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Biodegradation of organic solid waste by Actinomycetes consortium enriched with blackstrap molasses

R. Veeramani and K.K. Kavitha*

Department of Environmental and Herbal Science, Faculty of Science,

Tamil University, Thanjavur – 613010

ABSTRACT

Each household produces a significant amount of waste in the form of solid waste and sludge, which are improperly disposed. Management of solid waste is very big menace and challenge for the present situation. Hence this study was aimed to mitigate the organic portion MSW by using biological methods. Municipal Solid Waste (MSW) of Punnainallur (East) and Sreenivasapuram were taken up for the present study. A composite soil samples were collected from both study area. Physicochemical analysis of composite soil samples were performed, the results revealed that there is a huge nutrient content in Punnainallur (East) site. Actinomycetes species present in the soil samples were counted and isolated. There are 81 (3.24×10^{-6}) pale yellow colored actinomycetes colonies were enumerated at 10^{-6} dilution. The morphological and biochemical characterization of the isolates proved that the following actinomycetes species *Actinobispora sp*., *Agromyces sp*., *Actinosynnema sp*., and *Microtetrospora sp* were found in the tested soil samples. Predominant actinomycetes species were selected to prepare consortium and that consortium was activated by black strap molasses. Selected Actinomycetes Consortium (SAC) enriched with molasses were applied on the processed organic solid waste at different quantities (100 ml, 150 ml, 200 ml & 250 ml). After 30 days the biodegraded products were harvested and subjected to physicochemical and phytotoxic studies. The results revealed that solid organic waste biodegraded with 200 ml of SAC solution efficiently degraded organic waste and produce compost and which could not be toxic to plant and soil.

Keywords **–** Municipal Solid Waste, biodegradation, Selective Actinomycetes Consortium (SAC)

1. INTRODUCTION

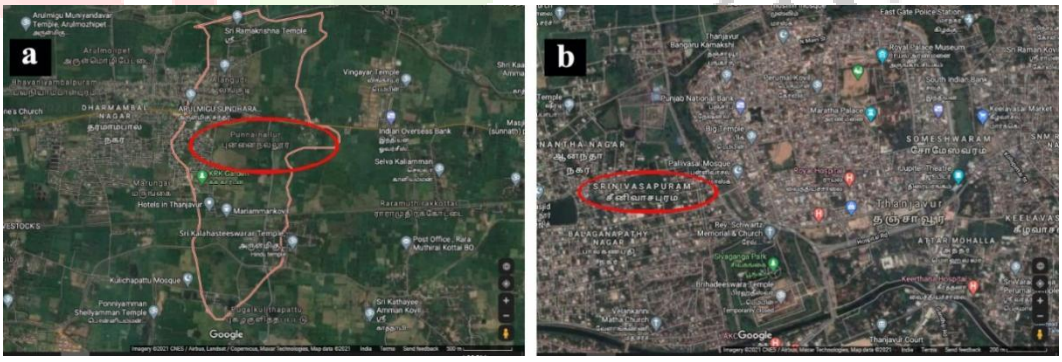
Municipal solid waste (MSW) is a very big menace to the present and future situation. Due to ever growing population the production of domestic waste per capita is high. Anthropogenic activities are the main reason for MSW generation in world wide. The developed countries adopted stringent methodologies to mitigate and manage the MSW. But poor and under developed countries face lot of issues to manage the MSW. Hence adopting low cost technologies should be helpful for reducing solid waste in economically weaken countries. The majority of urban solid wastes in developing countries have high levels of food waste and moisture content (Visvanathan, et al., 2004).The most common problems associated with improper management of solid waste include disease transmission, odour nuisance, atmospheric, land & water pollution, fire hazards, aesthetical nuisance & economic losses (Nethaji and Pynthamil, 2015). Hence managing the municipal solid waste in proper way is inevitable one. In view of that present study focused to find out the alternate solution to reduce the organic portion of the municipal solid waste.

2. MATERIALS AND METHODS

Study area

Thanjavur is a city in the south Indian state of Tamil Nadu with the population of 2.91 lakhs (as per 2011 census) people. Thanjavur city is spread over an area of 128 km² with the elevation of 88 m. The geographical coordinate of Thanjavur is 10.786999 latitude and the 79.137825 longitude. Municipal Solid Waste generated in Thanjavur city was dumped in different locations. The important locations where the MSW stored are Punnainallur (East) and Sreenivasapuram. These three places where considered as the hot spot for storing MSW in Thanjavur city (Figures 1a & b).

