochemical tests for protease, carbohydrase, lipase and cellulase activity of isolated microbes were carried out. The bacterial isolates, HU 2 and HB 2 were selected based on their extracellular enzyme activity efficiency and one fungal isolate (Asp) was selected based on its abundance during composting process. The isolated bacteria HU2 showed positive results for protease, carbohydrase, lipase and cellulase while bacterial isolate HB2 gave positive results for protease, carbohydrase andcelluloses activity.

3.3. Identification of Selected Microorganisms

Gram staining and negative staining were carried out for isolated two bacterial strains and the results obtained are presented in Table 3. Morphology and microscopic characterization of isolated fungal strains are present in Table 4.

Bacterial code	Gram reaction	Morphology	Endospore	Oxidase	Glucose fermentation	Lactose fermentation	Casein hydrolysis	Tentative organism
HU2	Positive	Rod	Present	-ve	+ve, no gas	+ve	-ve	*Bacillus* spp.
HB2	Negative	Rod	Absent	+ve	-ve	-ve	+ve	*Pseudomonas* spp.

Table 3: Biochemical and physiochemical characterization of bacterial isolates
**Identification based on the Bergey's manual of determinative bacteriology*

Isolate	Form	Elevation	Margin	Filament color	Conidiophore	Conidia shape	Surface	Tentative Fungus
Asp	Filamentous	Raised	Curved	Light brown	Large vesicle	Round	Smooth	*Aspergillus* spp.

Table 4: Morphological and microscopic characterization of isolated fungi
**Identification based on simplified fungi identification key, University of Georgia*

After obtaining the pure cultures of organic waste degrading bacterial (HU2 and HB2) and fungal isolate (Asp), organic waste degradation efficiency was examined for both the individual isolates and for a selected bacterial and fungal combination was done using gravimetric analysis.

3.4. Analysis of Physiochemical Properties

Treatment	C content	N content	C/N ratio
Initial	1110.7	38.3	29:1
T1	950.8	36.4	26:1
T2	420.6	28.3	15:1
T3	400.8	25.4	15:1
Control	958.8	28.8	33:1

Table 5: Changes in the C/N ratio during normal (control) and treatments

Samples were drawn from combination experiments and were analyzed for both physico-chemical parameters. The chemical analyses of the samples were performed on air-dried (29-30 ^{o}C for 4 days) samples.

The carbon content, the total nitrogen content and the carbon to nitrogen ratio were calculated as a compost maturity index. As an experimental set up, organic waste was mixed with saw dust evenly to facilitate the aerobic condition and to adjust the moisture content throughout the composting process. Aeration is the most important factor in composting systems[4]. Aeration helps in maintaining compost temperature and it facilitates the thermophilic decomposition of organic wastes.

Within 30 days, the C/N ratio at initial stage of 29:1 was reduced to 15:1 in treatments, 2 and treatment 3 due to the high efficiency in carbon and nitrogen decomposition. However, C/N ratio of 33:1 in the control does not showed a better decomposition of carbon and nitrogen content in organic waste due to the absence of increased amount of bacterial and fungal population. The C/N ratio below 20 means, that it indicates acceptable compost maturity[11]. It has been reported that during efficient composting, the C/N ratio is expected to decrease because of degradation of organic matter and mineralization[8]. Therefore, [5] stated that the C/N ratio cannot be used as the absolute indicator of compost maturity, since the values for well-composted materials show high variability, due to the characteristics of the waste used.

3.5. Analysis of Organic Waste Degradation

Analysis of the data obtained in the experiment was carried out using the SPSS 16 software. One-way ANOVA was performed to test whether there is a significant difference between the mean reductions of organic waste amount which was inoculated with individual isolates and to test whether, there is a significant difference between the mean reduction of organic waste amount with different combinations of bacterial and fungal suspensions.

3.5.1. Testing of organic waste degradation by individual isolates

The reduction of organic waste amount and % of degradation of residual waste was calculated. The values of mean reduction of organic waste and percentage of organic waste degradation by individual isolates are summarized in Table 5.

Isolate	Mean reduction of weight in organic waste ± SEM	Mean % of organic waste degradation
Control	356.67±8.82^{a}	35.33
B1	390.00±11.54^{*b}	39.33
B2	430.00±5.77^{*c}	42.50
B3	470.00±15.27^{*d}	46.83
U1	376.67±6.67^{*aeg}	37.00
U2	430.00±20.00^{*cf}	42.83
U3	480.00±11.54^{*d}	47.83
A1	363.33±18.56^{*beg}	38.33
A2	420.00±20.00^{*cf}	42.50
A3	430.00±20.00^{*cf}	43.83

Table 6: Degradation of organic waste by individual isolates

Note: Mean values that are indicated with an asterisk mark (*) are significantly different from the control and the mean values that share a letter are not significantly different from each other at 0.05 significant level.

*B indicates HB2 bacterial isolate *U indicates HU2 bacterial isolate *A indicates Asp fungal isolate
** 1, 2 and 3 indicate the increasing concentration of each isolate

Organic waste degradation in individual isolates was significantly different from different from the control at 0.05 significant level (P = 0.000) except U1 and control. However, each of these concentrations was not significantly different from each other. [11] also demonstrated that the highest decomposition activity in terms of weight loss and volume loss was shown by suspension of dump site sample which consisted of *Bacillus* sp. combination. Therefore, the bacterial and fungal combination was used to examine the biodegradation of organic waste under different densities of bacterial and fungal spore suspensions in the second step.

3.5.2. Testing of organic waste degradation by the bacterial and fungal combination under different densities

The values of mean reduction of organic waste and percentage organic waste degradation by the bacterial and fungal combination under different densities are summarized in Table 6.

