STUDY ON THE BACTERIOLOGICAL EXAMINATIONS OF PREPARED EFFECTIVE MICROORGANISM SOLUTIONS FROM NATURAL WASTES (VEGETABLE WASTES , COW DUNG AND SESAME MEAL CAKE)

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Abstract

In this research, EM (effective microorganism) solutions were prepared from waste of the vegetables by primary fermentation. The cow dung and commercial sesame meal cake were also applied. The preparation of EM solutions were carried out under two different conditions such as condition C1 (vegetable waste) and condition C2 (vegetable waste, cow dung and sesame meal cake) at different pH values. pH values of used solvent / solutions were 6.5 for purified water, 9.5 for slaked lime solution and 2.5 for lemon juice. During the fermentation, biogas was evolved. The volume of evolved gas were measured hourly till to five days. The microorganisms in EM solutions were isolated, cultured and characterized by cultural and microscopic morphology at department of biotechnology, Mandalay Technological University.

Keywords: effective microorganisms, vegetable waste, sesame meal cake, cow dung,

Introduction

Effective Microorganisms (EM) are mixed cultures of beneficial naturally-occurring organisms that can be applied as inoculants to increase the microbial diversity of soil ecosystem. They consist mainly of the photosynthesizing bacteria, lactic acid bacteria, yeasts, actinomycetes and fermenting fungi. These microorganisms are physiologically compatible with one another and can coexist in liquid culture. There is evidence that EM inoculation to the soil can improve the quality of soil, plant growth and yield (Kengo and Hui-lian, 2000).

The use of effective microorganisms in agricultural soil suppress soil-borne pathogens**.** These effective microorganism**s** also increases the decomposition of organic materials and consequently the availability of mineral nutrients and important organic compounds to plants (Singh *et al*., 2003).

In addition, EM enhances the activities of beneficial indigenous microorganisms, for example mycorrhizae which fix atmospheric nitrogen thereby supplementing the use of chemical fertilizer and pesticides. Improvement in soil fertility has significant positive effect on plant growth, flowering, fruit development and ripening in crops (Lévai *et al*., 2006). The concept of effective microorganisms (EM) was developed by Professor Teruo Higa, University of the Ryukyus, Okinawa, Japan (Higa, 1991; Higa and Wididana, 1991a). EM consists of mixed cultures of beneficial and naturally-occurring microorganisms that can be applied as inoculants to increase the microbial diversity of soils and plant. The inoculation of EM cultures to the soil/plant ecosystem can improve soil quality, soil health and the growth yield and quality of crops (Higa and James, 1994).

EM contains selected species of microorganisms including predominant populations of lactic acid bacteria and yeasts and smaller numbers of photosynthetic bacteria, actinomycetes and other types of organisms. All of these are mutually compatible with one another and can coexist in liquid culture (Higa, 1994).

EM is not a substitute for other management practices. It is, however, an added dimension for optimizing our best soil and crop management practices such as crop rotations, use of organic amendments, conservation tillage, crop residue recycling and biocontrol of pests. If used properly, EM can significantly enhance the beneficial effects of these practices (Higa and Wididana, 1991b).

Materials and Methods

Sample Collection

The natural waste materials such as vegetable wastes, cow dung and sesame meal cake were collected for the preparation of effective microorganism solution and production of biogas. Vegetable waste was collected from local market, Chanmyatharsi Township, Mandalay Region. Cow dung was collected from Taung Pyone Village, Madaya Township, Mandalay Region. Sesame meal cake was collected from Local market, Mandalay Region.

Vegetable waste samples were cut into small pieces and washed with water. Cow dung samples were dried under the sunlight. These cow dung samples were pounded and sieved to get the size of powder. Sesame meal cake were ground to get powder sample. Three solvent solutions (purified water (pH 6.5), lemon juice (pH 2.5) and slaked lime solution (pH 9.5) were prepared to add into vegetable waste (Figure 1).

(a) (b) (c) (d) (e)

Figure 1 (a) Vegetable waste, (b) Cow dang powder, (c) Sesame meal cake, (d) Lemon and (e) Slaked lime solution

Preparation of Effective Microorganism Solution and Production of Biogas from Vegetable Waste Only (Control, C)

Effective microorganism solution was prepared by using vegetable waste only. Six kilogram of small pieces of fresh vegetable wastes were put into the anaeroboic digester. The neck of the digester was entwined with teflon. The lid was also tightly sealed with the damp wheat. The gas delivery pipe was also set up as shown in Figure 2. While the preparation of effective microorganism solution, the biogas was evolved. The liberated biogas was collected by downward displacement of water. The amount of biogas produced was recorded 1 h interval till 5 h and also determined daily till 5 days. The prepared digester is shown in Figure 2. After production of biogas for 5 days, the anaerobic digester was tightly sealed kept for one month. After one month, the mixture in the anaerobic digester can be used as EM solution.