Figures 1 a & b: Satellite view of soil sampling sites Punnainallur (East) and Sreenivasapuram



Site selection

The sites were selected for soil sampling was determined as per the regulations prescribed by Ackerson et al., (2017). Moreover, the sampling locations were identified in consultation with municipal authorities responsible for the operation of the site to obtain a representative sample. The identified sites for the present study is MSW dumping areas and they were highly polluted with organic, inorganic and inert waste materials. The soil collected from MSW dumping area may contain the native microorganisms which effectively degrade the organic waste.

Soil sampling

Sampling for the present study was done in Wednesday, 24th January 2019. Sites were chosen with an emphasis on nearly-level, well-drained surfaces and, where possible, native vegetation. Each soil was sampled to a depth of 8 inches. Soil samples are collected by traveling in a zig-zag pattern collecting soils at each locations. Composite samples are a mixture of individual samples generally collected from multiple locations and mixed together to form a single composite sample. We may reduce the effects of soil heterogeneity by averaging soil properties over wider areas by integrating several sub samples into a single composite sample.

Processing of soil samples for analysis

Soil processing is experiment-dependent. The collected composite soil samples are subjected to air dry for several days. After removing plastic wastes, inert materials, rocks and plant debris, the dried sample is then crushed with a mortar and pestle and passed through an American Society for Testing and Materials (ASTM), where 2mm standard sieve is used to remove gravel. Soils that are processed by air drying and sieving can be stored at room temperature in a polythene bags.

Physicochemical analysis of soil samples

Soil is a very complex medium, and the physicochemical characteristics of a soil can give clear idea about soil profile. There are five main soil properties were analyzed in this study such as soil color, texture, moisture content, pH and Electrical Conductivity (EC). The important chemical characteristics like Organic Carbon (OC), Total Nitrogen, Available Potassium, Available Phosphorus, Exchangeable Calcium, Available Iron, Manganese, Zinc, Copper, Available Boron and Calcium carbonate also studied in this work. All the experiments were conducted as per the standardized procedures prescribed by Pansu, M., & Gautheyrou, J. (2006).

Enumeration and isolation of actinomycetes

Pretreatment

All soil Samples had been mixed with calcium carbonate & pretreated for 2-5days at 37°C. 1gm soil mixed with 0.1g Calcium carbonate & incubates at 37°C for 2-5 days. This pretreatment enhances the population of *Streptomyces* spp., in soil sample (Williams and Cross 1971).

Isolation of Actinomycetes

Isolation of soil Actinomycetes were performed by serial dilution and spread plate technique using isolation media and nutrient agar medium. 1g of soil sample was taken in 9 ml of distilled water and mixed properly. Serial dilution was made up 10^{-3} ml of the dilution sample was inoculates in the isolation medium plates from each dilution. The media are added to the tetracycline and ampicillin to inhibit microbial contamination respectively. Plates were incubated at both at 28°C and 37°C and monitored after 2-7 days. Streaking on isolation media plates led to purify actinomycetes colonies (Williams and Cross 1971).

Actinomycetes colony counting

To detect and measure the number of colonies created, the plate was carefully examined under the digital colony counter. The number of colonies was calculated according to dilution ratio and defined as the number of colony forming units (CFU) per milliliter (Williams and Cross 1971).

$$\text{CFU/ml} = \frac{\text{Number of colonies x dilution factor } (10^{-3} = 1000)}{\text{Volume of culture plate (25 µl convert into ml = 0.025 ml (25/1000))}}$$

Morphological identification

Isolates of soil microbes were observe under a high power magnifying lens and colony morphology was noted with respect to color, aerial mycelium, size and nature of colony, slide color and felling the consistency with a sterile loop (Williams and Cross 1971).

Microscopic observation

Morphological examination of the soil microbes was done by using cellophane tape and cover slip buried methods (Williams and Cross 1971). Gram staining, Lactophenol blue staining was performed to check the morphology of the cells and spore chain morphology was identified by cover slip culture technique.

Identification of actinomycetes strains

The identification of soil actinomycetes strain was carried out based on morphological characteristics and biochemical tests (Holt et al., 1994).

Development of Selective Actinomycetes Consortium (SAC)

The isolation was followed by identification and screening of actinomycetes species. The effective isolates of various actinomycetes species were mixed in different proportions to prepare actinomycetes consortia. The compatibility of the actinomycetes strains within the consortium was checked regularly using gram staining technique.

Activation of SAC

The consortia were activated within seven days by the supplementary material blackstrap molasses (Figure 2). The mixtures were prepared in the proportions of 6 liters pure water, 3 liter molasses and 1litre of SAC properly. Then, the mixtures were then poured into a clean plastic containers and sealed airtight, so that little air is left in the container. The containers were kept under shade at an ambient temperature of 24–26°C for 7-10 days. Afterwards a white layer on the top of the solution accompanied with a sweetish sour, rather pleasant smell was observed which a characteristic feature of the efficient SAC.

