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Formulation of Effective Microbial Consortia and its Application for Sewage Treatment

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Abstract

The present study was conducted for sewage treatment using effective microbial consortium. The Effective Microorganisms (EM) like *Lactobacillus, Pseudomonas, Aspergillus, Saccharomyces* and *Streptomyces* were isolated from respective sources. The microbial consortium was formulated using molasses as medium at pH 3.8 and incubated at 37°C for 3 days. The sewage treatment was carried out with the addition of 3 ml/l EM solution under aerobic condition. The BOD, COD, TDS and TSS were reduced by 85%, 82%, 55%, and 91% respectively after 3 days of treatment. The results showed that the formulated EM was efficient for sewage treatment and thereby it reduced the environmental impact.

Keywords: Effective Microorganisms; Molasses; Optimization; Sewage; BOD; COD; TDS; TSS

Abbreviation: EM: Effective Microorganisms

Introduction

Sewage treatment is one of the major problems faced by municipalities. Sewage is the wastewater comprising 99.9% water and 0.1% solid particles. The domestic sewage has high amount of organic and inorganic pollutants [1]. The untreated sewage causes foul smell [2]. The improper disposal of sewage causes pollution and destroys the aquatic organisms due to high organic content and biological oxygen demand (BOD) concentration [3]. So, the sewage has to be treated to reduce the environmental impact. The chemically treated water causes harmful effects due to toxic chemicals than the organisms which are originally present in the sewage [4]. The organisms present in wastewater degrade organic matter [5] and helps for further treatment. In conventional treatment method, bacteria remove the organic content of wastewater but the solid particle remains as sludge. The sludge can be used as fertilizer or incinerated, disposed into ocean or landfill. The conventional sewage treatment processes are expensive to operate and maintain [6] and causes pollution.

Effective Microorganism (EM) is the consortia 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 [7]. But later, it has its application in wastewater treatment [8]. The EM has its wide application in the field of agriculture, natural farming, livestock, gardening, composting [9], bioremediation [10], algal control and prawn culture. The EM suppresses soil borne pathogen and pest, promotes plant growth, improves soil fertility and yield of crops and used as feed additive for livestock. The EM treated sludge is used as fertilizer and the EM treated waste water is used in crop production as it is enriched with beneficial microorganisms [11].

The EM secretes organic acids and enzymes which acts on sewage and degrades complex organic matter into simpler ones [12]. The antioxidant substances produced by EM enhances the breakdown of solids and reduces the sludge volume [13]. Missouri river in Jefferson City, North America was polluted by run off from industries and cities and generates foul odour. The application of EM for one month reduced the foul odour [14]. In Thailand, EM was sprayed 3 to 4 times on 3000-4000 metric tons of garbage which were dumped daily at a site just outside Bangkok in Ladkra Bhan. The EM reduced the foul odour and flies [10].

The EM used in this study comprises *Lactobacillus, Pseudomonas, Aspergillus, Saccharomyces* and *Streptomyces.* 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 actinomycete. The bioactive substance produced by yeast promotes plant growth. *Pseudomonas* releases bioactive compounds which act on the sewage and precipitates or detoxifies the metal. *Aspergillus* decomposes organic matter rapidly and produces alcohol, esters and antimicrobial substances. Actinomycete produces antimicrobial substances from amino acids derived from organic matter for suppressing harmful fungi and bacteria.

The main objective of this study was to develop low cost and eco-friendly sewage treatment process using effective microbial consortia.

Materials and Methods

Collection of samples

The respective samples were collected for isolation of various microorganisms. The curd sample was used for isolation of *Lactobacillus.* The oil spilled soil and moist soil at the depth of 10 cm was aseptically collected in a sterile polythene bag from VIT University, Vellore, Tamil Nadu for isolation of *Pseudomonas* and *Streptomyces,* respectively. The dry yeast granules were used for isolation of *Saccharomyces.* The boiled rice sample was maintained in closed container for 3 days under sterile condition until the fungal mat was observed and used as inoculum for isolation of *Aspergillus.* The samples were refrigerated at 4°C for further use.

