The Bioscan 11(3): 1469-1472, 2016
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.com

OPTIMIZATION OF AERATION AND TIME PERIOD FOR THE IMPROVEMENT OF MICROBIOLOGICAL QUALITIES IN VERMIWASH

RUCHI SONI[1], RAMESHWAR KUMAR[2]* AND J. P. SAINI[3]
[1]Hill Area Tea Science Division,
CSIR-Institute of Himalayan Bioresource Technology, Palampur (H.P) - 176 061 INDIA
[2-3]Department of Organic Agriculture, Collage of Agriculture,
Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur (H.P) - 176 061, INDIA
e-mail: drrameshwar@gmail.com

KEYWORDS
Actinomycetes
Aerated vermiwash
Fungi
Phosphate solubilizing bacteria (PSB)
N_2 Fixers

Received on :
08.08.2016

Accepted on :
26.07.2016

***Corresponding author**

ABSTRACT
Vermiwash is an important ecofriendly approach in organic farming, which plays a critical role in sustainable agriculture. Therefore, the present study was aimed at the standardization of aeration and time period to achieve maximum population of beneficial microflora in vermiwash. Significantly higher microbial loads of N_2 fixers, actinomycetes, fungi and phosphate solubilizing bacteria i.e. 7.56, 6.78, 6.78 and 7.08 log cfu/ml, respectively in fresh vermiwash was obtained with 24 h of aeration at room temperature (20 ± 2^0C). Further one month study revealed that the maximum population counts for N_2 fixers (8.70 log cfu/ml) and actinomycetes (7.0 log cfu/ml) were obtained after 5 days incubation, while, fungi (6.59 log cfu/ml), PSB (7.20 log cfu/ml) and total microbes (9.54 log cfu/ml) showed maximum population at 10 days. A gradual reduction in microbial counts was observed after 15 to 30 days and aeration showed negligible effect on microbial population. Nutritional profile analysis of 10 days old aerated vermiwash showed that it was a rich source of carbon, nitrogen, iron, manganese and zinc. Our study concluded that vermiwash should be used upto 10 days of preparation with 24 h of proper aeration to attain maximum microbial population and nutrients.

INTRODUCTION

Organic farming is now becoming mainstream all over the world because it works in harmony with nature rather than against it. It has come up with a new hope to sustain agriculture in an ecofriendly manner which provides us good quality of food without any hazardous chemicals in it. Moreover, chemical fertilizers have played a significant role in the green revolution and providing the major plant nutrients, but unbalanced use of them, had led to reduction in soil fertility and environmental degradation. Organic farming is one of the many approaches believed to meet the purpose of sustainable agriculture. The most feasible strategies toward environmentally sustainable agriculture is to seek out microbes based preparations such as organic manures, biofertilizers etc. to salvage these compounds in place. They are renewable, pollution free and are of low cost (Gomiero *et al*., 2008; Brahmaprakash and Sahu, 2012).

Organic farmers use various approaches based on biofertilizer inputs and management practices to improve soil fertility. Vermicompost technology has an important place and it plays a critical role in organic farming. Vermiculture is a mixed culture containing soil beneficial microflora and an effective strain of earth worms *Eisenia foetida* (Mal *et al.,* 2013). Vermi composting refers to the process of composting in which plant beneficial microbes and nutrients are produced by means of excreta of earth worms. In recent times, the commercial vermin culturists have started promoting a product called vermiwash i.e. washing of warm and vermicompost. It is a brown colored leachate with pH around 7-7.7 and the preparation certainly has soluble plant nutrients apart from some organic acids, mucus of earthworms and microbes (Ndegwa and Thompson, 2001; Ansari and Sukhraj, 2010; Manyuchi *et al.,* 2013a, b). It contains beneficial microflora such as N_2 fixers, PSB, actinomycetes and fungi along with macro-micronutrients which stimulate the growth and yield of crops (Shivsubramanian and Ganeshkumar, 2004; Kaur *et al.,* 2015).

