academicJournals

Vol. 7(32), pp. 4117-4122, 9 August, 2013
DOI: 10.5897/AJMR12.1077
ISSN 1996-0808 ©2013 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Evaluation of phenol degradation by effective microorganism (EM) technology with EM-1

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Accepted 16 July, 2013

Effective microorganisms (EM) are a culture of co-existing beneficial micro-organisms predominantly consisting of lactic acid bacteria, photosynthetic bacteria, yeast, fermenting fungi and actinomycetes that are claimed to enhance the decomposition of organic matter which in turn improves soil fertility and toxic pollutants including petroleum hydrocarbons, among others. In the present study, EM-1 commercial formulation was evaluated for phenol degradation under batch condition with 250, 500, 750 and 1000 mg/l of synthetic phenol. The biomass concentration, residual phenol concentration, speficic growth rate and specific degradation rate was recorded at respective concentration under different time interval. EM-1 could tolerate all the tested concentration of phenol and the maximum specific growth rate and degradation rate was recorded at 250 mg/l of phenol supplemented media followed by 500, 750 and 1000 mg/l.

Key words: Effective microorganism, EM-1, phenol degradation.

INTRODUCTION

The technology of effective microorganisms was developed during the 1970's at the University of Ryukyus, Okinawa, Japan. Studies have shown that effective microorganism (EM) may have a number of applications, including agriculture, livestock, gardening and landscaping, composting, bioremediation, cleaning septic tanks, algal control and household uses (Jingchun et al., 2009). The practical application was developed by Professor Teuro Higa who devoted much of his scientific career to isolating and selecting different microbes for beneficial effects on soils and plants. Professor Teuro Higa found microbes that can co-exist in mixed cultures and are physiologically compatible with one another. When these cultures are introduced into the natural environment, their individual beneficial effects are greatly magnified in a synergistic fashion (Bajwa, 2005). A microbial inoculant containing many kinds of naturally occurring beneficial microbes called 'effective microorganisms' has been used widely in nature and organic farming (Chrispaul et al., 2010).The concept of effective microorganisms was developed by Japanese horticulturist, Teuro Higa, from the University of Ryukyus in Japan. Teuro Higa reported in the 1970s that a combination of approximately 80 different microorganisms is capable of positively influencing decomposing organic matter such that it reverts into a life promoting process. Studies have shown that EM may have a number of applications, including agriculture, livestock, gardening landscaping, composting, bioremediation, cleaning septic tanks, algal control and household uses (Javaid et al., 2008). The application of EM will improve soil and irrigation water. It can be used in seed treatment. It can be used to make organic sprays for the enhancement of photosynthesis and control of insects, pests and diseases (Sekaran et al., 2007). The use of EM for reducing volumes of sewage sludge has often been suggested as

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feasible in either wastewater treatment plants or on-site wastewater treatment systems such as septic tank and industrial effluents and effective decom-position of organic wastes residue into high nutritious compost using effective microorganisms has been reported (Namasivayam and Kirithiga, 2010, Namasivayam et al., 2011).

Phenol and phenolic compounds are ubiquitous pollutants to natural water resources from the effluents of a variety of industrial chemical such as refineries, phenol manufacturing, pharmaceuticals and industries of resin paint, dying, textile wood, petro chemical, pulp mill, cokin and coal gasification plants among others (Agarry et al., 2008). Phenol is known to cause severe health and environmental effects. Small, single releases of phenol do not stay long in the air (usually half is removed in less than 1 day), and usually do not stay in the soil for long periods (usually completely gone in 2-5 days), but can stay in water for longer than 9 days. Phenol will stay in the air, soil, and water for much longer times if a large amount of it is released at one time, or if a steady amount is released over a long time (Agarry, 2008). The potential of various organisms to metabolize organic compounds has been observed to be a potentially effective means in disposing of hazardous and toxic wastes (Anna, 1999; Parvanov, 2008). In the present study, EM-1 commercial formulation was evaluated for phenol degradation under batch condition with different initial concentration of phenol.

