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Formulation of Effective Microorganism [EM] to Analyse its Impact on Municipal Wastewater Management

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ABSTRACT

Discharging wastewater from households, industries, and other sources into the environment without treatment hurts the ecosystem as well as people's quality of life by causing negative impacts on all living species, including health concerns to humans such as diarrhoea which kills 3,50,000 Indian children annually and only 33% of India's urban wastewater is treated. The majority of energy is dedicated solely to the aeration process, which regulates the degradation of organic matter (sludge) found in wastewater, which is one of the most critical issues in wastewater treatment. As a result, the goal of this research is to develop long-term strategies for degrading organic materials using Effective Microorganisms Technology (EM). EM, also known as Effective Microorganism, is a carefully selected and proportioned liquid culture of fermenting microorganisms. In this study, three microorganisms were isolated from various sources, including soil, yogurt, and milk by serial dilution and spread plate technique, each isolate was made into a pure culture after incubation. They were identified by the VITEK method as *Pseudomonas aeruginosa*, *Lactobacillus sporogenes*, and *Candida albicans*. The microbial consortium was then formed by incubating all three organisms in a nutrient broth that had been modified. Each microorganism culture and formulated microbial culture were added to sewage samples taken in Erlenmeyer flasks. All samples were tested for physico-chemical parameters such as pH, BOD, COD, TDS, TSS, SO_4^{2-} and Cl- after a three-day incubation period at room temperature. The standard deviation method was used for statistical analysis and Microsoft Power BI was used to visualize the results. *Lactobacillus sporogenes* was the only culture that showed a significant reduction in all parameters except one when compared to other cultures that showed a reduction in most parameters except a few. As a result, *Lactobacillus* proved to be effective in wastewater treatment when compared with other cultures. Further research is required to determine its true potential.

Key words : *Effective microorganism technology, Wastewater treatment, Sustainability, Bioremediation, Microbial consortium.*

Introduction

According to the Central Pollution Control Board [CPCB] report 'Status of Sewage Treatment in India', Out of the total amount of sewage generated, only 21.3 percent is treated. Disposal of untreated sewage is the major cause of water pollution in India. As a result, water pollution management is a major concern today, and new technologies must emerge to speed up the sewage treatment process which must be both cost-effective and environmentally responsible. Bioremediation is the use of living

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organisms to remove pollution from the environment. To bio-remediate sewage water, effective microorganism technology can be used as one of the methods. Effective Microorganisms [EM] is a liquid culture of beneficial microorganisms primarily photosynthetic bacteria such as *Rhodopseudomonas palustris* and lactic acid bacteria such as *Lactobacillus plantarum* and yeasts, for example, *Saccharomyces cerevisiae* and other microorganisms (Higa 1991). It was initially developed to boost soil activity. Today, it is widely used in a variety of applications such as wastewater management, solid waste management, animal husbandry, organic farming, and many others. The EM principle is the transformation of a degraded ecosystem containing harmful microorganisms into one with beneficial microorganisms. It secretes various enzymes, organic acids, and other substances to break down the sludge in wastewater, reduces its volume, and also reduces foul odour by destroying the putative microorganisms present in the sludge (Higa, 1998).

The use of effective microorganisms in wastewater treatment can reduce the levels of BOD, COD, TSS, TDS, and other unwanted substances (S. Karthick Raja Namasivayam *et al.*, 2011). Domestic wastewater treated with EM showed reductions in physicochemical and biological parameters (Doraipandian Kannan *et al.,* 2012). A microbial consortium of effective microorganisms [EM] was developed, and it demonstrated efficacy in sewage water treatment (Monica *et al.,* 2011).

In this study, three groups of microorganisms were isolated and formed into a consortium, and their efficacy in municipal wastewater treatment was investigated.

Materials and Methods

Sample Collection

In order to isolate various kinds of microorganisms, different samples were used. Rhizosphere soil was collected in a sterile plate for the isolation of *Pseudomonas* species. Milk and yogurt were used to isolate *Lactobacillus* and *Candida* species.

Isolation of Effective Microorganisms

All the samples were serially diluted in 10- fold dilutions and the spread plate technique was done by 0.1ml of serially diluted soil sample onto *Pseudomonas* isolation agar.