Vermiwash is a rich source of macro-micronutrients so it acts as a suitable medium for various beneficial microbes such as N_2 fixers and PSB (Chattopadhyay, 2014). So, the application of the vermiwash into the soil significantly influences the biogeochemical cycles of nitrogen and phosphorus with the help of these beneficial microbes. In the ecosystem, a mixed population of microbes such as bacteria, actinomycetes and fungi is also essential to accelerate enzymatic degradation of organic material present in nature so as to make them available to the plants (Trivedi and Bhatt, 2006).

The growth of beneficial microbes depends upon various physical and chemical factors such as aeration, time, pH, temperature carbon-nitrogen sources and minerals (Das *et al*., 2014; Nosrati *et al*., 2014; Vyas *et al*., 2014). In a liquid

medium such as vermiwash, aeration and time play a significant role in the proper growth of microbes because with the passage of time oxygen drops below a specific level, nutrients can be depleted and other microbes of non interest may grow up. Therefore, study of aeration and time period is an initial and important step to improve growth of effective microbes present in the liquid manures such as vermiwash so that farmers may be able to attain maximum benefits of it. Very less information is available in the literature about the effect of aeration and time on the microbiological quality of liquid manures like vermiwash which may be helpful in achieving the maximum growth of microbes. Keeping in view the above factors, the objective of our study was to evaluate the effect of aeration and time on microbial loads of beneficial microbes such as N_2 fixers, actinomycetes, PSB, and fungi present in the vermiwash.

MATERIALS AND METHODS

Preparation of vermiwash

The present study was undertaken in the Department of Organic Agriculture, College of Agriculture, CSKHPKV, Palampur, located in the mid-hills and sub-humid agro-climatic zone (32°6'N, 76°18'E) of Himachal Pradesh, India. Vermiwash was prepared at room temperature as described earlier by Chadha *et al*., (2012). Two pitchers with small holes at their bottoms fitted to each other with the help of rubber pipe. Among those, the first pitcher was filled with 2-3 inches layer of sand along with a layer (5-10 cm) of dry biomass and a thick covering of (10 cm layer) of cow dung. As the material was filled up to 2/3 of pitcher, 200-300 adult earthworms (*Eisenia foetida*) were added and was hanged with the help of plastic rope under a shady area. Another pitcher filled with water was placed above the pitcher mentioned above so that water got trickled drop wise in the pitcher having earthworms. Another empty pitcher was placed below it to collect the brown colored leachate, vermiwash.

Effect of aeration on microbial population of vermiwash

5L vermiwash sample was subjected to 48 h aeration with the help of aeration pump. pH of vermiwash was determined with the help of digital pH meter make Eutech Cyberscan. Microbial population of vermiwash after 1 h, 2 h, 4 h, 24h and 48h aeration was enumerated in terms of log cfu/ml on their respective media *i*.e. Jensen's Medium (for N_2 fixers count), Actinomycetes Isolation Agar (for actinomycetes count), Potato Dextrose Agar (for fungal count), Pikovaskaya's Agar (for PSB count) and Nutrient Agar (for total microbial count) by using serial dilution and plate count method (Vieira and Nahas, 2005). The plating was done in triplicates. The media components used were of analytical grade (AR) obtained from Merck limited - India, Sigma-Aldrich Inc. USA, and Hi Media Laboratories, Bombay, India.

Effect of time period on microbial population of vermiwash

For one month, 5L vermiwash samples with interval of five days, were subjected to 24 h of aeration at room temperature (20 ± 2°C).The microbial population count was analyzed as discussed above. Simultaneously control of non-aerated vermiwash samples were also tested for microbial count along with the aerated samples.

Determination of organic carbon, nitrogen and micronutrients in vermiwash

Determination of organic carbon in 24 h aerated 10 days old vermiwash was done by digestion method given by Walkley and Black, (1934). Nitrogen content was estimated by Kjeldahl's method (AOAC, 1990). Determination of micronutrient contents was done by DTPA (Diethylene Triamine Penta - acetic acid) method (Lindsay and Norvell, 1978).