MATERIALS AND METHODS

Effective microorganisms (EM-1)

The liquid culture of the EM-1 used in the study was supplied by Environ Biotech and contained a mixture of lactic acid bacteria *Lactobacillus plantarum* (1.0 x 10^4 CFU/ml) yeast with (1.0 x 10^5 CFU/ml) *Candida utilis, Actinomycetes Streptomyces albus* (3.0 X 10^3 CFU/ml), fermenting fungi *Aspergillus oryzae* (1.1 x 10^5 CFU/ml). EM solution is a yellowish liquid with a pleasant odour and sweet sour taste with a pH of 3 and stored in cool place without refrigeration.

Activation of EM-1

EM is available in a dormant state and requires activation before application and the activation was performed as per the instruction given by manufacturer. Activation involves the addition of 20 l of water and 2 kg of Jaggery (pure cane sugar) to 1 l of dormant EM. The mixture was poured into a clean airtight plastic container with no air left in the container. The container was stored away from direct sunlight at ambient temperatures for 8 to 10 days. Gas was released every day until fermentation was complete. During the period of activation, a white layer of actinomycetes forms on the top of the solution accompanied by a pleasant smell. The pH is also a determining factor; the pH of the EM should be below 4.0.

Phenol degradation study

1 L of Ramsay media was prepared and sterilized by autoclaving. After sterilization different concentrations of phenol such as 250, 500, 750 and 1000 mg (sterilized by filtrations) was added into sterile medium. 250 mg/l of EM-1 was added into the respective flasks and kept at 35°C under 150 rpm. Every 12 h, the biomass concentration was estimated using the dry weight method.

Biomass estimation

The biomass concentration was estimated using the dry weight method. 50 ml sample of culture broth was withdrawn from the bioreactor and centrifuged (Gllenkamp centrifuge) at 5000 rpm for 15 min in plastic centrifuge tubes. The supernatant was transferred into small bottles and stored at 4°C for phenol estimation. The pellets were re-suspended in de-ionized water and recentrifuged. The supernatant was decanted and pellets rinsed off from the tube into a pre-weighed 1.2 µm pore filter paper (Whatman GF/C). The filter paper was then dried in an oven at 105°C for between 12 – 24 h, cooled in a dessicator at room temperature and re-weighed until a constant dry weight was obtained. The difference between the pre-weighed filter paper and the second weight was used to estimate the dry weight of the biomass.

Phenol estimation

Phenol concentration was determined quantitatively by a colorimetric method, using 4-aminoantipyrine as colour reagent. These analyses were performed according to the procedures described in standard method for the estimation of water and waste water (Greenberg et al., 1992).

Estimation of specific growth rates and specific degradation

Specific growth rates of the culture at different phenol concentrations were calculated as per the following relationship (Kovari and Elgi, 1998)

$$\mu = \frac{1}{X}\frac{dX}{dt}$$

Where, *X* is biomass concentration (mg/L) at time, *t* (h) and μ is the specific growth rate (h^{-1}).

The specific degradation rate was calculated from the plot of the negative logarithm of S/S_o vs. time which is a straight line with a slope equal to q, the phenol degradation rate (Hirayama et al., 1994).

RESULTS AND DISCUSSION

Microbial inoculants containing many kinds of naturally occurring beneficial microbes called ‘effective microorganisms’ has been used widely in nature and organic farming. The concept of effective microorganisms was developed by Japanese horticulturist Teuro Higa from the University of Ryukyus, Japan. Teuro Higa reported in the 1970s that a combination of approximately 80 different microorganisms is capable of positively influencing decomposing organic matter such that it reverts into a life promoting process. Studies have shown that EM may