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Isolation of effective microorganisms

The curd sample and oil spilled soil sample were serially diluted, 10^{-4}, 10^{-5} and 10^{-6} dilutions of sample were inoculated on de Man Rogosa Sharpe Agar and King's B Agar and incubated at 37°C for 24 hours to isolate *Lactobacillus* and *Pseudomonas,* respectively. The moist soil sample was serially diluted, 10^{-3}, 10^{-4} and 10^{-5} dilutions were inoculated on Kenknight's Agar and incubated at 37°C for 3 days to isolate *Streptomyces*. The obtained inoculum from rice was inoculated on Czapek's Dox Agar by hyphal tip technique and incubated at 28°C for 3 days to isolate *Aspergillus*. The loop full of inoculum was inoculated on Potato Dextrose Agar and incubated at 37°C for overnight period to isolate *Saccharomyces*. The obtained colonies were subcultured to get pure culture as described by Cappuccino and Sherman [15].

Characterization of effective microorganisms

The isolates were identified by morphological and biochemical studies. Biochemical tests like catalase test, oxidase test, IMViC test, sugar fermentation tests, Triple Sugar Iron test, urease test and hydrolysis tests were performed as described by Cappuccino and Sherman [15].

Formulation of EM

The isolated microorganisms were cultured together in a medium (molasses) at various pH, temperature and concentration of molasses. The optimal physical conditions for formulating EM was analysed by culturing microbial consortia at pH of 6.5-8, temperature of 28°C and 37°C and molasses concentration of 1-10%.

Sewage treatment using EM

The raw sewage sample was collected from VIT University, Vellore, Tamil Nadu. The floating particles were removed from sample and collected in a clean container. The container was washed using sodium hypochlorite and water followed by rinsing of sample before collection. 20 litres of sewage water was collected, divided into six equal parts and maintained one as control and rest five for inoculating different concentrations of EM. The pH, total dissolved solids (TDS), total suspended solids (TSS), biological oxygen demand (BOD) and chemical oxygen demand (COD) of sample were analysed according to the standard protocol of APHA [16] within 2 hours of collection. Then the formulated EM solution was added to sewage at various concentration ranged from 1-10 ml/l. The EM inoculated water was analysed daily to determine the effect of EM in treating sewage.

Statistical analysis

All the experiments were done in triplicates. The data was analysed statistically using Microsoft Excel 2007 and reported as mean ± standard deviation (SD).

Results and Discussion

Characterization of EM

The isolated microorganisms were characterized according to Bergey's manual (Table 1). Erdogrul and Erbilir [17] stated that *Lactobacillus* is gram positive rods, catalase and oxidase negative. *Pseudomonas* was identified as gram negative motile rods and showed positive for catalase, oxidase and citrate tests [18]. Praveen and Jain [19] reported that *Streptomyces* is gram positive rods and can hydrolyse casein. The species of *Streptomyces* exhibited variation in colour of substrate mycelium depending on the media composition [20].

Formulation of EM

Effect of pH and temperature: The growth of EM was observed at pH of 6.5 to 8 and temperature of 28°C and 37°C. The *Pseudomonas* may grow in a wide pH range of 4-10 at 27°C and 37°C but the optimal condition is pH 8 and 37°C [21]. The fungal species isolated from Antarctic soil was observed to grow at temperature between 4°C and 35°C and exhibited variation in growth pattern [22]. Praveen and Jain [19] stated that *Streptomyces sampsonii* shows its growth at pH of 5-10 and temperature of 15-42°C.

Effect of molasses concentration: The growth of microbial consortia was observed at various molasses concentration of 1-10%. The lowest concentration of molasses facilitated the growth of EM and the increased concentration inhibited the growth and survival of EM. It is observed from Table 2 that 1% to 3% of molasses is favourable for

