



Effective Microbial Technology: A low cost option for treatment and reuse of organic wastes

• **Ayushi Sinha** • **Soumya Shree**
• **Sonal Suman**

Received : November 2014

Accepted : March 2015

Corresponding Author : Sonal Suman

Abstract : *Effective Microorganisms Technology refers to the use of beneficial microorganisms used to degrade the organic wastes. This increases the yield of crops and improves the efficiency of the soil. Different concentrations of jaggery, 2%, 6%, 10% were prepared and inoculated with four different microbes isolates (*Actinomycetes*, *Aspergillus sp.*, *Lactobacilli* and *Yeast*) each of them having a particular pH, 7.9, 6.5, 7 and were maintained at particular temperatures, 37°C, 26°C and room temperature respectively for a week. The best growth of these Effective Microorganisms was observed at a temperature of 26°C and at pH value of 6.5 in a medium containing 6% jaggery solution. Based on the results obtained from solid waste treatment, it was concluded that the use of EM*

technology has desirable effect by significant increase in organic matter, organic carbon, ammonia and phosphate content of waste in first week but there was decrease in organic matter and organic carbon in second week. The chloride, ammonia and phosphorous content of waste increased in the second week of incubation.

Keywords: *Effective Microorganisms, organic matter, waste, Estimation.*

Ayushi Sinha

B.Sc. III year, Industrial Microbiology (Hons.),
Session : 2012-2015, Patna Women's College,
Patna University, Patna, Bihar, India

Soumya Shree

B.Sc. III year, Industrial Microbiology (Hons.),
Session : 2012-2015, Patna Women's College,
Patna University, Patna, Bihar, India

Sonal Suman

Guest Faculty, Deptt. of Industrial Microbiology,
Patna Women's College, Bailey Road,
Patna – 800 001, Bihar, India.
E-mail : sonal.micro89@gmail.com

Introduction:

Effective Microorganism (EM) is the consortium of beneficial and naturally occurring microorganisms which are not chemically synthesized or genetically modified. The EM technology was developed by Professor Dr. Teruo Higa at University of Ryukus, Okinawa, Japan in 1970s. The EM solution is the blending of effective microorganisms in molasses at low pH. Initially EM was developed to increase the crop yield by enhancing the soil activity (Higa and Parr, 1994). The use of these effective microorganisms leads to an increase in the nutrient content of the soil such as increase in nitrogen, potassium, organic carbon,

organic matter and a slight increase in chloride content. The EM secretes organic acids and enzymes which acts on sewage and degrades complex organic matter into simpler ones. The antioxidant substances produced by EM enhances the breakdown of solids and reduces the sludge volume (Higa and Chinen, 1998). The EM used in this study comprises of *Lactobacillus*, *Aspergillus*, *Saccharomyces* and *Actinomyces*. The lactic acid bacteria enhance the breakdown of organic matter such as lignin and cellulose. Yeast produces antimicrobial substances and their metabolites are used as substrate for lactic acid bacteria and *Actinomyces*. The bioactive substance produced by yeast promotes plant growth. *Aspergillus* decomposes organic matter rapidly and produces alcohol, esters and antimicrobial substances. *Actinomyces* produce antimicrobial substances from amino acids derived from organic matter for suppressing harmful fungi and bacteria. Therefore, enhancing the chemical properties of organic waste that can also be used as biofertilisers and to develop low cost and eco-friendly waste treatment process using effective microbial consortia.

Materials and Methods:

Isolation of Effective Microorganisms : Four different strains of microorganisms were isolated from different sources on selective media. *Lactobacillus* was isolated from curd on Rogosa media. *Aspergillus* and *Actinomyces* were isolated from moist rhizospheric soil on Czapeks Dox Agar and Starch Casein Agar media, respectively. Yeast was isolated from yeast granules on Potato Dextrose Agar media. The plates were incubated at 37°C for 24 hours (*Lactobacillus*), 26°C for 48 hours (*Aspergillus* and Yeast) and 26°C for 5 days (*Actinomyces*). The growth obtained for different isolates were revived after every week (Aneja, 2013).

Preparation of EM solution: Different concentrations of jaggery i.e. 2%, 6%, 10% are prepared each having pH, 7.9, 6.5, 7 and were maintained at temperatures, 37°C, 26°C and room temperature respectively for a week. The prepared jaggery solutions were inoculated with isolated microorganisms and were incubated at 37°C, 26°C and room temperature for a week. The product formed was known as EM solution (Monica et al., 2011).

Preparation of waste bags and its inoculation with EM solution: Four bags of equal dimensions were filled with equal amounts of organic waste. The bags were inoculated with different concentrations of EM solutions and were sealed properly. The whole process and incubation was performed at room temperature. The incubation of these inoculated waste bags was allowed for two weeks (Monica et al. 2011).