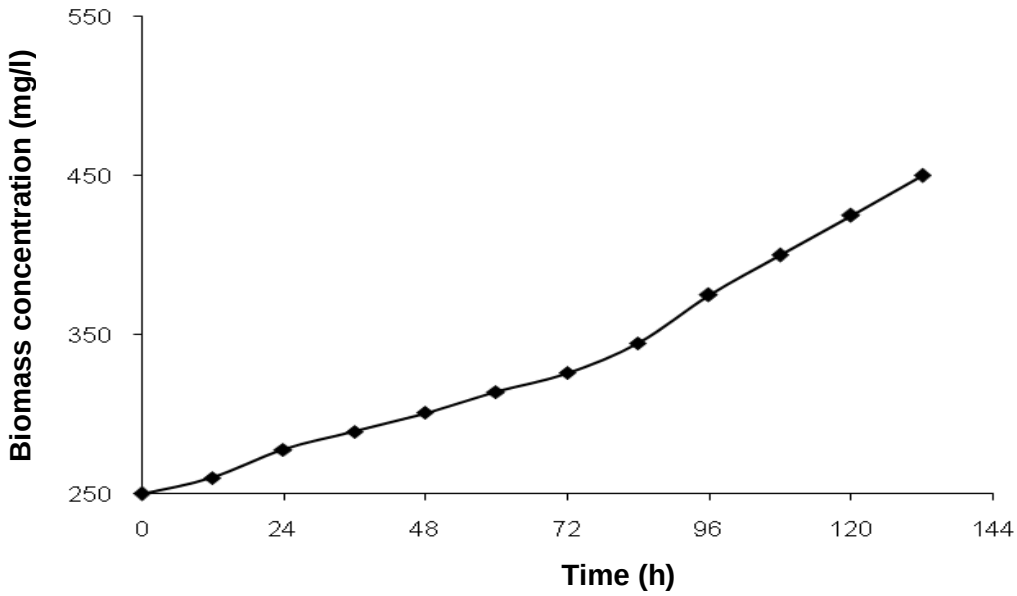

Figure 1. Biomass concentration at 250 mg/l of initial phenol concentration.

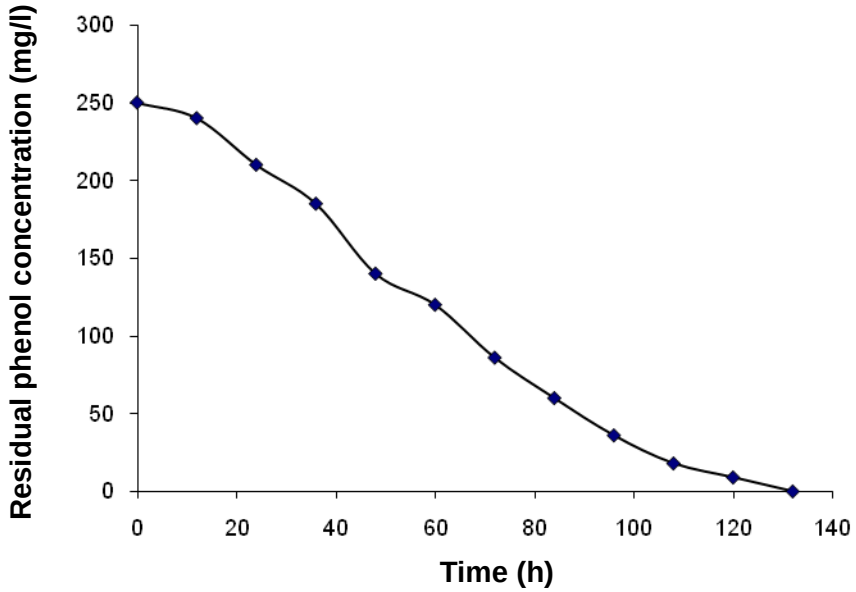

Figure 2. Residual phenol concentration at 250 mg/l initial phenol concentration.

have a number of applications, including agriculture, livestock, gardening landscaping, composting, bioremediation, cleaning septic tanks, algal control and household uses. The application of EM will improve soil and irrigation water. It can be used in seed treatment. It can be used to make organic sprays for the enhancement of photosynthesis and control of insects, pests and diseases. But less report are available on degradation of organic pollutants by effective microorganism. In the present study, EM-1 commercial formulation was evaluated for phenol degradation under batch condition.

