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# BIOREMEDIATION OF SEWAGE USING SPECIFIC CONSORTIUM OF MICROORGANISMS

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## ABSTRACT

Municipal sewage problems are more complex as the volume of the wastewater is large and it requires huge area for treatment. The objective of this work is to evaluate a set of microbial consortium for domestic wastewater treatment. Lab scale bioreactors using specific consortium was applied for the domestic wastewater treatment. The study was conducted to test individual abilities of microbial genera and as consortia with an ultimate aim of achieving safer environmental standards. However, the key component of the research study is to confirm the role of microbial flora in treatment of municipal sewage.

**KEYWORDS:** Ministry of Urban Development, ASP, UASB

## INTRODUCTION

The shortage of water resources has become a globally serious problem. Sewage after appropriate treatment and the reuse of the recycled water have become the consensus of all over the world (Qin *et al.,* (2013). With growing population, advanced agricultural practices, industrialization, urbanization and multiple use of water has increased the demand for water. Due to daily human activity and also various agricultural and industrial operations, wastewater is produced in enormous quantity. Due to lack of proper management and treatment facilities most of the urban wastewater generated in Indian cities is discharged into natural aquatic systems without treatment. According to the manual of the Central Public Health and Environmental Engineering Organization (CPHEEO), Ministry of Urban Development, in India the per capita per day water requirement is 100 lpcd. Domestic wastewater treatment has become a remarkable aquatic environmental problem for all over the world. Due to non availability of cheaper methods and higher cost of treatment plants, municipalities are releasing untreated domestic wastewater in to aquatic bodies like ponds and lakes, where it is causing eutrophication (Patel and Kanungo 2012). The treatment of wastewater is accomplished by four basic methods or techniques i.e. physical, mechanical, biological and chemical. Most of the wastewater is treated in industrial scale wastewater treatment plants (WWTPs) which may include physical, chemical and biological treatment processes (Narmada and Mary selvam kavitha (2012).

According to Pradip *et al.* (2012), Conventional wastewater treatment technologies such as Activated Sludge Process (ASP), Up-flow Anaerobic Sludge Blanket React (UASB) and other land based treatment technologies are presently used in India. Sarala (2012), reported that electrocoagulation method is promising in wastewater treatment. The objective of the sewage treatment is to produce a disposable effluent without causing harm to the surrounding environment and also prevent pollution (Khopkar, 2004).

According to Ottengraf (1983), the great advantage of the heterogeneous microbial population present in the biofilter is the excellent ability to survive long periods (up to 2 months) without activity, provided that periodic aeration is ensured. Adamse *et al.* (1984), stated that the composition of activated sludge treating municipal sewage comprises *Pseudomonas, Flavobacterium, Alcaligens, Acinetobacter and Zooglea sp.* Other researchers have found *Pseudomonas,*

*Acinetobacter sp* and *Enterobacteriaceae* to be the dominant bacteria (Kappesser *et al*., 1989). Under oxygen restriction conditions, the dominant species are from the genera *Acinetobacter, Aeromonas* and *Flavobacterium* (Autheunisse and Koene, 1987). *Nitrosomonas* and *Nitrobacter* are chemolithotrophic bacteria present in activated sludge process (Hughes and Stafford, 1976). According to Narmada and Mary selvam kavitha (2012), the main species involved in effective waste water treatment include Lactic acid bacteria - *Lactobacillus plantarum, L. casei, Streptoccus lacti,* Photosynthetic bacteria – *Rhodopseudomonas palustrus, Rhodobacter spaeroide,* Yeasts – *Saccharomyces cerevisiae, Candida utilis,* Actinomycetes – *Streptomyces albus, S. griseus* and Fermenting fungi – *Aspergillus oryzae, Mucor hiemalis* etc.,

## MATERIALS AND METHODS

Sewage sample was collected according to standard procedures from APHA (1998). Microorganisms with unique characteristics of degradation, Bio-remediation and transformation were selected and obtained from NCL, Pune and IMTECH, Chandigarh. In the present study, *Bacillus megatherium* (NCIM 2104), *Nitrobacter sps.,* (NCIM 5062), *Nitrosomonas sps.,* (NCIM 5071), *Pseudomonas denitrificans* (NCIM 2038), *Chromatium sps.,* (NCIM 2336), *Bacillus mucilaginosus* (NBDC), *Lactobacillus acidophilus* (NCIM 2285), *Rhodococcus terrae* (NCIM 5126), *Bacillus licheniformis* (MTCC 2450), *Thiobacillus ferrooxidans* (MTCC 2361) were used as candidates in the consortium.