RESULTS AND DISCUSSION

From last two decades, researchers are searching for a cost effective technology which should be ecological sustainable with no hazardous effects on environment and useful to the society too. Our study has focused on vermiwash which is a simple, cost effective and eco-friendly approach in organic farming. It contains macro micronutrients and beneficial microbes which have important plant growth promoting attributes and antagonistic activities (Khidrapure *et al.,* 2015). The microbes present in organic manures have specific physic-chemical conditions such as temperature, pH, aeration rate, time and carbon-nitrogen source for their growth. They also show their activities at appropriate growth conditions, so there is a need to optimize these conditions for their growth and better expression of beneficial traits (Chavan *et al.,* 2013).In the present study, effect of aeration and different time intervals on microbial population of vermiwash was studied. The pH of vermiwash samples was ranged from 7-7.4 which is nearer to neutral and optimum for the growth of majority of microbes as stated by various researchers also (Kalra *et al.,* 2010; Manyuchi *et al*., 2013a).

Microbial load in fresh vermiwash sample aerated for different intervals of time

Fresh vermiwash sample was subjected to aeration for different time periods to evaluate maximum microbial growth.

Table1: Effect of aeration on microbial population of fresh vermiwash sample

Microorganisms	Population count (log cfu/ml) Aeration period				
	1 h	2 h	4 h	24 h	48 h
N_2 Fixers	7.0 ±0.29	7.0±0.46	7.26±0.03	7.56±0.03	7.43±0.06
Actinomycetes	6.0 ±0.29	6.30±0.17	6.30±0.23	6.78±0.06	6.30±0.58
Fungi	5.30±0.12	5.0±0.29	6.30±0.12	6.78±0.06	5.48±0.01
Phosphate solubilizing bacteria	6.70±0.12	6.70±0.58	7.30±0.15	7.08±0.01	6.78±0.01
Total Microbial Count	8.50±0.29	8.90±0.06	9.0± 0.58	9.70±0.58	9.0± 0.29

*values are the mean of three replicates, ± = standard error of the mean

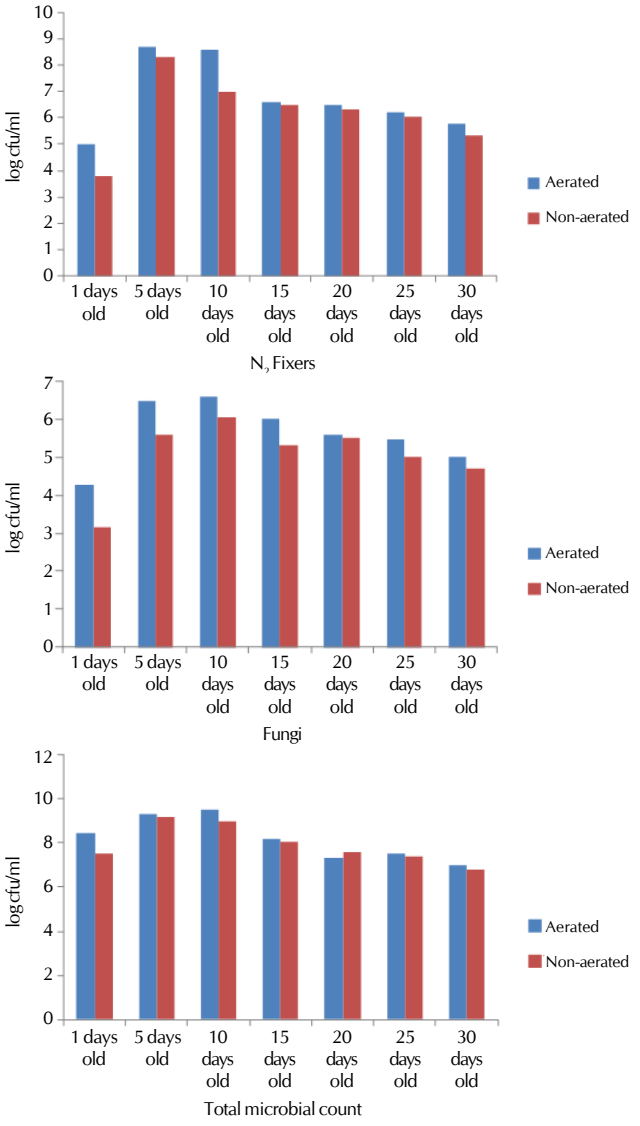

Figure 1: Effect of different time periods on microbial population of aerated and non-aerated vermiwash