Figure 2 The production of biogas

Preparation of Effective Microorganism Solutions and Production of Biogas from Vegetable Waste with Purified Water (C_1W)

Six kilogram of small pieces of fresh vegetable wastes and one liter of purified water were put into the anaerobic digester. The amount of biogas produced was recorded hourly and also recorded daily till 5 days. After production of biogas for 5 days anaerobic digester was tightly sealed and kept for one month. After one month, the mixture in the anaerobic digester can be used as EM solution (C_1W).

Preparation of Effective Microorganism Solutions and Production of Biogas from Vegetable Waste with Lemon Juice (C_1A)

Six kilogram of small pieces of fresh vegetable wastes and one liter of lemon juice (pH 2.5) were put into the anaerobic digester. The amount of biogas produced was recorded hourly and also recorded by daily till 5 days. After production of biogas for 5 days, the anaerobic digester was tightly sealed and kept for one month. After one month, the mixture in the anaerobic digester can be used as EM solution (C_1A).

Preparation of Effective Microorganism Solutions and Production of Biogas from Vegetable Waste with Slaked Lime Solution (C_1B)

Six kilogram of small pieces of fresh vegetable wastes and one liter of slaked lime solution (pH 9.5) were put into the anaerobic digester. The amount of biogas produced was recorded hourly and also recorded daily till 5 days. After production of biogas for 5 days, the anaerobic digester was tightly sealed kept for one month. After one month, the mixture in the anaerobic digester can be used as EM solution (C_1B).

Preparation of Effective Microorganism Solutions and Production of Biogas from Vegetable Waste, Cow Dung and Sesame Meal Cake with Purified Water (C_2W)

Two kilogram of small pieces of fresh vegetable waste, two kilogram of cow dung and two kilogram of sesame meal cake were put into the anaerobic digester by successive layers and one liter of purified water was added into the anaerobic digester. The biogas was evolved and the gas production was checked. The amount of biogas was recorded hourly and also determined daily till 5 days. After production of biogas for 5 days, the anaerobic digester was tightly sealed and kept for one month. After one month, the mixture in the anaerobic digester can be used as EM solution (C_2W).

Preparation of Effective Microorganism Solutions and Production of Biogas from Vegetable Waste, Cow Dung and Sesame Meal Cake with Lemon Juice (C_2A)

Two kilogram of small pieces of fresh vegetable waste, two kilogram of cow dung and two kilogram of sesame meal cake were put into the anaerobic digester by successive layers and one liter of lemon juice was added into the anaerobic digester. The biogas was evolved and the gas production was checked. The amount of biogas was recorded hourly and also determined daily till 5 days. After production of biogas for 5 days, the anaerobic digester was tightly sealed and kept for one month. After one month, the mixture in the anaerobic digester can be used as EM solution (C_2A).

Preparation of Effective Microorganism Solutions and Production of Biogas from Vegetable Waste, Cow Dung and Sesame Meal Cake with Slaked Lime Solution (C_2B)

Two kilogram of small pieces of fresh vegetable waste, two kilogram of cow dung and two kilogram of sesame meal cake were put into the anaerobic digester by successive layers and one liter of slaked lime solution was added into the anaerobic digester. The biogas was evolved and the gas production was checked. The amount of biogas was recorded hourly and also determined daily till 5 days. After production of biogas for 5 days, the anaerobic digester was tightly sealed and kept for one month. After one month, the mixture in the anaerobic digester can be used as EM solution (C_2B).

Isolation and Characterization of Microorganisms

The microorganisms were isolated from the prepared EM solutions and commercial EM solutions were characterized by cultural morphology and microscopic morphology at Department of Biotechnology, Mandalay Technological University.

Results and Discussion

Production of Biogas

While the preparation of effective microorganism solution, the biogas was evolved. The amount of bio gas was determined for hour by hour till five hours. The amount of biogas was also recorded by daily till five days. The results are described in Tables 1 and 2 and Figure 3.

Table 1 Production of Biogas (Hourly)

No.	**Time taken (hour)**	**Volume of collected biogas (mL)**						
		C	**C_1W**	**C_1A**	**C_1B**	**C_2W**	**C_2A**	**C_2B**
1	1	800	1800	1600	1500	1400	1200	2500
2	2	700	400	800	100	600	800	1300
3	3	600	200	400	-	300	500	300
4	4	300	100	300	-	200	100	100
5	5	200	100	100	-		-	-
Total		**2600**	**2600**	**3200**	**1600**	**2500**	**2600**	**4200**

Table 2 Production of Biogas (Daily)