Various physico chemical parameters were considered for the present study. They are pH, temperature, electrical conductivity, total solids, total suspended solids, total dissolved solids, alkalinity, hardness, chlorides, oil and grease, sludge volume index (SVI), chemical oxygen demand (COD), biological oxygen demand (BOD), Nitrogen – total nitrogen (TN), nitrites ($NO_2$), nitrates ($NO_3$) and ammonia ($NH_3$), phosphorus (as 'P'), sulphides ($H_2S$). The sewage sample was analyzed for fifteen successive days to standardize the values. Analysis was done according to American Public Health Association methods (APHA, 1998). The physico-chemical parameters are vary day to day. Because, sewage flows from residential dwellings, commercial establishments and other facilities where individual water using activities create an intermittent flow of wastewater that can vary widely in volume and degree of pollution. Standard deviation was calculated for all the parameters and results were tabulated.

Bacterial adaptation studies were carried out using bacteria grown in respective media i.e., nutrient broth, *Nitrobacter* medium, *Nitrosomonas* medium, MRS medium and *Thiobacillus* medium in increasing concentration of sterilized sewage. The various concentration of sterilized sewage includes 5 %, 10 %, 20 %, 30 %, 40% 50% 60%, 75%, 90% and 100%. The transfer of culture from one concentration to the next was made when the respective bacteria was in the exponential phase of growth.

Cultures were screened for tolerance in sterilized sewage. The active cultures containing respective quantities of nutrient media and sterilized sewage were incubated in erlenmeyer flasks (250 ml) at 30$^{o}$C, 200 rpm for 12 hours. The turbid sample (3 ml) was pipetted aseptically for $OD_{600}$ measurements. When the culture has reached exponential phase, the culture was transferred into erlenmeyer flasks (250 ml) containing sterilized sewage in increased concentration at 30$^{o}$C, 200 rpm.

Bacterial survival rate was determined in sterilized sewage by serial dilutions of the bacterial cultures obtained from sterilized sewage fed broths were made and 0.1 ml each of the respective cultures was plated onto nutrient agar plates using the spread plate technique. The plates were then incubated at 30$^{o}$C for 24 hours before enumeration of the colonies were formed.

Sewage sample was collected and transferred to 10 plastic systems (tub) of 7 litres capacity. Adapted cultures were grown in sewage mixed nutrient broth. Cultures, each of 7ml was transferred to respective system containing raw sewage. One system containing was raw sewage was not inoculated with any culture and kept it as blank. The process was allowed for 24 hours. Efficiency of microorganism for pollutant removal was considered based on the BOD removal efficiency. Based on the removal efficiency a consortium of individual microorganisms was prepared in specific ratio to use as inoculums for further experimentation.

For optimization studies, four identical rectangular reactors were designed and fabricated with galvanized iron sheet. The size of the reactor was 66,600 $cm^3$. The length, breadth and height were predetermined and they are 60 cm, 30 cm and 37 cm respectively. Each reactor was open type and designed to accommodate the volume of 50 litres of sewage sample and more than 16 litre of air. 50 litres of sewage sample was considered as working sample. In the present study out of four reactors one reactor was used as blank and remaining three were used as triplicate for sewage sample. The experimental design was batch type and work was carried out one after the other for each variable parameter of the work.

Optimization studies were conducted for concentration of inoculum, hydraulic retention time for sewage remediation. For determining the optimum culture or inoculum concentration different concentration of inoculums (0.05%, 0.1%, 0.2%, 0.3%, 0.4% and 0.5%) were taken and added to reactors containing raw sewage sample. Culture was not added to reactor which was kept as blank.

Reactors were filled with fresh sewage sample. Pure cultures of inoculum (consortium) were added to three reactors and one reactor was kept as blank. 50ml of fresh inoculum was prepared using sterilized sewage fed broth and used for experimentation. In the present study, settling tank was not used hence, the volume of reactor Vt is equal to volume of reactor Vr.