EM-1 tolerated all the concentration of phenol and the growth rate was gradually increased. Figure 1 shows biomass concentration with respect to time at initial phenol concentration of 250 mg/l. It was found that the lag period was 12 h and the complete degradation occurred at 132 h as shown in the Figure 2. The specific growth rate of biomass and degradation of phenol was found to be 0.004

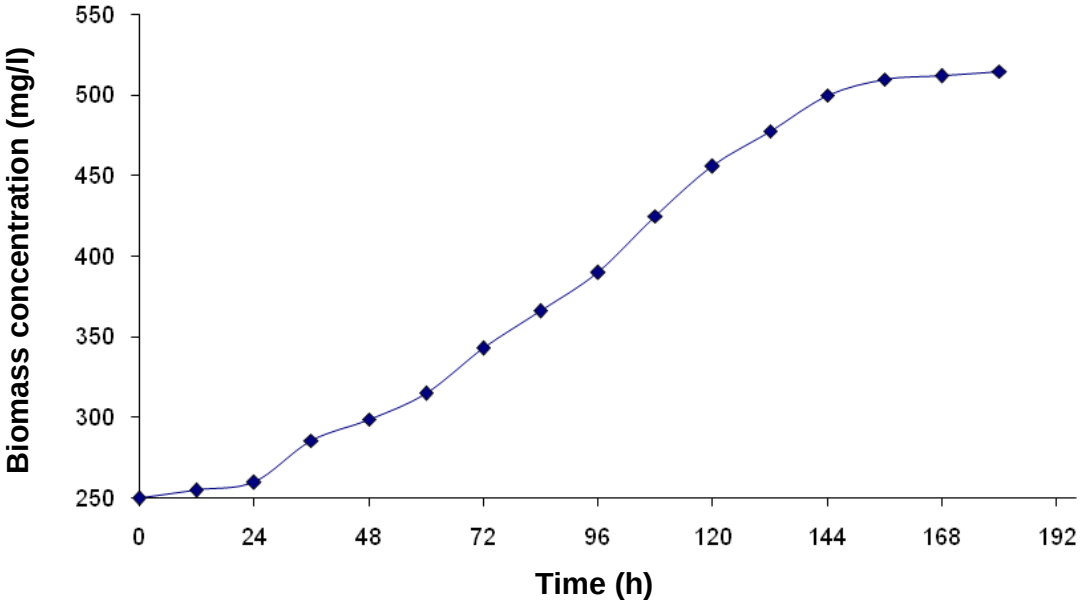

Figure 3. Biomass concentration at 500 mg/l of initial phenol concentration.

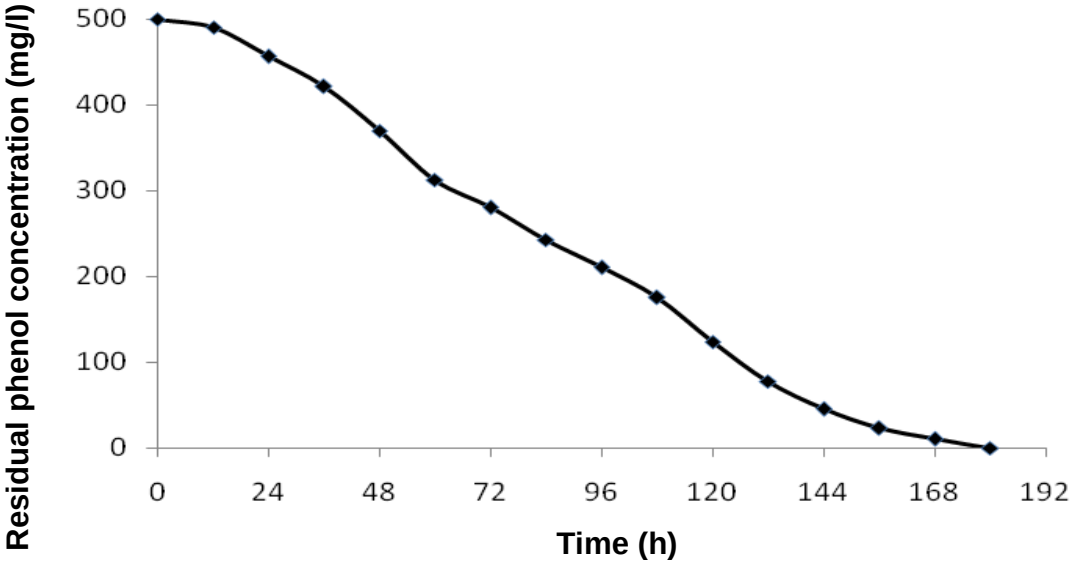

Figure 4. Residual phenol concentration at 500 mg/l initial phenol concentration.