No.	Time taken (day)	Volume of collected biogas (mL)						
		C	C_1W	C_1A	C_1B	C_2W	C_2A	C_2B
1	1	3500	5000	5200	2000	4200	3600	6200
2	2	2600	3200	3500	-	200	1200	1000
3	3	1200	1300	300	2000	-	200	-
4	4	100	700	-	-	-	-	600
5	5	100	700	-	-	-	300	-
Total		**7500**	**10900**	**9000**	**4000**	**4400**	**5300**	**7800**

C = Control = Vegetable Wastes only
C_1W = Vegetable Wastes with Purified Water
C_1A = Vegetable Wastes with Lemon Juice
C_1B = Vegetable Wastes with Slaked Lime Solution
C_2W = Vegetable Wastes, Cow Dung and Sesame Meal Cake with Purified Water
C_2A = Vegetable Wastes, Cow Dung and Sesame Meal Cake with Lemon Juice
C_2B = Vegetable Wastes, Cow Dung and Sesame Meal Cake with Slaked Lime Solution

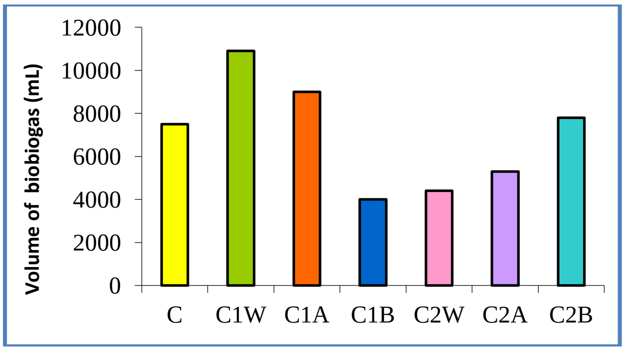


Figure 3 Total volume of biogas (daily) from various conditions

According to this table, the highest amount of biogas was evolved from vegetable waste with purified water.

Isolation and Characterization of Microorganisms in Prepared Effective Microorganism and Commercial Effective Microorganism Solutions

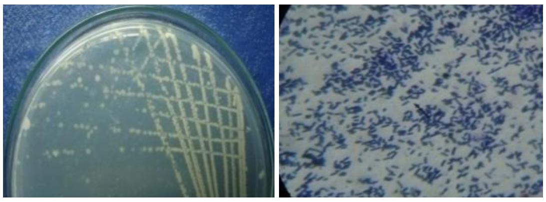
The microorganisms were isolated from prepared effective microorganism solution and commercial effective microorganism solutions were characterized according to cultural and microscopic morphology at Department of Biotechnology, Mandalay Technological University. The results are presented in Tables 3-17 and Figures 4-10.

Table 3 Cultural Morphology of C_1W (Vegetable Waste with Purified Water)

Sample	Cultural Morphology					
	Shape	Color	Opacity	Elevation	Size (mm)	Family
Condition-C_1W (Liquid)	Circle	Pale yellow	Opaque	Convex	2	Bacillaceae
	Circle	Pale yellow	Opaque	Convex	0.2	Bacillaceae
Condition-C_1W (Residue)	Irregular	Yellow	Opaque	Raised	2	Bacillaceae
	Irregular	Cream	Opaque	Flat	4	Bacillaceae
	Circle	Pale yellow	Opaque	Convex	0.5	Bacillaceae
	Circle	Pale yellow	Opaque	Convex	2	Streptomyceae

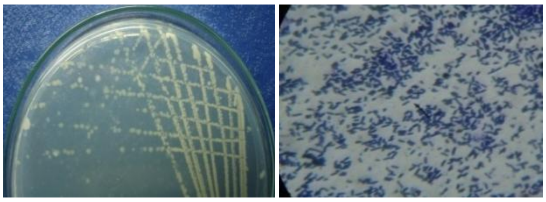
Table 4 Microscopic Morphology of C_1W(Vegetable Waste with Purified Water)

Sample	Microscopic Morphology				
	Shape	Size (μm)	Gram Stain	Spore +/–	Family
Condition-C_1W (Liquid)	Rod	2 × 4	+	+	Bacillaceae
	Rod	1× 2-4	+	-	Bacillaceae
Condition-C_1W (Residue)	Rod	1.5×2.5-2	+	-	Bacillaceae
	Rod	0.5 × 2-3	+	-	Bacillaceae
	Rod	1 × 2-4	+	-	Bacillaceae
	Rod	1.5 × 4-7	+	-	Streptomyceae



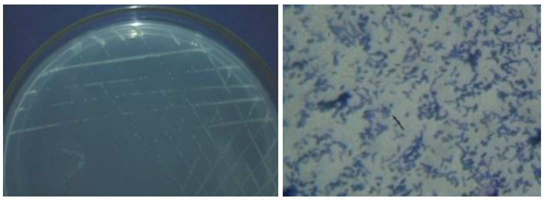
(a) *Bacillaceae* [C_1W liquid)