Waste application: 500 gms of soil was autoclaved at 121.5°C for 15 minutes. This sterilized soil was transferred into four small sterilized beakers in equal amounts. These beakers were then inoculated with equal amounts of different EM inoculated wastes. Moong seeds were allowed to be grown at proper moisture and light availability. A control was set which had only soil and was devoid of fermented wastes. The seeds were allowed to grow, after 3 days they were uprooted and there lengths were noted.

Physical analysis: Analysis of the EM inoculated waste was done for weight, pH and temperature at 0hr (initial) and for 2nd week of incubation of the waste samples.

Chemical analysis:

Estimation of Organic matter and Organic carbon : The estimation was done as reported by Black (1954). The organic carbon was calculated as follows:

$$\text{Organic carbon content of sample (in \%)} = \frac{10 \times (B - T) \times 0.003 \times 100}{B \times W}$$

Where;

T = volume of ferrous ammonium sulphate solution used for sample titration (ml)

B = volume of ferrous ammonium sulphate solution used for blank titration (ml)

W = weight of the sample

Formula for calculation of organic matter:

Organic matter % = Organic carbon X 1.724

Where 1.724 is Van Bemelan factor.

Estimation of Ammonia : Ammonia was estimated following the method of Hetrick and Whitney, (1986).

Estimation of Phosphorus : The spectrophotometric determination of Inorganic Phosphate in waste was carried out by Molybdenum method (Kale and Anthappan, 2012).

Estimation of Chlorine : The determination of chloride was done by titrating the sample with silver nitrate, following the standard protocol given by W.H.O, (1989).

Results and Discussion:

Different biochemical tests were performed on isolated *Lactobacillus*, *Yeast*, and *Actinomycetes*. The overall results are presented in Table 1.

Table 1. Overall biochemical tests for different isolates

| S.No | Microorganisms | Biochemical tests | Results |
|------|----------------------|---|---|
| 1. | <i>Lactobacillus</i> | Casein hydrolysis Fermentation test a) Sucrose b) Lactose c) Glucose | Positive Slightly positive Positive Positive |
| 2. | <i>Saccharomyces</i> | Amylase production Fermentation test a) Sucrose b) Lactose c) Glucose | Positive Positive Positive Slightly positive |
| 3. | <i>Actinomycetes</i> | Citrate utilization Catalase test | Positive Positive |

Physical analysis of solid waste : The EM inoculated wastes were analyzed at 0 hr and 2nd week for their weight, pH, and temperature. Following were the results obtained (Tables 2 and 3).

Table 2. Wet weight of the initial (without EM solution) sample

| S. No | Samples | Total weight | Weight of paper | Weight of sample |
|-------|--|--------------|-----------------|------------------|
| 1. | Dry leaves (mango and Guava) | 50g | 5g | 45g |
| 2. | Kitchen waste (onion, potato, banana peels; and some leftover of green vegetables) | 100g | 5g | 95g |
| 3. | Wheat and rice bran | 80g | 5g | 75g |

Table 3. Dry weight of the initial (without EM solution) sample

| S. No | Samples | Total weight | Weight of paper | Weight of sample |
|-------|--|--------------|-----------------|------------------|
| 1. | Dry leaves (mango and Guava) | 30g | 5g | 25g |
| 2. | Kitchen waste (onion, potato, banana peels; and some leftover of green vegetables) | 70g | 5g | 65g |
| 3. | Wheat and rice bran | 50g | 5g | 45g |

- Weight of the bag prepared = 215.5g
- Weight of the plastic bag (Initial) = 500mg

pH- The waste sample was at pH 7, i.e., at neutral pH.

Observations of solid waste after 2 weeks of incubation are as follows:

Weight - The weight of the sample decreased as shown in Table 4. The microbes acted on waste and degraded them to simpler compounds.

Table 4. Weight of the sample bags after two weeks

| Samples | 2% | 6% | 10% | C |
|---------|------|------|------|------|
| Weight | 196g | 180g | 192g | 205g |

- Weight of the plastic bag (Initial) = 500mg

pH- The pH of the waste sample was varying from the initial because of the action of microorganism on the waste samples as shown in Table 5. There was a high change in pH of concentration 6%. The pH was decreased from 7 to 2-3 by fermenting molasses which was in correlation with the work conducted by Monica et al. 2011. The pH from this point onwards was constant as the organism utilized the entire energy source and there was no further growth of organism. The pH of 10% and 2% increased from 7 to 8-9 (Table 5), which indicates the growth of microorganisms that could survive in alkaline condition, which mainly consisted of bacteria.

Table 5. pH of the sample bags after two weeks

| Samples | 2% | 6% | 10% | C |
|---------|-----|-----|-----|-------|
| pH | 7-8 | 2-3 | 8-9 | 7-7.5 |

Chemical analysis of solid waste

Analysis of the EM inoculated waste was done for organic matter, organic carbon, chloride, phosphate and ammonia. The analysis was done for 0hr, 1st week and 2nd week incubation of the waste samples. Following observations were obtained (Fig. 1-5).