and 0.026 h^{-1}, respectively. Figure 3 shows the change in biomass concentration with time at 500 mg/l initial phenol concentration. The graph showed that lag period slightly increased to 24 h, which indicated that growth of EM-1 was affected by initial phenol concentration. The specific growth rate and degradation was observed to be 0.004 and 0.020 h^{-1}. Complete degradation of 500 mg/l occurred in 168 h as shown in Figure 4. At 750 and 1000 mg/l of the lag phase was 36 and 48 h, respectively as observed in Figures 5 and 6. It took nearly 204 and 252 h for the complete degradation of phenol as shown in Figures 7 and 8. Phenol had shown significant inhibitory effect on growth of microorganism at higher concentrations (Kumar et al., 2005; Stoilova et al., 2006). The specific growth rate and specific degradation rate was found to be 0.005 and 0.012 h^{-1}, respectively at 750 mg/l of initial phenol concentration. At 1000 mg/l of initial phenol concentration, specific growth rate and specific degradation rate was found to be 0.004 and 0.012 h^{-1}. The results showed that specific growth rate and specific

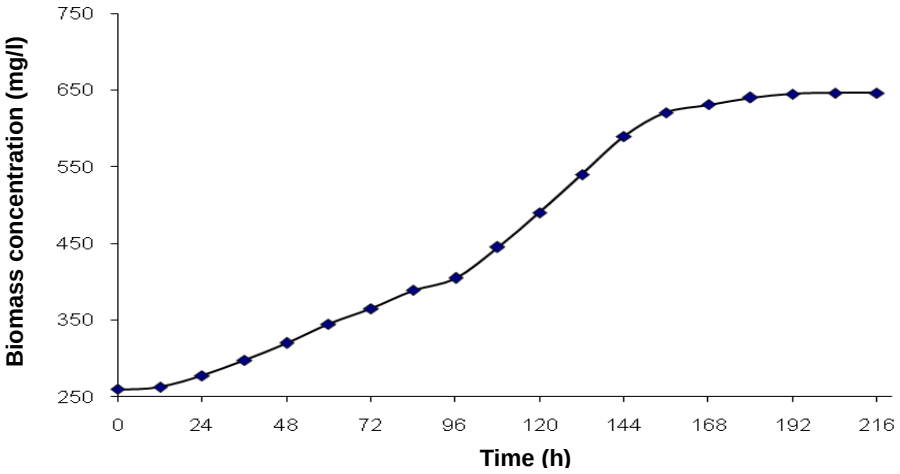

Figure 5. Biomass concentration at 750 mg/l of initial phenol concentration.

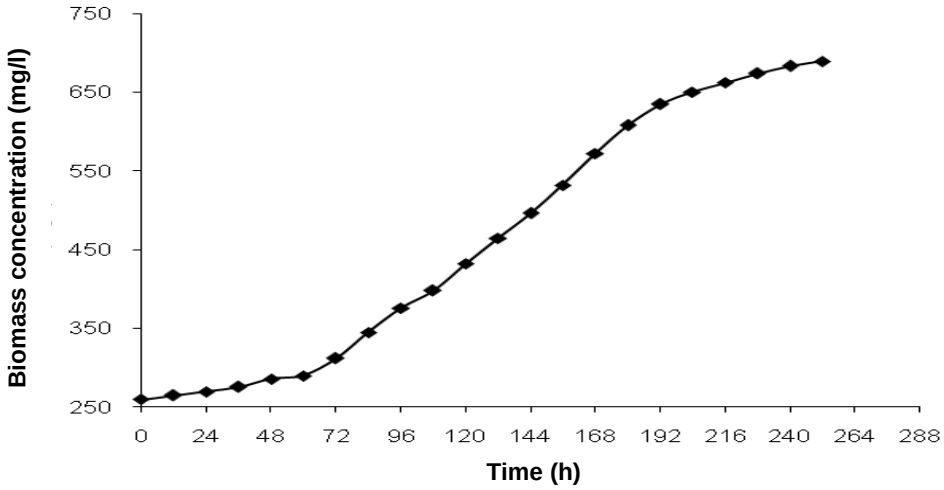

Figure 6. Biomass concentration at 1000 mg/l of initial phenol concentration.