Significantly higher populations of N_2 fixers (7.56 log cfu/ml), actinomycetes (6.78 log cfu/ml), fungi (6.78 log cfu/ml), PSB (7.08 log cfu/ml) and total microbial count (9.70 log cfu/ml) were obtained after 24 h of aeration (Table 1). Further aeration up to 48 h did not show any significant difference in the growth of beneficial microbes present in vermiwash. Maintenance of physical and chemical conditions is important so as to obtain good quality of liquid manure and aeration plays a significant role in it (Pant *et al.*, 2011). So, continues aeration may be one of the reasons to increase the growth of beneficial microbes in vermiwash. In general, forced aeration in any organic manure or compost is advantageous as it achieves higher efficiency by combining aeration with agitation (Sundberg, 2005).

Comparison of microbial loads in aerated and non-aerated vermiwash in different intervals of time

One month study was conducted to compare microbial loads of beneficial microflora present in aerated and non-aerated

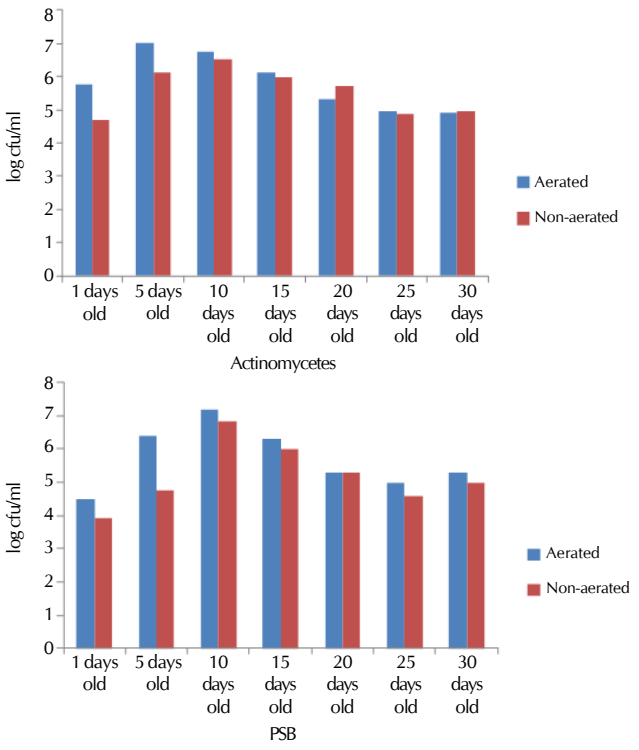

Table 2: Nutritional profile of 10 days old vermiwash with 24 h aeration

Parameters	Value
1. Carbon (%)	1.9± 0.35
2. Nitrogen (%)	2.5±0.17
3. Micronutrients (ppm)	
Fe^{2+}	0.31±0.04
Mn^{2+}	0.06±0.02
Zn^{2+}	0.03±0.01

*values are the mean of three replicates, ± = standard error of the mean

vermiwash. Twenty four hours aerated vermiwash sample showed maximum counts for N_2 fixers (8.7 log cfu/ml) and actinomycetes (7.0 log cfu/ml) after 5 days of incubation. Moreover, maximum microbial loads for fungi (6.59 log cfu/ml) and PSB (7.20 log cfu/ml) along with total microbial load (9.54 log cfu/ml) was found in 10 days old aerated sample (Fig 1). It is contributed to the fact that vermiwash is a rich source of micro-macronutrients which continuous supplies substrates that influences the growth of microbes present in it (Durga and Ramasubramanian, 2015; Kaur *et al.*, 2015). A considerable difference in the population of beneficial microbes could be seen between aerated and non-aerated samples throughout the month. It emphasized the fact that aeration contributed in proper supply of oxygen that enhanced the metabolism of the microbes which was directly correlated with their increased growth.