(b) *Bacillaceae* [C_1W liquid)



(c) *Bacillaceae* [C_1W Residue]

(d) *Bacillaceae* [C_1W Residue]



(e) *Bacillaceae* [C_1W Residue]

(f) *Stretomyceae* [C_1W Residue]

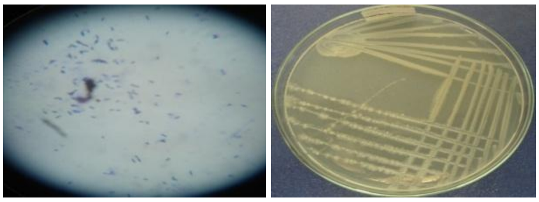
Figure 4 Cultural and microscopic morphology of the C_1W condition (vegetable waste with purified water)

Table 5 Cultural Morphology of Condition –C_1A (Vegetable Waste with Lemon Juice)

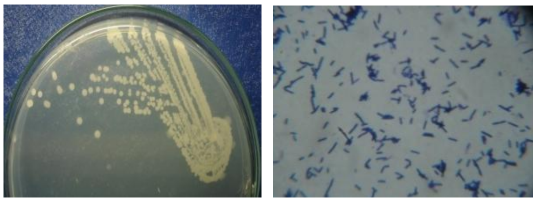
Sample	Cultural Morphology					
	Shape	**Color**	**Opacity**	**Elevation**	**Size (mm)**	**Family**
Condition-C_1A (Liquid)	Filamentous	Creamy	Opaque	Raised	Small	Streptomyceae
Condition-C_1A (Residue)	Circle	Pale	Opaque	Putrinate	2	Streptomyceae
	Circle	Pale	Opaque	Convex	0.5	Streptomyceae
	Irregular	Pale	Opaque	Flat	3	Streptomyceae

Table 6 Microscopic Morphology of C_1A (Vegetable Waste with Lemon Juice)

Sample	Microscopic Morphology				
	Shape	**Size (µm)**	**Gram Stain**	**Spore +/–**	**Family**
Condition-C_1A Liquid	Y - Rod	1 × 1-2	+	–	Streptomyceae
Condition-C_1A Residue	Rod	1.5 × 4-7	+	–	Streptomyceae
	Rod	1 × 2-2.5	+	–	Streptomyceae
	Rod	1 × 2	+	–	Streptomyceae

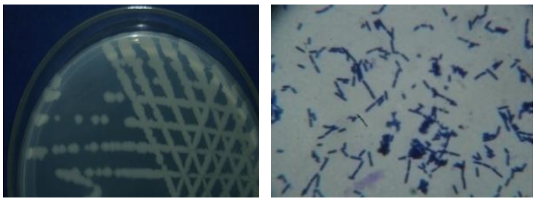


(a) *Stretomyceae* [C_1A, Liquid]



(b) *Stretomyceae* [C_1A, Residue]

(c) *Stretomyceae* [C_1A, Residue]



(d) *Stretomyceae* [C_1A, Residue]

Figure 5 Cultural and microscopic morphology of the C_1A condition (vegetable waste with lemon juice)

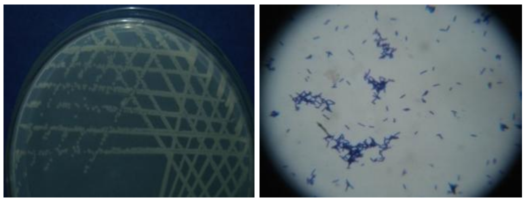
Table 7 Cultural Morphology of C_1B (Vegetable waste with Slaked Lime Solution)

Sample	Cultural Morphology					
	Shape	Color	Opacity	Elevation	Size (mm)	Family
Condition-C_1B Liquid	Irregular	Pale	Opaque	Flat	2	Streptomyceae
	Circle	Pale	Opaque	Convex	0.5-0.7	Bacillaceae
Condition-C_1B Residue	Circle	Pale	Opaque	Convex	0.5	Streptomyceae
	Circle	Pale	Opaque	Convex	0.5	Bacillaceae
	Irregular	Yellow	Opaque	Flat	2.5	Bacillaceae

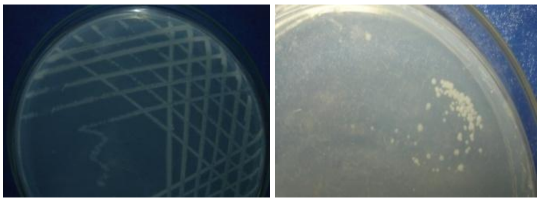
Table 8 Microscopic Morphology of C_1B (Vegetable Waste with Slaked Lime Solution)