After 24 hours of incubation, sample was withdrawn and analyzed for various physico-chemical parameters by American Public Health Association (APHA, 1998) standard methods. The percentage of biological oxygen demand (BOD) removal was determined.

The Hydraulic retention time (HRT) also known as hydraulic residence time or t (tau), is a measure of the average length of time that a soluble compound remains in a constructed bioreactor.

Hydraulic retention time is the volume of the aeration tank divided by the influent flow rate:

$$HRT = \frac{\text{Volume of aeration tank}}{\text{Influent flow rate}}$$

Mathematically it can be expressed as $\theta = V_r / Q$

$V_r$ = Volume of the reactor

Q = Influent flow rate

where using SI Units volume is in [$m^3$] and influent flow rate is in [$m^3/h$]. HRT is usually expressed in hours (or) sometimes in days.

Reactors were filled with fresh sewage sample. Optimized concentration of inoculum was added to the experimental reactor and one reactor was kept as blank. Samples were withdrawn at every six hour intervals i.e., 6 hours,

12 hours, 18 hours and 24 hours. Samples were analyzed for various physico-chemical parameters and results were tabulated.

## RESULTS AND DISCUSSIONS

Sewage sample was collected and analyzed for 15 successive days. The mean and standard deviation were calculated for all parameter values. The physico-chemical parameters of the raw sewage are as follows: pH was ranging from 7.1 – 8.05 with mean of 7.48 and standard deviation of $\pm$ 0.28. Electric conductivity was ranging from 2300 – 2900 MMhos/cm$^2$ with mean of 2564 and standard deviation of $\pm$ 201.28. Temperature was ranging from 22 – 29$^0$C with mean of 25.7 and standard deviation of $\pm$ 2.02. Total suspended solids were ranging from 345 – 410 mg/lit with mean of 376.26 and standard deviation of $\pm$19.65. Volatile suspended solids were ranging from 113 – 170 mg/lit with mean of 149.53 and standard deviation of $\pm$ 17.22. Chlorides were ranging from 125 – 201 mg/lit with mean of 165.66 and standard deviation of $\pm$ 21.76. Total hardness was ranging from 355 – 455 mg/lit with mean of 386.33 and standard deviation of $\pm$ 28.37. Alkalinity was ranging from 400 – 560 mg/lit with mean of 494.2 and standard deviation of $\pm$ 49.80.

COD was ranging from 460 – 550 mg/lit with mean of 504.66 and standard deviation of $\pm$ 25.88. BOD was ranging from 110 – 220 mg/lit with mean of 173.2 and standard deviation of $\pm$ 37.62. Total Nitrogen was ranging from 39.4 – 56.1 mg/lit with mean of 46.84 and standard deviation of $\pm$ 6.04. Total Phosphorus was ranging from 5.92 – 12.0 mg/lit with mean of 8.53 and standard deviation of $\pm$ 1.9. Oil and grease was ranging from 28 – 50.4 mg/lit with mean of 34.42 and standard deviation of $\pm$ 7.85. Sludge volume index was ranging from 110 – 140 ml/g SS with mean of 127.13 and standard deviation of $\pm$ 7.85.

For isolating the bacteria from adapted nutrient culture broth (fed with 90% sterilized sewage) the nutrient agar plates were inoculated with 1ml inoculum from $10^{-8}$ dilution by pour plate method. The samples were observed for growth after 24 hours of incubation. Bacterial colonies (viable count) were observed in nutrient agar plates and *Nitrobacter* sps., showed more colony forming units i.e. 45 X $10^8$ when compared to other microorganisms in the present study. It was followed by *Nitrosomonas* sps., *Pseudomonas denitrificans*, *Bacillus mucilaginosus*, *Chromatium* sps., *Bacillus licheniformis*, *Bacillus megatherium*, *Rhodococcus terrae*, *Lactobacillus acidophilus*. But *Thiobacillus ferrooxidans* has not shown any colony on agar plate. *Thiobacillus ferrooxidans* grows at pH 2.5 and it might be the reason for absence of growth on nutrient medium plates of near to neutral pH.