Figure 2: Black strap molasses

Preparation of municipal waste for decomposition

Municipal wastes were collected from primary municipal deposit points around Tanjore and all the secondary ingredients which included wood pieces, polythene, plastics, etc., were removed. Only organic portion of the municipal wastes was considered as a final substrate for treatment which was designated as "Municipal organic waste."

Ex situ biodegradation of Municipal organic waste

The segregated municipal organic waste subjected to aerobic ex situ biodegradation in which organic wastes are biodegraded inside the composting pin. There are two plastic pins of uniform size were taken and it was named as T and C.

Municipal organic waste treated with SAC solution supplemented with molasses

5 kg of municipal organic waste was placed inside the 6 different pins. Subsequently 100 ml, 150 ml, 200 ml and 250 ml of SAC solution supplemented with blackstrap molasses was sprayed over the waste in the pins. The municipal organic wastes in the 6^{th} pin considered as a control, hence it was not treated with SAC solutions. Then, pins were covered with mosquito net to avoid flies attack and prevent maggot's formation. The wastes in the pins are mixed properly once in a day and SAC solutions were sprayed twice in a week. The final products were extracted after 30 days of biodegradation and examined further.

Analysis of biodegraded product

The harvested biodegraded products from pins were subjected to physico chemical analysis as per the method prescribed by Tandon (1993).

Phyto toxic study

The phytotoxicity of biodegraded organic waste was determined using a seed germination assay. The 2 g of biodegraded municipal organic waste was diluted in 30% deionized water for this experiment. Then, the extract (with 85% moisture content) was kept it for 2 hours without any disturbance. There after the extract was centrifuged at 6000 rpm for 15 minutes and the supernatant was filtered with Whatman filter paper. 3 ml of filtered extract was poured in the five petri plates and 10 viable VRI 2 ground nut seeds (Parentage: Spanish

bunch JL 24 x CO_2) were placed on the each petri plates. Now the seeds were incubated in room temperature for 72 hours (Cesaro, Belgiorno and Guida, 2015; Luo et al., 2018). After the incubation the germinated seeds were identified and the percentage of germination was calculated by using below formula:

$$\text{Germination \%} = \frac{\text{Number of Seeds Germinated}}{\text{Total Number of Seeds}} \text{ X } 100$$

3. RESULTS AND DISCUSSION

In the present work, the soil sample was collected from Municipal Solid Waste dump sites at two different locations namely Punnainallur (East) and Sreenivasapuram MSW disposal sites. The collected MSW soil samples were designated as site-A and site-B. The collected soil samples were processed and subjected to physico-chemical analysis. The soil analysis results obtained was correlated with the findings of Saritha *et al.*, (2014) and Uma, Prem Sudha & Murali (2016). Moreover the current study proved that the soil collected from Punnainallur MSW disposal site (site-A) contain higher nutrient factors than Sreenivasapuram MSW disposal site (site-B) (Table 1).

Table 1: Physical and chemical characteristics of two different soil samples

S. No.	Parameters	Site - A	Site - B
1	pH	7.2 ± 0.07	6.5 ± 0.05
2	EC (ms/cm)	0.797 ± 0.0005	0.794 ± 0.0008
3	CEC (meq/ds^{-1})	13.57 ± 0.005	13.42 ± 0.003
4	Nitrate (ppm)	4.8 ± 0.08	4.4 ± 0.05
5	Phosphorous B1 (ppm)	144.4 ± 0.05	143.7 ± 0.05
6	Phosphorous B2 (ppm)	192 ± 0.47	193 ± 0.47
7	Potassium (Exchang) (ppm)	32 ± 0.48	29 ± 0.47
8	Calcium (Exchang) (ppm)	173.5 ± 0.47	172.7 ± 0.47
9	Magnesium (Exchang) (ppm)	306 ± 0.42	301 ± 0.47
10	Sulphur (Available) (ppm)	123.1 ± 0.06	122.5 ± 0.05
11	Sodium (Exchang) (ppm)	483 ± 0.41	481 ± 0.46
12	Zinc (Available) (ppm)	0.7 ± 0.05	0.6 ± 0.08
13	Manganese (Available) (ppm)	7.03 ± 0.005	6.8 ± 0.005
14	Iron (Available) (ppm)	18.87 ± 0.004	18.77 ± 0.005
15	Copper (Available) (ppm)	1.04 ± 0.005	1.01 ± 0.004
16	Boron (Available) (ppm)	0.2 ± 0.05	0.1 ± 0.04
17	Organic Matter (OM) (%)	3.16 ± 0.008	3.14 ± 0.005

Each value represents mean ± SEM of 3 samples

The processed soil samples were serially diluted and cultured in the laboratory to identify the dominant actinomycetes and this study results revealed that there are 126 white colored colonies (5.04 x 10^6 CFU/ml) enumerated in site-A soil sample where as there are 81 pale yellow colored colonies (3.24 x 10^6 CFU/ml) enumerated in site-B soil sample (Table 2).