Treatment	Mean reduction of weight in organic waste ± SEM	Mean % of organic waste degradation
Control	453.33±27.28	45.33
T1	560.00±25.16^{*a}	56.00
T2	640.00±11.54^{*b}	64.00
T3	666.67±29.02^{*b}	66.67

Table 7: Degradation of organic waste by bacterial and fungal combination

Note: Mean values that are indicated with an asterisk mark (*) are significantly different from the control and the mean values that share a letter are not significantly different from each other at 0.05 significant level.

Graphical representation of the reduction of organic waste by individual isolates, bacterial and fungal combination and the mean percentage of degradation of organic waste, under different concentrations of microorganism suspensions are shown in figure 3.1 and 3.2 respectively.

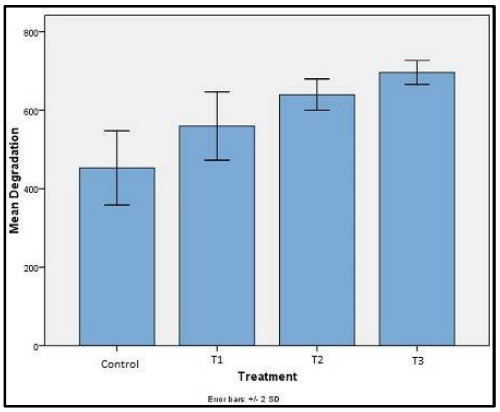

Figure 1: Mean weight loss of organic waste treated with different combination of inocula

It was observed that the mean reduction in weight loss was increased when the densities of microorganisms increase. The highest mean reduction in weight was observed in the treatment 3 and the lowest mean reduction in weight was observed in the samples of control (figure 1).

As in the second experiment, the highest mean reduction in weight was observed in the samples which were inoculated with highest concentration of bacterial or fungal isolate individually (figure 3.2).

When considering the bacterial and fungal combination, bacteria use up a variety of enzymes to break down organic material by oxidizing it and providing them with the resources for their growth and reproduction. A bi-product of the oxidation process is the fact that the heat generates the ideal conditions for more thermophilic microorganisms and efficiency of composting process[9]. The decomposition rates and pathogen reduction in composting are increased steadily as temperature rise to thermophilic temperatures. Therefore organic waste degrading bacterial sp. combination would result better performance in composting. Although the fungi are effective at breaking down tough debris that the bacteria unable to continue the decomposition process. Hence both the bacterial and fungal combinations result in the highest decomposition rates by reducing the time taken for composting[3].

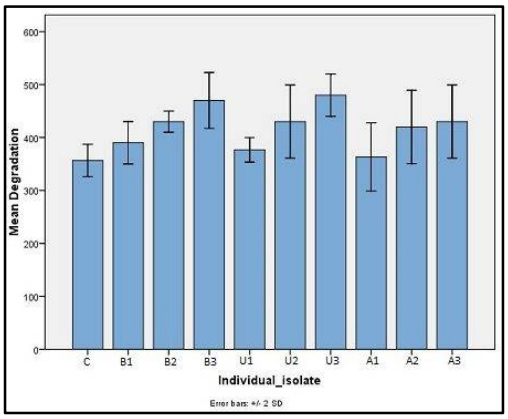

Figure 2: Mean weight loss of organic waste treated with different isolates with different densities.B3, U3 and A3 is the highest concentration of HB2, HU2 and Asp respectively

According to the results obtained in one-way ANOVA test for bacterial and fungal combination with different concentrations, the mean values for control and T1 (P = 0.004), control and T2 (P = 0.000), control and T3 (P = 0.000), T1 and T2 (P= 0.026) and T1 and T3 (P = 0.012) are significantly different respectively. Therefore, increasing the concentration of bacterial and fungal concentration has an effect on the degradation. But, there was no effect on degradation when increased bacterial and fungal concentration from three fold-level to four-fold level due to the fact that, there is no significant difference between the mean reduction in T2 and T3 (P = 0.730). Ego, it can be concluded that the organic waste degradation has been facilitated by the addition of increasing amount of bacterial and fungal concentrations and other internal and external factors.

According to the results obtained, the bacterial and fungal combination (T3) which consisted of four fold increased concentration of HB2, HU2 and Asp (McFarland 5 and $1.2x10^9$ spores/ml) was identified as the best combination of bacterial and fungal concentration with the highest mean reduction and the mean percentage of degradation of organic waste.

The bacterial isolates in the combination were identified by biochemical tests of each bacterial isolate. The biodegradation efficiency of the same bacteria has been reported by several authors.[16] have reported that *Pseudomonas* sp. could degrade 48.15% of municipal waste and also demonstrated a 65.12% of organic waste degraded using *Bacillus* sp.

isolated form dump site. Therefore, in this study organic waste degradation was examined by using a bacterial and fungal consortium including *Bacillus* sp., *Pseudomonas* sp. and *Aspergillus* sp. respectively in the second experiment.

4. Conclusion

In this study, two bacterial isolates and one fungal isolate were used to analyze the organic waste degradation by measuring the weight loss, the carbon content, the nitrogen content and C/N ratio. Organic waste degradation was tested by using both bacterial and fungal species separately and as a combination. The combination including highest densities showed the lowest carbon content, the nitrogen content, the C/N ratio and the highest weight loss of organic waste. The identified bacteria were *Bacillus* sp. and *Pseudomanas* sp. by observing the morphological characteristics and analyzing the results of biochemical reactions. The fungal species was identified as *Aspergillus* sp. by observing the morphological characteristics.
The combination of *Bacillus* sp., *Pseudomanas* sp. and fungus *Aspergillus* sp. could be used as efficient organic waste degraders.

5. Nomenclature

- cfu - Colony Forming Unit
- ºC - Degree of Celsius
- ml - milliliter

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