Moreover, further decrease in microbial counts was found and aeration showed almost no effect on their populations when samples were kept for 15 to 30 days at room temperature. The growth of microbes is one of the most familiar examples of important dynamical processes in ecology. Incubation time also plays an important role in rapid growth of their population,

even when they are grown in their low densities in nutrient rich environment (Davis *et al.,* 2005). Further increase in incubation period reduced the growth of all microbes, which may be due to the nutrient limitation as microbial population requires optimum level of nutrients for their growth. Second obvious factor may be accumulation of toxic by-products in the culture medium as a result of prolonged incubation period.

Nutritional status of vermiwash sample

Vermiwash is excellent source of macro-micro nutrients as studied previously by others researchers also (Sundararasu and Jeyasanker, 2014; Durga and Ramasubramanian, 2015, Kaur *et al.,* 2015). Ten days old 24 h aerated vermiwash sample was examined for important macro-micro nutrients. It was found that vermiwash contained significant quantities of carbon (1.9%), nitrogen (2.5%), and micronutrients viz. iron (0.3 ppm), manganese (0.06 ppm) and zinc (0.03 ppm) (Table 2). The growth of microbes in vermiwash is contributed to the biomass present in it which is a collection of complex materials as stated by Zambare *et al*. (2008). It is further digested by secretary enzymes of earthworms and the resultant simpler substances is the best suitable media for the growth of N_2 fixers, PSB, actinomycetes and fungi which are helpful for plant growth and its development. Gandhi and Shivakumar, (2010) and Khidrapure *et al*. (2015) stated that the improvement in growth, yield and quality parameters of the different crops by the application of vermicompost and vermiwash are correlated to the macro-macronutrients and beneficial microflora present in them.

REFERENCES

Ansari, A. A. and Sukhraj, K. 2010. Effect of vermiwash and vermicompost on soil parameters and productivity of okra (*Abelmoschus esculentus*) in Guyana. *Afr. J. Agric. Res*. **5:** 1794-1798.

AOAC. 1990. Official Methods of Analysis of the Association of Official Analytical Chemists. 15th edition, Arlington VA, pp 1058-1059.

Brahmaprakash, G. P. and Sahu, P. K. 2012. Biofertilizers for sustainability. *J. Indian Inst. Sci.* **92:** 1-10.

Chadha, S., Rameshwar, Ashlesha, Saini, J. P. and Paul, Y. S. 2012. Vedic krishi: sustainable livelihood option for small and marginal farmers. *IJTK*. **11:** 480-486.

Chattopadhyay, A. 2014. Effect of vermiwash and vermicompost on an ornamental flower, *Zinnia sp. J. Horticulture*. **1:** 1-4.

Chavan, V., Joshi, S. and Pejaver, M. 2013. Comparative study of microbial population in vermicompost and biocompost in relation with physicochemical parameters. *National Conference on Biodiversity: Status and Challenges in Conservation* –FAVEO. **1:** 245-248. ISBN: 978-81-923628-1-6.

Das, S. K., Avasthe, R. K. and Gopi, R. 2014. Vermiwash: use in organic agriculture for improved crop production. *Pop. Kheti*, **2:** 45-46.

Davis, K. E. R., Joseph, S. J. and Janssen, P. H. 2005. Effects of growth medium, inoculum size and incubation time on culturability and isolation of soil bacteria. *Appl. Environ. Microb.* **71**: 826–834.

Durga, S. and Ramasubramanian, V. 2015. Quantification of micro and macro nutrients from different types of vermiwashes. *Indian Journal of Science*. **15:** 50-58.

Gandhi, A. and Sivakumar, K. 2010. Impact of vermicompost carrier based bioinoculants on the growth, yield and quality of rice (*Oryza sativa* l.) c. v. nlr 145. *The Ecoscan*. **4:** 83-88.

Gomiero, T., Paoletti, G. M. and Pimentel, D. 2008. Energy and environmental issues in organic and conventional agriculture. *Crit. Rev. Plant Sci.* **27**: 239-254.