Table 2: Microbial population dynamics of two different soil samples

S. No	Sample	Number of colonies	Colony color	Results (CFU/ml)
1	Site - A	126	White	5.04 x 10^6
2	Site - B	81	Pale yellow	3.24 x 10^6

Actinomycetes strains present in the colonies were identified through morphological and biochemical characterization. The results revealed that the actinomycetes present in the both soil samples are *Actinobispora sp*., *Agromyces sp*., *Actinosynnema sp*., and *Microtetrospora sp* (Table 3) and these findings correlated with the findings of Faith Efosa Oviasogie, Christopher Uche Ajuzie & Uyiosa Glory Ighodaro (2010) and Saha & Santra (2014).

Table 3: Biochemical characterization of Actinomycetes isolates

S. No	Identified Actinomycetes strain
A	*Actinobispora* sp.
	Agromyces sp.
B	*Agromyces* sp.
	Actinosynnema sp.
	Microtetrospora sp

The potent actinomycetes consortium was prepared from the actinomycetes isolates by the method prescribed by Jeya Bharathi *et al.*, (2017). There after actinomycetes consortium is activated by using blackstrap molasses. Blackstrap molasses can act as a carbon source for actinomycetes growth. A week after bio-activation of actinomycetes consortium. Selective Actinomycetes Consortium (SAC) solution was applied on the municipal organic waste at different quantity (100, 150, 200 and 250 ml). After 30 days biodegradation the end products were harvested and subjected to physico chemical analysis. The result explain that the municipal organic waste biodegraded with SAC solution activated with blackstrap molasses showed high nutrient value when compare to control (Tables 4).

Table 4: Physical and chemical characteristics of Municipal Organic Waste biodegraded by different concentrations of SAC solution supplemented with blackstrap molasses

S. No.	Parameters	Control	SAC solution supplemented with molasses (after 30 days)			
			100 ml	150 ml	200 ml	250 ml
1	pH	6.1 ± 0.05	7.1 ± 0.05	7.3 ± 0.05	7.5 ± 0.05	8.3 ± 0.05
2	EC (mS/cm)	3.11 ± 0.008	4.15 ± 0.008	4.25 ± 0.008	4.37 ± 0.005	5.39 ± 0.009
3	Nitrogen (%)	1.3 ± 0.05	2.5 ± 0.05	2.6 ± 0.05	2.7 ± 0.05	3.0 ± 0.05
4	Phosphorous (%)	1.45 ± 0.009	1.65 ± 0.009	1.74 ± 0.008	1.81 ± 0.009	2.0 ± 0.05
5	Potassium (%)	0.42 ± 0.008	0.69 ± 0.008	0.77 ± 0.009	0.80 ± 0.05	1.2 ± 0.09
6	Carbon (%)	54 ± 0.27	65 ± 0.27	76 ± 0.27	88 ± 0.27	93 ± 00
7	C:N ratio	21:1	24:1	27:1	30:1	31:1

Each value represents mean ± SEM of 3 samples

The maturity and toxicity of the biodegraded products were determined through seed germination assay. The seed germination assay results showed that the municipal organic waste biodegraded with 200 ml of SAC solution with blackstrap molasses exhibit higher maturity rate without phytotoxic (Table 5).

Table 5: Results of seed germination assay (VRI 2 ground nut seeds) for evaluating the phyto toxicity of biodegraded municipal organic waste

S. No.	No. of seeds	Test	Quantity of SAC solution				Control (C)
			100 ml	150 ml	200 ml	250 ml	
1	10	T1	70 ± 0.4	80 ± 0.4	90 ± 0.4	70 ± 0.4	80 ± 0.4
2	10	T2					
3	10	T3					
4	10	T4					

Each value represents mean ± SEM of 3 samples

This findings were similar with the findings of the researchers Kalaivani, Amiya Kumar & Shanthi (2011) and Premalatha *et al.*, (2017). The results of the current study showed that the municipal organic solid waste biodegraded with a selective actinomycetes consortium activated with blackstrap molasses (200 ml) could make potent and nutritive compost product and which may be used as a bio compost.

4. CONCLUSION

This study is an eco-friendly approach to the organic solid waste pollution abatement. The methods adopted in this study is cost effective and provide better solution to the pollution problem. Biodegradation organic solid waste produce large quantities of nutrition rich compost product and it may generate revenue also create job opportunity for economically weaken people. In a nutshell this study may mitigate the pollution in one hand and generate revenue in other hand.

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