The purpose of adaptation is to allow bacterial community to rapidly adapt to their new environment (Eva and Springaely, 2003). There are several mechanisms, or combinations by which microbial communities can adapt to their environment. Firstly, there can be an increase in population size of those organisms that tolerate or even degrade the compound by induction of appropriate genes. Secondly, the cells can adapt through mutations of various kinds, such as single nucleotide changes or DNA rearrangements that result in resistance to or degradation of the compound. Thirdly, they may acquire genetic information from either related or phylogenetically distinct populations in the community.

### Performance of Individual Microbial Species

The studies on bioremediation capabilities each microorganism in terms of BOD removal reveals that, BOD reduced by 41.67% in the presence of *Bacillus megatherium*, 46.34% in the presence of *Nitrosomonas sp.,* 41.43% in the presence of *Nitrobacter sps.*, 43.3% in the presence of *Pseudomonas denitrificans*, 37.14% in the presence of *Chromatium* sps., 43.10% in the presence of *Bacillus mucilaginous*, 33.79% in the presence of *Lactobacillus acidophilus*, 36.41% in the presence of *Bacillus licheniformis*,28.26% in the presence of *Rhodococcus terrae* and 5.23% in the presence of *Thiobacillus ferrooxidans.*

Phosphorus is recognized as one of the major nutrients required by living organisms, involved in vital physiological process. At the same time it can also be considered as a pollutant in the concentration are high under specific environmental conditions. It contributes to increase eutrophication process of lakes and natural waters (Usharani *et al.*, 2009). The possible entry of this ion into aquatic environment is through household sewage water. Min jin *et al*. (2005) reported 90% of COD removal efficiency using microorganism *Bacillus megatherium* as candidate in the consortium for bioremediation of sewage. Usharani *et al*. (2009), observed the phosphate removal efficiency of 38-55% by *bacillus sps.,* from wastewater. Usharani *et al*. (2009), further reported that there is a 92.5% of phosphate removal by using consortium which include *Bacillus* sps., *Pseudomonas* sps., and *Enterobacter* sps., Ioana *et al*. (2010), *Bacillus megatherium* has bio-accumulative properties of some heavy metals such as lead, arsenic, cadmium are of biosolubilization of phosphate, silica etc., Min Jin *et al*., (2005), reported that the ammonical nitrogen was removed with the efficiency rate of 99% by using the organism *Nitrobacter europea* at the rate of 2.5 X $10^6$ and *Nitrobacter winogradskyi* at the rate of 4.5 X $10^5$. In nitrification, nitrosofying-bacteria (e.g. *Nitrosomonas*) oxidize ammonia to nitrite and nitrofying-bacteria (e.g. *Nitrobacter*) oxidise the nitrite to nitrate. Nitrifying bacteria are slow growing (under optimum conditions Td = 8 h for *Nitrosomonas*, 10 h for *Nitrobacter* (Bock *et al*., 1986) and are therefore easily washed out of conventional suspension culture systems, such as activated sludge, where the prevailing conditions often result in doubling times of 1-3 days. Hence, to operate a high rate nitrification process, some form of biomass retention is required. Although biomass retention is the chief operational characteristic of traditional trickling filters, a high cell concentration cannot be achieved because of the large, inactive volume occupied by the biomass support material. If the Td (doubling time of microorganisms) is more than 4hours or 8 hours then the quality of the wastewater may not change significantly in a stipulated time. Hence it is essential to add suitable external micro flora to the reactor to achieve parameters of desired levels.

The denitrifying microorganisms reduce nitrite and nitrate to molecular nitrogen. Margarida *et al*., (2003) achieved 72-84% of nitrogen removal from the treatment reactor with the help of denitrifying microorganisms. Holm and Vennes (1970), reported that 24.45 % decrease of BOD, 100% removal of $H_2S$ from 2mg/litre concentration to zero in the sewage treatment using purple sulfur bacterial population. According to Deng *et al*., (2003) *Bacillus mucilaginosus* produce heat stable polysachharide biofloculant. Bacteria can utilize the nutrients in the culture medium to synthesize high molecular weight polymers internally within the cell under the action of specific enzymes and these polymers can be excreted and exist in the medium or on the surface of the bacteria as capsule. Hence, the action of bacteria converts the simple substances in their environment into complex polymers that can be used as flocculant. In wastewater treatment, flocculation is an easy and effective method of removing suspended solids (SS). Since bioflocculants can be nontoxic, harmless and without secondary pollution, they have a great potential for use in those industries.