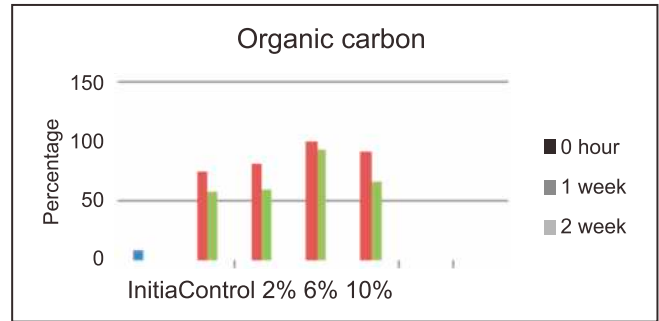


Fig 1. Graph showing percentage of organic carbon in waste

The organic carbon content increased in the first week of incubation and decreased in the second week of incubation but in both cases 6% EM inoculated waste showed maximum value (99.6 and 92.04) as compared to control (75.44 and 58.83).

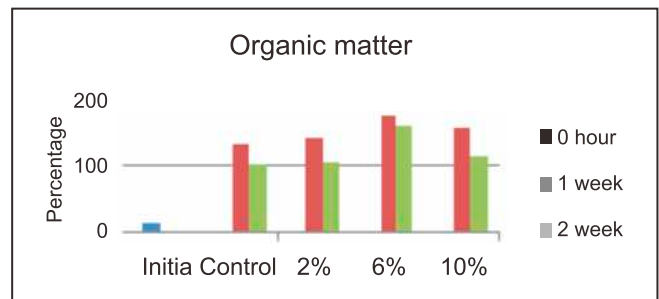


Fig 2. Graph showing percentage of organic matter in waste

The organic matter content increased in the first week of incubation and decreased in the second week of incubation but in both cases 6% EM inoculated waste showed maximum value (171.7 and 158.67) as compared to control (130.06 and 101.42).

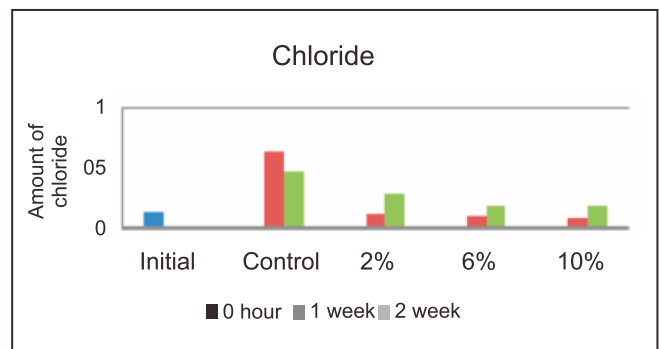


Fig 3. Graph showing amount of chloride in waste

There was a considerable increase in the chloride content of all the EM inoculated organic wastes. In both the weeks 6% EM inoculated waste showed considerable growth in content (0.0994 and 0.3479) as compared to control (0.6319 and 0.4615) which is slightly higher.

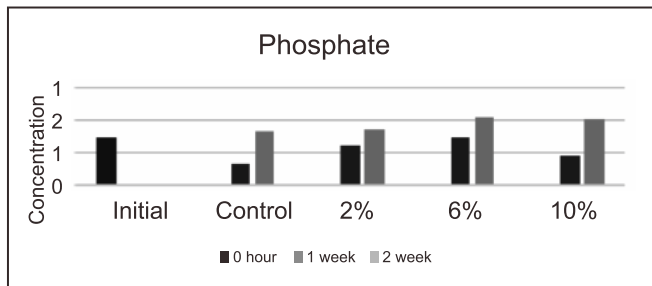


Fig 4. Graph showing concentration of phosphate in waste

There was a considerable increase in the phosphate content of all the EM inoculated organic wastes. In both the weeks 6% EM inoculated waste showed maximum value (1.40 and 2.10) as compared to control (0.60 and 1.60).

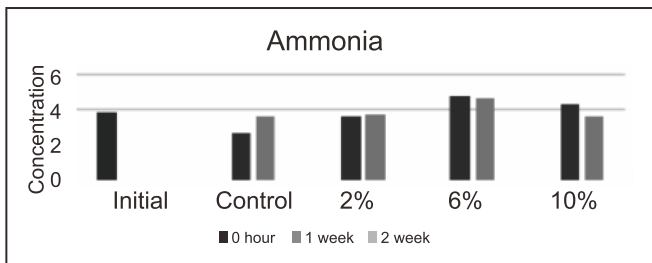


Fig 5. Graph showing concentration of ammonia in waste

There was a considerable increase in the phosphate content of all the EM inoculated organic wastes. In both the weeks 6% EM inoculated waste showed maximum value (4.70 and 4.80) as compared to control (2.70 and 3.60).