Sample	Microscopic Morphology				
	Shape	Size (µm)	Gram Stain	Spore +/–	Family
Condition-C_1B Liquid	Rod	1 × 2	+	+	Streptomyceae
	Rod	2 × 3-5	+	_	Bacillaceae
Condition-C_1B Residue	Rod	1 × 1.5-2	+	_	Streptomyceae
	Rod	0.5 × 2-3	+	_	Bacillaceae
	Rod	1.5 × 4-7	+	_	Bacillaceae

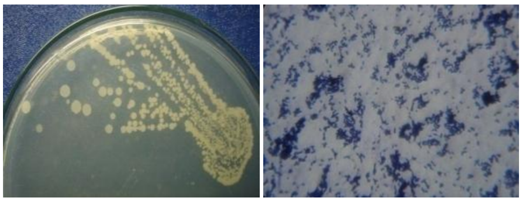
(a) *Strepomyceae* [C_1B, Liquid]



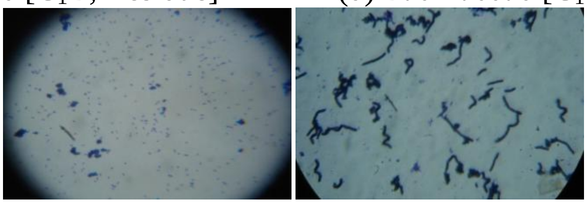
(b) *Bacillaceae* [C_1B, Liquid]



(c) *Strepomyceae* [C_1B, Residue]



(d) *Bacillaceae* [C_1B, Residue]



(e) *Bacillaceae* [C_1B, Residue]

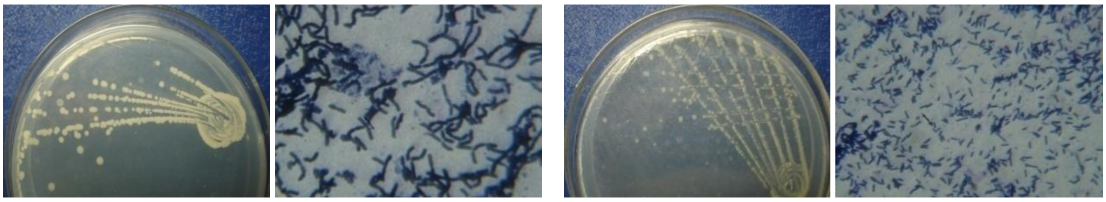
Figure 6 Cultural and microscopic morphology of the C_1 B condition (vegetable waste with slaked line solution)

Table 9 Cultural Morphology of C_2W (Vegetable Waste, Cow Dung, Sesame Meal Cake with Purified Water)

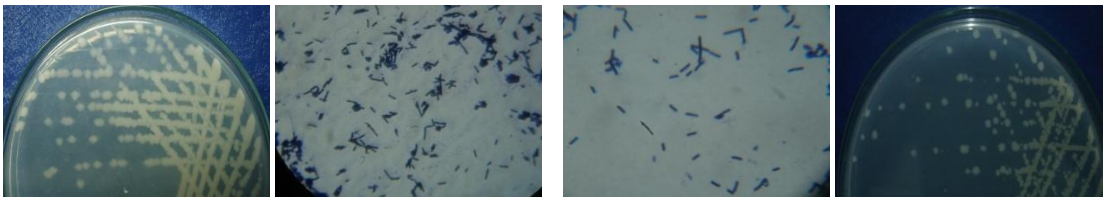
Sample	Cultural Morphology					
	Shape	**Color**	**Opacity**	**Elevation**	**Size (mm)**	**Family**
Condition-C_2W Liquid	Circle	Pale	Opaque	Convex	2.5	Streptomyceae
	Circle	Pale	Opaque	Convex	1	Bacillaceae
	Spindle	Pale	Opaque	Flat	3	Bacillaceae
Condition - C_2W Residue	Irregular	Pale	Opaque	Convex	1.5-2	Streptomyceae
	Circle	Pale	Opaque	Umbonate	2	Streptomyceae

Table 10 Microscopic Morphology of C_2W (Vegetable Waste, Cow Dung, Sesame Meal Cake with Purified Water)

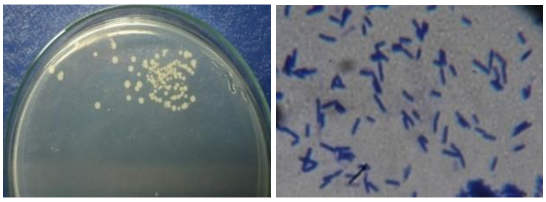
Sample	Microscopic Morphology				
	Shape	**Size (µm)**	**Gram Stain**	**Spore +/–**	**Family**
Condition -C_2W Liquid	Rod	2 × 4-7	+	_	Streptomyceae
	Rod	0.5 × 2-3	+	_	Bacillaceae
	Rod	1 × 2-3	+	_	Bacillaceae
Condition- C_2W Residue	Rod	1 .5× 3-5	+	_	Streptomyceae
	Rod	1.5 × 6	+	_	Streptomyceae