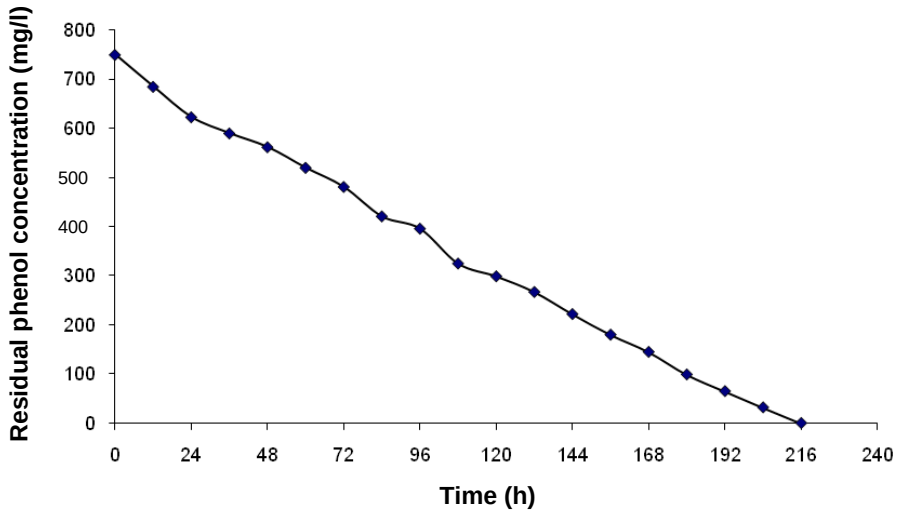

Figure 7. Residual phenol concentration at 750 mg/l initial phenol concentration.

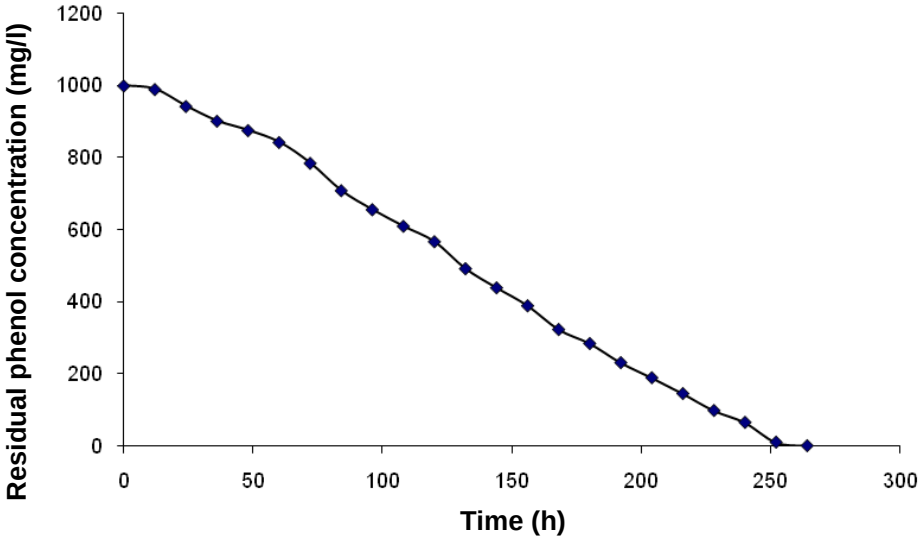

Figure 8. Residual phenol concentration at 1000 mg/l initial phenol concentration.

degradation rate decreased as the initial concentration of phenol increased due to inhibition effect of phenol at higher concentration. The present study reveals EM technology could be recommended as possible candidate for phenol biodegradation. Further studies with field trials will be helpful to exploit EM technology for organic pollutant degradation.

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