The results of the present study further confirmed that solid waste samples, organic matter, ammonia, phosphate and chloride content were significantly increased as reported by previous researcher (Higa and Parr, 1994; Higa 1995).

Based on the results obtained from solid waste treatment, it was concluded that the use of EM technology has desirable effect by causing significant increase in organic matter, organic carbon, ammonia and phosphate content of waste in first week but there was decrease in organic matter and organic carbon in second week. The chloride content of waste increased in the second week of incubation. There was a slight increase in ammonia and phosphorus in second week of incubation relative to the first week. The results obtained for phosphate, organic carbon and organic matter were in co-relation with the work conducted by Kale and Anthappan, (2012).

The grown seeds were uprooted and their length was noted as given in Table 6. According to the observation the moong seeds planted in the container consisting of the soil mixed with 6% EM waste showed the maximum plant length as also reported by Higa and Parr, (1994).

Table 6. Measured length of moong seedlings for each sample

| S.No. | Samples | Length of seedlings |
|-------|---------|---------------------|
| 1. | 2% | 6.1 |
| 2. | 6% | 10.7 |
| 3. | 10% | 9.5 |
| 4. | C | 5.3 |

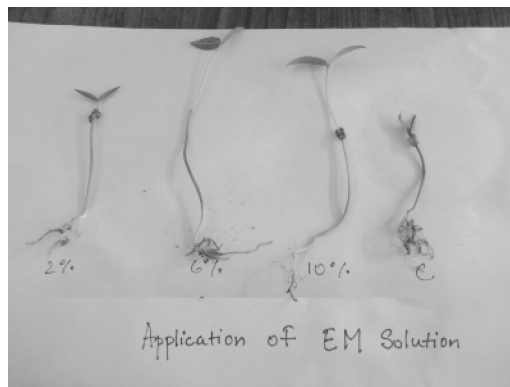


Fig 6. Moong seedlings showing maximum growth in 6% EM inoculated waste and minimum growth in control

In Fig 6, it was observed that the seedling growth was maximum in soil inoculated with 6% EM waste which had the maximum content of organic carbon, phosphate and ammonia which is suitable for the growth of seedlings. The uninoculated soil does support the growth of moong seedlings but addition of waste comprising of EM solution enhances the growth rate and soil fertility. (Chaudhary and Iqubal, 2006).

Acknowledgement :

We extend our gratitude to the former Principal, Dr. Sister Doris D'Souza A.C. and Prof. Sheila Bedi, Head, Dept. of Botany and Coordinator, Dept. of Industrial Microbiology, Patna Women's College for their constant support. We are thankful to Mr. Vijay, our laboratory staff, for his help throughout this project.

References:

Aneja K.R. (2013). *Experiments in Microbiology, Plant Pathology and Biotechnology* Fourth revised edition. New age international limited.

Black C.A. (1954). *Soil plant relationship* (Monograph) Iowa State University Press, Iowa State College, Ames. *John Wiley and Sons, Inc., New York, NY*.

Chaudhary M.S. and M. Iqubal. (2006). *Soil Fertility Improvement with EM for Vegetable Crops*. EM Database. EM Technology Network, Inc.

Hetrick J.H and Whitney R.M. (1986). *Determination of Nitrogen in milk by Direct Nesslerization of the digested sample*. American Dairy Science Association, Dean Milk Company, *Rockford, Illinois*. pp 111-122.

Higa T, Parr JF (1994). Beneficial and Effective Microorganisms for a Sustainable Agriculture and Environment. *International Nature Farming Research Centre, Atami, Japan*. pp16.

Higa, T. (1995). *Effective microorganisms: Their role in Kyusei Nature Farming and sustainable agriculture*. In J.F. Parr, S.B. Hornick, and M.E. Simpson (ed.) *Proceedings of the Third International Conference on Kyusei Nature Farming*. U.S. Department of Agriculture, Washington, D.C., USA.

Higa T, Chinen N. (1998). *EM Treatments of Odor, Waste Water and Environmental Problems* College of Agriculture University of Ryukyus Okinawa Japan.

Kale D.K. and Anthappan P.D. (2012). *Solid waste management by use of Effective Microorganisms Technology*, *Asian Journal. Experimental Science*, 26(I):5-10.

Monica S., Karthik L., Mythili S. and Sathiavelu A (2011). *Formulation of Effective Microbial Consortia and its Application for Sewage Treatment*. *Journal of Microbial & Biochemical Technology* pp 051-055.

W.H.O., Geneva (1989). *Water Quality Determination of Chloride*. International Organization for Standardization, ISO 9297:1989.