(a) *Streptomyceae* [C_2W, Liquid] (b) *Bacillaceae* [C_2W, Liquid]



(c) *Bacillaceae* [C_2W, Liquid] (d) *Streptomyceae* [C_2W, Residue]



(e) *Streptomyceae* [C_2W, Residue]

Figure 7 Cultural and microscopic morphology of the C_2W condition (vegetable waste, cow dung, sesame meal cake with purified water)

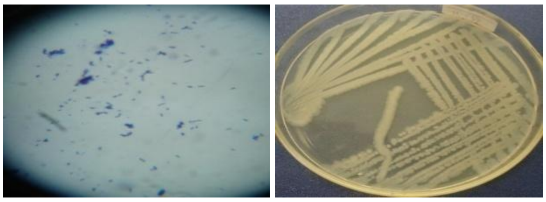
Table 11 Cultural Morphology of C_2 A (Vegetable Waste, Cow Dung, Sesame Meal Cake with Lemon Juice)

Sample	Cultural Morphology					
	Shape	**Color**	**Opacity**	**Elevation**	**Size (mm)**	**Family**
Condition-C_2 A Liquid	Circular	Creamy	Transparent	Raised	Very Small	Latobacillaceae
	Irregular	yellowish	Opaque	Flat	Normal	Bacillaceae
Condition-C_2 A Residue	Irregular	Creamy	Opaque	Flat	Irregular	Bacillaceae
	Filamentous	Creamy	Opaque	Raised	Small	Streptomyceae

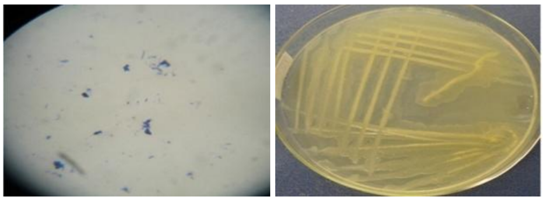
Table 12 Microscopic Morphology of C_2A (Vegetable Waste, Cow Dung, Sesame Meal Cake with Lemon Juice)

Sample	Microscopic Morphology				
	Shape	**Size (µm)**	**Gram Stain**	**Spore +/–**	**Family**
Condition-C_2 A Liquid	Double Rod	1 × 1-1.5	+	–	Lactobacillaceae
	Rod Chain	1 × 2-3	+	+	Bacillaceae
Condition-C_2 A Residue	Rod Cluster	1 × 1-2	+	–	Bacillaceae
	Double Rod	0.8-1 × 1-1.5	+	–	Streptomyceae

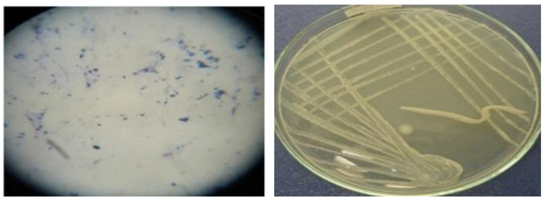
(a) *Latobacillaceae* [C_2A, Liquid]



(b) *Bacillaceae* [C_2A, Liquid]



(c) *Bacillaceae* [C_2A, Residue]



(d) *Streptomyceae* [C_2A, Residue]

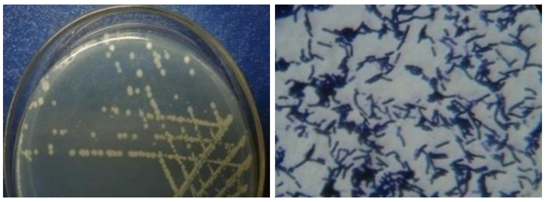
Figure 8 Cultural and microscopic morphology of the C_2A condition (vegetable waste, cow dung, sesame meal cake with lemon juice)

Table 13 Cultural Morphology of C_2B (Vegetable Waste, Cow Dung, Sesame Meal Cake with Slaked Lime Solution)

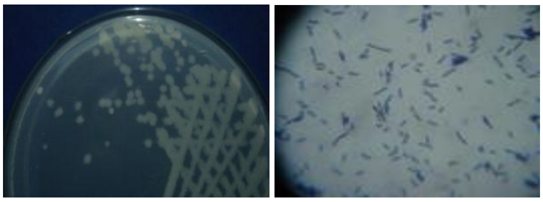
Sample	**Cultural Morphology**					
	Shape	**Color**	**Opacity**	**Elevation**	**Size (mm)**	**Family**
Condition- C_2B Liquid	Circle	Yellow	Opaque	Convex	1.5	Streptomyceae
	Irregular	Cream	Opaque	Flat	2	Streptomyceae
	Irregular	Yellow	Opaque	Convex	1	Corynebacterium
Condition- C_2B Residue	Circle	Pale	Opaque	Convex	3	Streptomyceae
	Circle	Pale	Opaque	Flat	1	Streptomyceae