The removal efficiency of BOD from wastewater by *Lactobacillus acidophilus* was more than 33% and it was clearly shown the formation of extracellular polysaccharide (EPS). Asha and Sharma (2010), used *Lactobacillus acidophilus* for the removal of As (III) from arsenic containing wastewater. In the present study, Lactobacillus sps was selected as a candidate of consortium for sewage treatment because of extensive formation of EPS which is essential for biofilm formation. According to Shih *et al.* (2001), *Bacillus licheniformis* can be used as bioflocculant. *R. terrae* were transferred to *Gordona* genera and named as *Gordonia terrae* (Collins *et al*., 1988; Stackebrandt *et al*., 1988). Nocentini *et al*. (2000), reported that *Gordonia terrae* shows a very appreciable capability of degrading pristane and squalene, which, for their high degree of branching, are considered extremely recalcitrant to biodegradation and often remain in the environment as residual contaminants after bioremediation.

Ron and Rosenberg (2001), reported that *Gordonia* sp. shows a complex change in cell surface properties during growth on hydrocarbons. These strains can use surface active compounds to regulate their cell surface properties to attach and detach from surfaces such as hydrocarbons. By the presence of biosurfectant properties the microorganisms *Rhodococcus terrae* was selected for the study. But the results were not promising by using the strain. Even though the strain is capable of degrading aromatic compounds, its role is beyond the scope of present study. Hence it was not considered as candidate for the bioremediation of domestic wastewater. Overall performance for removal of pollutants from sewage was also not acceptable mode. Hence, it was not considered as a candidate of the consortium for the bioremediation of sewage.

**Significance of Bacterial Inoculation**

Amit *et al*. (2003), reported that the bacterial inoculation from external source is essential. Without a start up culture, requires a long period of time and may therefore cause significant losses and environmental harm due to discharge of nitrogen rich effluents. It is evident that the role of *Nitrosomonas & Nitrobacter* sps in sewage degradation is more. Moreover, the Td (doubling time) for these two organisms are also more than 8 hours. Hence the concentration of these two microorganisms were doubled i.e., at the rate of 20% each and remaining six microorganisms at the rate of 10% each. Experiments were conducted using this ratio.

The optimization studies revealed that the percentage of removal efficiency was more at a particular concentration of every parameter. For each parameter the condition at maximum removal efficiency obtained was taken into consideration as optimized condition of that parameter for further experiments.

**Optimization of Concentration of Inoculum**

The results of removal efficiency of pollutant in terms of BOD in the domestic wastewater at various concentration of inoculum of consortium were tabulated. The studies on bioremediation capabilities of consortium inoculation in terms of BOD reduction was 56.12% at the concentration of 0.05% (or) 500 ppm, 61.55% at the concentration of 0.1%, 63.80 at the concentration of 0.2%, 64.44% at the concentration of 0.3%, 65.13% at the concentration of 0.4% and 66.16% at the concentration of 0.5%. Results of various other physico-chemical parameters were tabulated (Table 1).

As the quantity of wastewater is more, it is found that using more than 0.5% inoculum is not feasible. Hence, 0.2% of inoculum is considered as optimized concentration. It is opined that instead of using high concentration of inoculum we have to screen, isolate and enumerate high efficiency strains of microorganisms. Nadirah *et al*. (2008), reported that 61% removal of BOD, 97% COD, 86% removal of ammonia, 71% removal of total suspended solids, 50% removal of nitrate and 53% removal of oil and grease using *Pseudomonas putida*, *Pseudomonas fluorescence*, *Xanthobacter sps*., and *Rhodoccus sps.*, for treatment of domestic wastewater. Min Jin *et al*. (2005), reported that 91.7% COD removal and 99% ammonical-nitrogen removal efficiency using *nitrosomonas europea, nitrobacteria windogradskyi*, *Bacillus licheniformis, Bacillus megatherium, Bacillus sphaericus* for the treatment of domestic sewage and membrane bioreactors in 5 hours hydraulic retention time. Balaji *et al*. (2005), reported that 71% of BOD removal using cow dung as the source of microorganisms with dosing of 3% and 18hours HRT during the experiments conducted for treatment of tannary industry wastewater. According to Prasad and Manjunath (2010), lipid content removal and 99% of BOD removal can be obtained using 1% of bacterial consortium. Deng *et al*.(2003), reported that 85.5% of TSS removal and 68.5% COD removal using 0.01% of *Bacillus mucilaginosus* as inoculant for biofloculating material for the treatment of starch wastewater. Graphical representation is made for various physico-chemical parameters like total suspended solids,

chemical oxygen demand, biochemical oxygen demand, nitrogen, phosphorus and hydrogen sulphide. Because they are major pollution parameters in which decrease / increase is the index of treatment process (Figure 1).