Characteristics	*Lactobacillus*	*Pseudomonas*	*Saccharomyces*	*Aspergillus*	*Streptomyces*
Colony morphology	White, mucoid	Fluorescent, mucoid	Creamy white, mucoid, smooth	Black fuzzy mat	White, dry, powdery
Cell shape	Rods	Rods	Ovoid budding cells	Conidia arising from conidiophore	Filamentous rods
Gram stain	Gram positive	Gram negative			Gram Positive
Motility	Non-motile	Motile			
Catalase	-	+			
Oxidase	-	+			
Indole production	-	-			
Methyl Red	-	-			
Voges-Proskauer	-	-			
Citrate utilization	-	+			
Glucose fermentation	+	-	+	-	
Lactose fermentation	+	-	+	-	
Sucrose fermentation	+	-	+	-	
Triple Sugar Iron	Acid butt, acid slant	Alkaline butt, alkaline slant			
Urease	-	+			
Casein hydrolysis					+
Tyrosine hydrolysis					+
Xanthine hydrolysis					+

+ = positive, - = negative

Table 1: Characteristics of effective microorganisms.

EM. The growth of *Lactobacillus* and *Saccharomyces* was observed even at highest molasses concentration of 10%. The growth inhibition may be due to osmotic pressure created by molasses.

Effect of incubation period: The incubation period has greatest effect on microbial consortia formulation. At longer incubation period, the growth of microorganisms was inhibited due to depletion of nutrients, accumulation of toxic end products and change in pH. The optimal incubation period was 72 hours as growth of all the five organisms was observed (Table 3).

The pH is an important parameter for preparation of EM solution. Figure 1 depicts the variation in pH of EM solution during incubation. The pH was decreased from 7 to 2.9 in 5 days of incubation by fermenting the molasses. After 5 days of incubation, the pH was constant as the organisms utilised the entire energy source and there was no further growth of organisms. The organisms was not able survive at high acidic pH; hence EM solution was used after 3 days of incubation (pH 3.8).

Analysis of EM treated sewage

Biological oxygen demand: The EM reduced the BOD of sewage from 374.5 to 55.9 mg/l with mean reduction of 85%. The EM showed the effective result when compare to control while treated at a concentration of 3 ml/l for 3 days. The control showed the decrease in BOD from 374.5 to 248.6 mg/l in 5 days (Figure 3). The acetogenic bacteria strain BP103 reduced the BOD by 58.5–82.2%

Name Of Organism	1%	2%	3%	4%	5%	10%
Lactobacillus	+	+	+	+	+	+
Pseudomonas	+	+	+	+	-	-
Aspergillus	+	+	+	+	+	-
Saccharomyces	+	+	+	+	+	+
Streptomyces	+	+	+	-	-	-

\+ = present, - = absent

Table 2: Effect of molasses concentration.

Name Of Organism	Day 1 (24 hrs)	Day 2 (48 hrs)	Day 3 (72 hrs)	Day 4 (96 hrs)	Day5 (120 hrs)
Lactobacillus	+	+	+	+	+
Pseudomonas	+	+	+	-	-
Aspergillus	+	+	+	+	-
Saccharomyces	+	+	+	+	+
Streptomyces	-	+	+	-	-

\+ = present, - = absent

Table 3: Effect of incubation period.

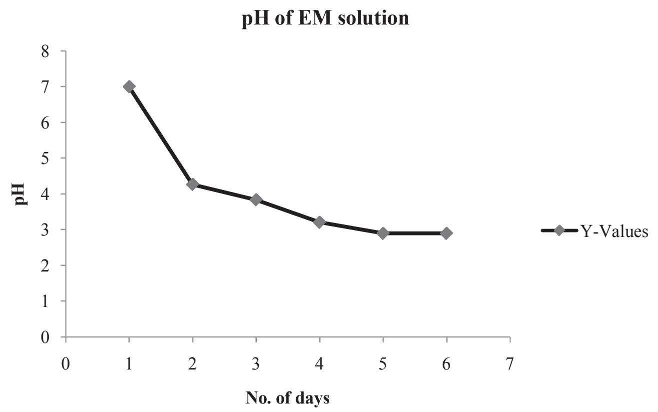

Figure 1: Impact of incubation period on pH of EM solution.