Table 14 Microscopic Morphology of C_2B (Vegetable Waste, Cow Dung, Sesame Meal Cake with Slaked Lime Solution)

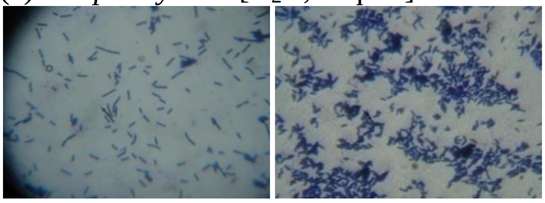
Sample	**Microscopic Morphology**				
	Shape	**Size (µm)**	**Gram Stain**	**Spore +/–**	**Family**
Condition- C_2B Liquid	Rod	2 × 4-6	+	_	Streptomyceae
	Rod	2 × 3.5	+	_	Streptomyceae
Condition- C_2B Residue	Rod	0.5-1 × 2-3	+	_	Cornyebacterium
	Rod	1 × 3	+	_	Streptomyceae
	Rod	1-1.5×3-4	+	_	Streptomyceae



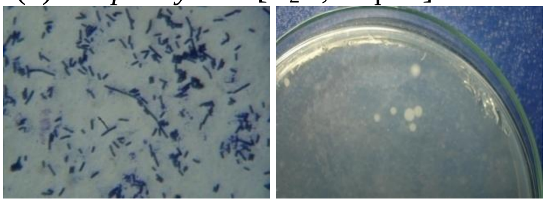
(a) *Streptomyceae* [C_2B, Liquid]



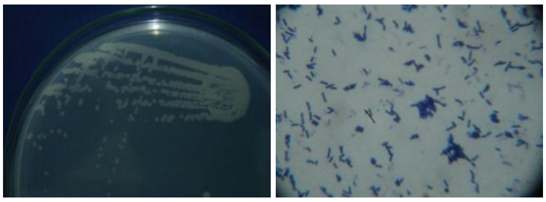
(b) *Streptomyceae* [C_2B, Liquid]



(c) *Cornyebacterium* [C_2B, Residue]



(d) *Streptomyceae* [C_2B, Residue]



(e) *Streptomyceae* [C_2B, Residue]

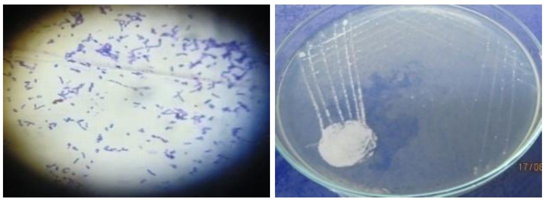
Figure 9 Cultural and microscopic morphology of the C_2 B condition (vegetable waste, cow dung, sesame meal cake with slaked lime solution**)**

Table 15 Cultural Morphology of Commercial EM

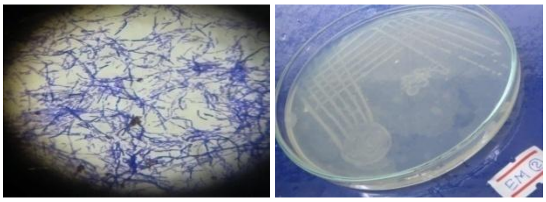
Sample	**Cultural Morphology**				
	Shape	**Color**	**Elevation**	**Size**	**Family**
Commercial EM	Irregular	White (Opaque)	Raised	0.5-1	Bacillaceae
	Irregular	Creamy	Flat	1-1.5	Bacillaceae
	Irregular	White	Flat	1.5	Bacillaceae

Table 16 Microscopic Morphology of Commercial EM

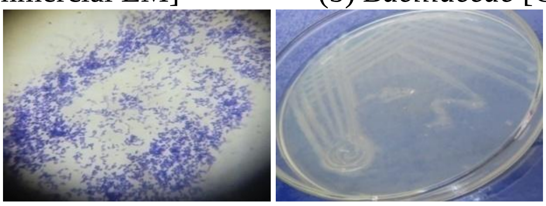
Sample	Microscopic Morphology			
	Shape	Size	Gram stain	Family
Commercial EM	Rod	2 × 2-3	+	Bacillaceae
	Rod	2-3 × 3-5	+	Bacillaceae
	Short rod	1-2 × 2-3.5	+	Bacillaceae



(a) *Bacillaceae* [Commercial EM]



(b) *Bacillaceae* [Commercial EM]