Kalra, A., Chandra, M., Awasthi, A., Singh, A. K. and Khanuja, S. P. S. 2010. Natural compound enhancing growth and survival of rhizobial inoculants in vermicompost based formulation. *Biol. Fert. Soils*. **46:** 521-524.

Kaur, P., Bhardwaj, M. and Babbar, I. 2015. Effect of vermicompost and vermiwash on growth of vegetables. *Res. J. Animal, Veterinary and Fishery Sci.* **3:** 9-12.

Khidrapure, G., Vasudevan, S. N., Janagoudar, B. S., Sreenivas A. G., Rao, S. and Doddagoudar, S. R. 2015. Orgo priming: an innovative seed quality enhancement technique in rice cv sonamasoori. *The Ecoscan*. **9:** 403-406.

Lindsay, W. L. and Norvell, W. A. 1978. Development of a DTPA soil test for zinc, iron, manganese and copper. *Soil Sci. Soc. Am. J*. **42:** 421-428.

Mal, S., Chattopadhyay, G. N. and Chakrabarti, K. 2013. Compost quality assessment for successful organic waste recycling. *The Ecoscan*. **3:** 199-203.

Manyuchi, M. M., Phiri, A., Muredzi, P. and Chitambwe, T. 2013a. Comparison of vermicompost and vermiwash bio-fertilizers from vermicomposting waste corn pulp. *World Acad. Sci. Eng Technol*. **7:** 368-371.

Manyuchi, M. M., Kadzungura, L., Phiri, A. and Muredzi, P. 2013b. Effect of vermicompost, vermiwash and application time on *Zea mays* growth. *IJSET*. **2:** 638-641.

Ndegwa, P. M. and Thompson, S. A. 2001. Integrating composting and vermicomposting in the treatment and bioconversion of bio solids. *Bioresource Technol*. **76:** 107-112.

Nosrati, R., Owlia, P., Saderi, H., Rasooli, I. and Malboobi, M. A. 2014. Phosphate solubilization characteristics of efficient nitrogen fixing soil *Azotobacter* strains. *Iran J Microbiol*. **6:** 285–295.

Pant, A., Radorich, T. J. K., Hue, N. V. and Arancon, N. Q. 2011. Effect of vermicompost tea (aqueous extract) on pak choi yield, quality and on soil biological properties. *Compost Sci. Util.* **19:** 279-292.

Shivsubramanian, K. and Ganeshkumar, M. 2004. Influence of vermiwash on biological productivity of marigold. *Madras Agricultural Journal*. **91:** 221-225.

Sundararasu, K. and Jeyasankar, A. 2014. Effect of vermiwash on growth and yield of brinjal, *Solanum melongena* (eggplant or aubergine). *AJST*. **5:** 171-173.

Sundberg, C. 2005. Improving compost process efficiency by controlling aeration, temperature and pH. Doctoral thesis. Swedish University of Agricultural Sciences, Uppsala. ISBN 91-576-6902-3.

Trivedi, R. and Bhatt, S. A. 2006. Phosphatase activity in semi arid soils of Pathan. *AJMBES*. **8:** 303-305.

Vieira, F. C. S. and Nahas, E. 2005. Comparison of microbial numbers in soils by using various culture media and temperatures. *Microbiological Research* **160:** 19-202.

Vyas, P., Rahi, P., Chadha, B. S. and Gulati, A. 2014. Statistical optimization of medium components for mass production of plant growth-promoting microbial inoculant *Pseudomonas trivialis* BIHB 745 (MTCC5336). *Indian J. Microbiol*. **54:** 239-241.

Walkley, A. and Black I. A. 1934. An examination of digestion method for determining soil organic matter and a proposed modification of the chromic acid titration method**.** *Soil Sciences*. **37:** 29-37.

Zambare, V. P., Padul, M. V., Yadav, A. A. and Shete, T. B. 2008. Vermiwash: biochemical and microbiological approach as ecofriendly soil conditioner. *ARPN J. Agri. Biol. Sci.* **3:** 1-5.