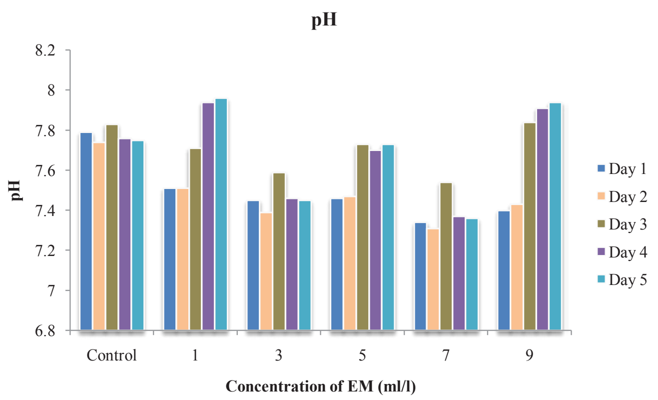

Figure 2: Effect of EM treatment on pH of sewage.

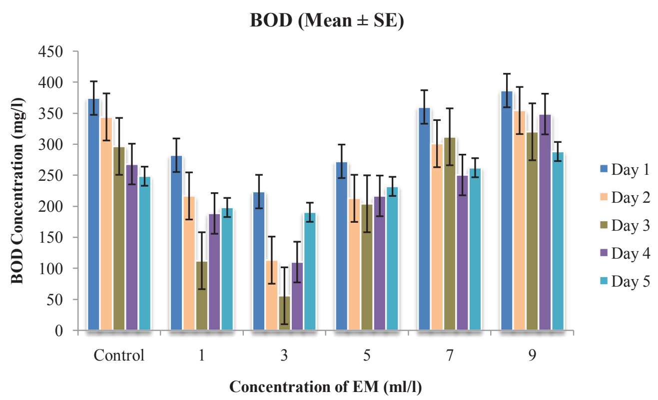

Figure 3: Effect of EM treatment on BOD reduction of sewage.

[23]. Mongkolthanaruk and Dharmsthiti [24] formulated bacterial consortium including *Pseudomonas, Bacillus* and *Acinetobacter* using molasses for treating lipid rich wastewater and the consortium reduced BOD from 448 to 72 mg/l. Kumar [25] used the bacterial consortium of *Pseudomonas aeruginosa*, *Bacillus megaterium* and *Stenotrophomonas maltophilia* for treating paper and pulp mill effluent and observed BOD reduction from 87 to 89%.

Chemical oxygen demand: The EM reduced the COD of sewage from 570.4 to 99.8 mg/l with mean reduction of 82%. The EM reduced the COD effectively while treated at concentration of 3 ml/l for 3 days. The control showed the decrease in COD from 570.4 to 409.3 mg/l in 5 days (Figure 4). The EM reduced the COD of wastewater from Nestle and Trebor companies to 76% in 11 days at a concentration 1 ml/l [26]. The acetogenic bacteria strain BP103 reduced the COD by 35.5–71.2% [23]. Stanley [27] reported that whey disposed from cheese manufacturing industry was treated using Kluyveromyces fragilis which reduced the COD by 29% and 37% in 16 and 20 hours, respectively after the growth of culture. Kumar [25] used the bacterial consortium of *Pseudomonas aeruginosa*, *Bacillus megaterium* and *Stenotrophomonas maltophilia* for treating paper and pulp mill effluent and observed COD reduction from 67% to 71%. The consortium of five white-rot fungi, *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Lentinus edodes*, *Trametes versicolor* and S22 removed 71% of lignin content and 48% of COD from wastewater [28].

Total dissolved solids: The EM reduced the TDS of sewage from

2460 to 1084 mg/l with mean reduction of 55%. The EM showed the effective reduction of TDS while treated at concentration of 3 ml/l for 3 days. The control showed the decrease in TDS from 2460 to 2309 mg/l in 5 days (Figure 5).

Total suspended solids: The EM reduced the TSS of sewage from 486.6 to 43.3 mg/l with mean reduction of 91%. The EM showed the effective reduction of TSS while treated at concentration of 3 ml/l for 3 days. The control showed the decrease in TSS from 486.6 to 433 mg/l in 5 days (Figure 6). The acetogenic bacteria strain BP103 reduced the total solid content by 59.1% [23]. Okuda and Higa [8] used EM to reduce the total solid content of wastewater by 94%.