(c) *Bacillaceae* [Commercial EM]

Figure 10 Cultural and microscopic morphology of commercial EM Solution)

Table 17 Microorganisms and their Family from the Prepared and Commercial EM Solutions

No.	Condition	No. of Isolated Microorganism	Family
1	C_1w (liquid)	2	Bacillaceae
2	C_1w (Residue)	4	Three- Bacillaceae, Streptomyceae
3	C_1B(Liquid)	2	Streptomyceae, Bacillaceae
4	C_1B (Residue)	3	Streptomyceae, two- Bacillaceae
5	C_1A(Liquid)	1	Streptomyceae
6	C_1A(Residue)	3	Three- Streptomyceae
7	C_2W (Liquid)	3	Streptomyceae, two- Bacillaceae
8	C_2W (Residue)	2	Two-Streptomyceae
9	C_2A (Liquid)	2	Lactobacillaceae, Bacillaceae
10	C_2A(Residue)	2	Bacillaceae, Streptomyceae
11	C_2B(liquid)	3	Two-Streptomyceae, Cornyebacterium
12	C_2B(Residue)	2	Two-Streptomyceae
13	Commercial EM	3	Bacillaceae

For condition C_1, prepared EM solutions contain families of Bacillaceae and Streptomyceae. For condition C_2, prepared EM solutions consist of Bacillaceae, Streptomyceae, Cornyebacterium and Lactobacillaceae. This means that adding materials such as cow dung (humic substance support) and sesame meal cake (protein support) can supply more effective microorganisms. Commercial EM solution contains families of Bacillaceae.

Conclusion

In this research, EM (effective microorganism) solutions were prepared from waste of vegetables, cow dung and sesame meal cake. While EM solution were prepared by primary fermentation, biogas was evolved. Two conditions such as C_1 (vegetable waste) and C_2 (vegetable waste, cow dung and sesame meal cake) with different pH values were performed and the amount of biogas produced were determined hourly and daily till 5 days. The highest amount of biogas was evolved from condition C_1W(vegetable waste with purified water). The microorganisms that contained in EM solutions were found to be Bacillaceae**,** Streptomyceae**,** Lactobacillaceae and Cornyebacterium. Commercial EM solution contains only Bacillaceae. Therefore using prepared EM solutions are suitable for agriculture for reducing the dependency on chemical fertilizers and pesticides, for solving all kinds of environmental problems such as water, air and soil pollution, for recycling of kitchen waste into valuable organic materials. The evolved biogas can also be used as renewable energy source.

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References

Higa, T. (1991). *Effective Microorganisms: A Biotechnology for Mankind*. In Parr, J.F., Hornick, S.B. and Whitman C.E. (ed.). Proceedings of the First International Conference on Kyusei Nature Farming. Washington, D.C., U.S. Department of Agriculture, p.8-14.

Higa, T. (1994). *Effective Microorganisms: A New Dimension for Nature Farming*. In Parr, J.F., Hornick, S.B. and Simpson M.E. (ed.). Proceedings of the Second International Conference on Kyusei Nature Farming. Washington, D.C., U.S. Department of Agriculture, P. 20-22.

Higa, T. and James, F. (1994). "Beneficial and Effective Microorganisms for a Sustainable Agriculture and Environment". *International Nature Farming Research Center*, Atami, Japan, pp 4-7

Higa, T. and Wididana, G. N. (1991a). *The Concept and Theories of Effective Microorganisms*. In Parr, J.F., Hornick, S.B. and Whitman C.E. (ed.). Proceedings of the First International Conference on Kyusei Nature Farming. Washington, D. C., U.S. Department of Agriculture, p.118-124

Higa, T. and Wididana, G. N. (199lb). *Changes In the Soil Microflora Induced by Effective Microorganisms*. In Parr, J.F., Hornick, S.B. and Whitman C.E. (ed.). Proceedings of the First International Conference on Kyusei Nature Farming. Washington, D. C., U.S. Department of Agriculture, p.153-162

Kengo, Y. and Hui-lian, X. (2000). "Properties and Applications of an Organic Fertilizer Inoculated with Effective Microorganisms". *Journal of Crop Production*, Vol **3**(1), pp 255-268.

Lévai, L., Veres, S.Z., Makleit, P., Marozsán, M. and Szabó, B. (2006). "New Trends in Plant Nutrition". *Proceedings of 41st Croatian and 1st International Symposium on Agriculture*.

Singh, D.S., Chand, S, Anvar, M. and Patra (2003). "Effect of Organic and Inorganic Amendment on Growth and Nutrient Accumulation by Isabgol (*Plantagoovata*) in Sodic Soil Under Greenhouse Conditions". *J. Med. Arom. Plant Sci*., Vol. 25, pp. 414-419