Fig. 1. Microbial cultures

Next, 0.1 ml of serially diluted yogurt and milk sample were spread onto Lactobacillus MRS agar and Sabouraud dextrose agar onto the respective plates. All the plates were incubated at 37 °C for 24 hours. After incubation, each of the isolated colonies were sub-cultured to obtain the pure cultures respectively.

Identification of Effective Microorganisms

Preliminary studies were done by performing gram staining technique to identify morphology. And all the isolates were identified by the VITEK method.

Formulation of Effective Microorganisms Consortium

The nutrient broth was modified by adding dextrose onto it to support the coexistence of these microorganisms in the same culture. All the microorganisms were inoculated separately onto the broth and incubated at 37 °C for 24 hours. After incubation, 5 ml from each of the cultures were transferred onto fresh broth together and incubated at 37 °C for 24 hours. Then optical density for all the cultures was measured using a colorimeter and found to be 0.80 for *Pseudomonas aeruginosa*, 0.68 for *Lactobacillus sporogenes,* 1.15 for *Candida albicans*, and 0.82 for Effective Microorganisms consortium.

Treatment of sewage using Effective Microorganisms and their formulation

A sewage sample was collected from Chennai Metropolitan Water Supply and Sewerage Board [CMWSSB]. 500 ml of sewage sample was taken in a series of 12 sterile Erlenmeyer flasks containing triplicates for each of the cultures [R1, R2&R3]. To all the flaks, 50 ml of appropriate cultures were added and incubated at room temperature for 3 days. After incubation, all the samples were analysed for physicochemical parameters like pH, BOD (Biochemical Oxygen Demand), COD (Chemical Oxygen Demand), TDS (Total Dissolved Solids), TSS (Total Suspended Solids), SO_4^{2-} (Sulphate), and Cl- (Chloride) as per APHA 23rd standards edition 2017.

Fig. 2. Experimental Setup

Data Visualization and statistical analysis

The data obtained from wastewater analysis was statistically analysed by using standard deviation (SD) and it was visualized using Microsoft Power BI.

Results and Discussion

Following the isolation of microorganisms using the spread plate technique, preliminary studies were conducted using the gram staining technique, which revealed Gram-positive Bacilli and Gram-negative Bacilli. By performing the VITEK method, they were identified as *Lactobacillus sporogenes*, *Pseudomonas aeruginosa*, and *Candida albicans*. After treating wastewater with EM for 3 days at room temperature, Physicochemical parameters of wastewater such as pH, BOD, COD, TDS, TSS, Cl-, SO_4^{2-} were analysed. All experiments were performed in triplicate, the mean was calculated and statistical analysis was performed using standard deviation. Comparisons of before and after treatment with EM culture were performed and they were visualized using Microsoft Power BI. From the data, it was found that *Lactobacillus sporogenes* culture (Figure 4) significantly reduced all parameters, BOD decreased from 390mg/l to 167.3 mg/l with a mean reduction of 57%, COD decreased from 1186 mg/l to 680mg/l with a mean reduction of 42%, TSS decreased from 97 mg/l to 63.7 mg/l with a mean reduction of 34% but Cl- increased from 335 mg/l to 373 mg/l with a mean increase of 11%. *Pseudomonas aeruginosa* culture (Figure 3) reduced most but a few parameters, BOD decreased from 390 mg/l to 358 mg/l with a mean reduction of 8.2%, TSS decreased from 97mg/l to 55.3 mg/l with a mean reduction of 42%, COD increased from 1186 mg/l to 1624 mg/l with a mean increase of 36% and Cl- increased from 335 mg/l to 360.7 mg/l with a mean increase of 7.6%. *Candida albicans* culture (Figure 5) reduced BOD, TSS, and SO_4^{2-} by 5.6%(390 to 277.7 mg/l), 38.4%(97 to 59.7mg/l), 41.9% (31 to 18 mg/l),

But COD and TDS increased by 13.8% (1186 to 1350 mg/l) and 7.6%(1283 to 1381 mg/l) respectively. In the microbial consortium (Figure 6), except for COD, TDS, and Cl-, all other parameters were reduced. BOD decreased by 1%(390 to 384 mg/l), TSS decreased by 32.6%(97 to 65.3 mg/l), and SO_4^{2-} by