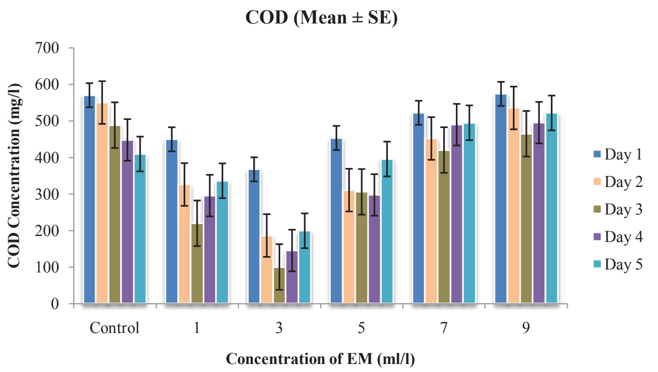

Figure 4: Effect of EM treatment on COD reduction of sewage.

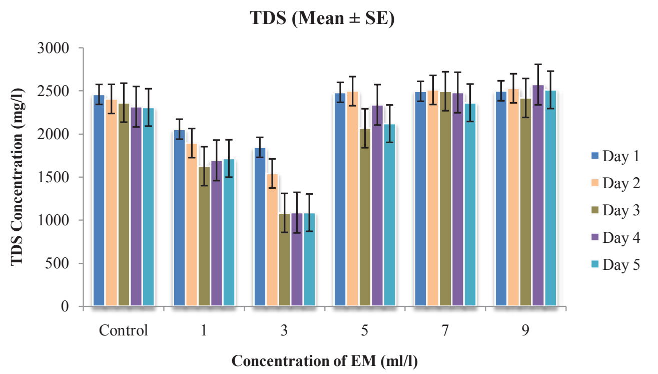

Figure 5: Effect of EM treatment on TDS reduction of sewage.

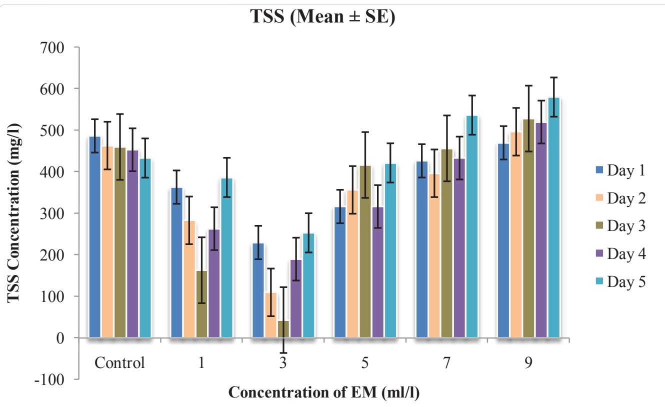

Figure 6: Effect of EM treatment on TSS reduction of sewage.

pH: The EM did not show any considerable change in pH of sewage. The fluctuation in pH was due to the natural environmental factors (Figure 2).

At higher concentration of EM, the BOD and COD was increased due to high microbial population. Hence 3 ml/l is the efficient concentration of EM for the effective treatment of sewage. After 3 days of treatment, the dissolved oxygen was decreased due to depletion of nutrients. So the treated water has to be left for chlorination.

The white rot fungi and brown rot fungi in presence of glucose reduced the BOD and COD of wastewater. If *Streptomyces* is cultured along with these fungi there was increase in the decolourisation to 85% [29]. The microorganisms exhibit efficient treatment in consortium than the sole organism.

The COD, BOD, TDS and TSS reduction of domestic wastewater by sedimentation, aeration, activated sludge and sand filter was 92.17%, 97.66%, 32.38% and 97.58%, respectively [30]. The sludge released by these process causes environmental impact and also it is expensive. But there is no release of sludge in EM treatment and the sewage can be treated economically.

Conclusion

The Effective Microbial consortium was formulated and its efficiency for sewage treatment was studied. The results showed that the EM treatment of sewage reduced BOD, COD, TDS and TSS by 85%, 82%, 55% and 91% respectively. The malodour and turbidity of sewage was reduced. The treatment process is highly viable and economical. The EM treated water is non-toxic and safe to dispose as it contains beneficial microorganisms. The EM reduces the environmental impact of conventional methods.

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