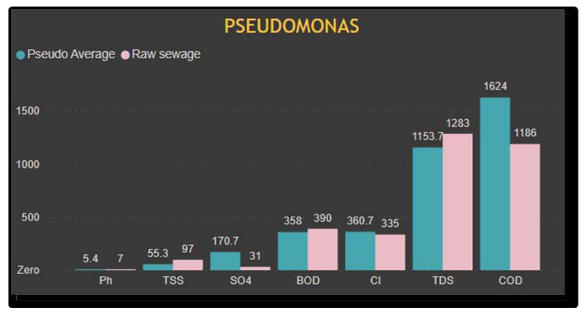

Fig. 3. Comparison of *Pseudomonas aeruginosa* before and after Treatment.

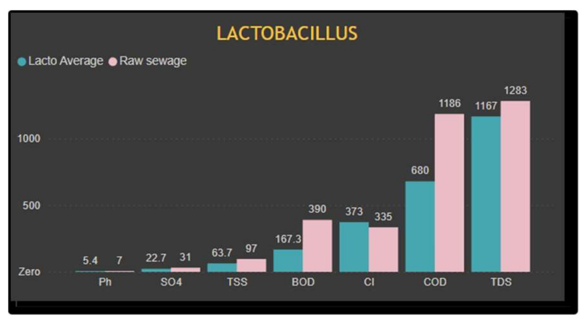

Fig. 4. Comparison of *Lactobacillus sporogenes* before and after Treatment.

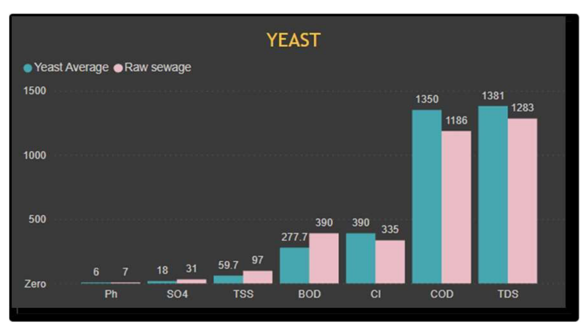

Fig. 5. Comparison of *Candida albicans* before and after Treatment.

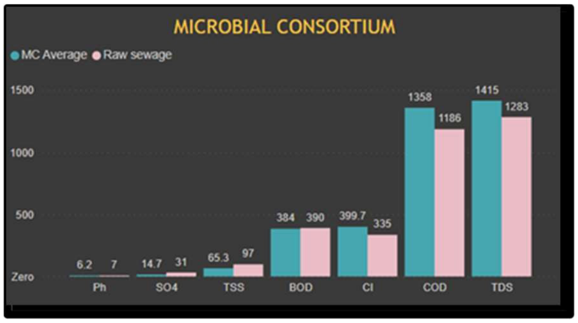

Fig. 6. Comparison of microbial consortium before and after Treatment.

52.6% (31 to 14.7 mg/l). Overall, COD decreased in the *Lactobacillus* treatment, TDS decreased with *Lactobacillus* and *Pseudomonas* treatment, SO_4^{2-} decreased in all treatments except *Pseudomonas*, and Cl- increased in all treatments. It is a fundamental study that used condensed versions of more in-depth ones. The potential causes for the increase in some parameters include the concentration of EM used and their interactions with native microorganisms, only one concentration of EM was used in this study so there may be either an increase in microbial population which in turn may contribute to increasing in parameters like COD, Cl-, TDS, and SO_4^{2}-.

Szymanski Monica *et al.* (2011) developed a microbial consortium and investigated its use in sewage treatment and discovered that EM treatment of sewage considerably reduced BOD, COD, TDS, TSS, and other contaminants Szymanski *et al.* (2003) concluded that when applied for solid treatment on septic tanks, EM had very little effect on reducing suspended solids and some effect on some parameters. Finally based on this study, the conclusion was drawn that among the formulation of Effective Microorganisms, *Lactobacillus sporogenes* has a significant impact on almost all parameters so it is considered effective in wastewater bioremediation and the other two microorganisms, *Pseudomonas aeruginosa*, and *Candida albicans* showed a reduction in some parameters while increasing in others. Further research is needed to determine the true effectiveness of EM.

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