**Optimization of Hydraulic Retention Time (HRT)**

The maximum removal efficiency of BOD was obtained with 12 hours HRT. The results of removal efficiency of BOD at different HRT were tabulated. The treatment of wastewater by the addition of 0.2% inoculum results in the decrease of BOD by 32.11% for 4 hours HRT, 46.42% for 8 hours HRT, 60.87% for 12 hours HRT, 62.25% for 16 hours HRT, 63.87% for 20 hours HRT and 63.9% for 24 hours HRT. Results of various other physico-chemical parameters were tabulated (Table 2).

Hashmi Imran (2007), achieved the mean removal efficiency of COD at the rate of 87% after 24 hours of treatment using activated sludge. Chuang *et al*. (1997), reported that high HRT may helps in the production of heterotrophic biomass and finally results in readily biodegradable COD from the sewage. Abbas *et al*. (2008), proved the denitrification ability of the bioreactor using immobilized methyl cellulose at a very low hydraulic retention time i.e., 3 hours. Dempsey *et al*. (2005), studied the performance of pilot scale expanded bed for removal of ammonia, suspended solids and carbonaceous COD using activated sludge for 42 days operation with low loading rates. They obtained the results of wastewater treatment i.e., 56% BOD removal and 62% of TSS removal. Shanableh *et al*. (1997), stated that high HRT helps the polyphosphate accumulating biomass to dominate the bioreactor system. Total nitrogen removal also increased with increase of HRT. According to Shanableh *et al*. (1997), when hydraulic retention time is higher than 8 hours total nitrogen removal efficiency will decrease. Practically wastewater treatment plants have to be designed to meet a number of conditions that are influenced by flow rates, wastewater characteristics and combination of both. The development and forecasting of average daily flow rates are necessary to determine the design capacity as well as the hydraulic requirements of the treatment system. In the present study, 0.2% (2000 ppm) inoculum rate and 12 hours HRT were considered as optimized to use for further experimentations (Figure 2).

**Table 1: Effect of Consortium for Domestic Sewage Treatment**

| S.No. | Physico-Chemical Parameters* | % Removal of Pollutant Compared to Un Inoculated Reactor | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0.05% | 0.1% | 0.2% | 0.3% | 0.4% | 0.5% |
| 1 | pH | - | - | - | 0.1 ↑ | 0.13 ↓ | 0.1 ↓ |
| 2 | Electric conductivity | 0.87 ↑ | 3.25 ↓ | 0.47 ↓ | 11.9 ↓ | 22.01 ↓ | 14.36 ↓ |
| 3 | Temperature | - | - | 0.5°C ↓ | 1.0°C ↑ | 1.0°C ↓ | - |
| 4 | Total suspended solids | 55.45 ↓ | 58.89 ↓ | 61.17 ↓ | 62.93 ↓ | 63.79 ↓ | 64.21 ↓ |
| 5 | Volatile suspended solids | 49.63 ↓ | 54.87 ↓ | 50.14 ↓ | 51.73 ↓ | 58.84 ↓ | 62.20 ↓ |
| 6 | Chlorides | 48.61 ↓ | 48.26 ↓ | 48.65 ↓ | 46.75 ↓ | 47.54 ↓ | 46.63 ↓ |
| 7 | Hardness | 32.76 ↓ | 64.76 ↓ | 40.84 ↓ | 41.83 ↓ | 34.93 ↓ | 25.50 ↓ |
| 8 | Alkalinity | 30.93 ↓ | 35.34 ↓ | 40.76 ↓ | 39.24 ↓ | 39.68 ↓ | 39.22 ↓ |
| 9 | Chemical oxygen demand | 56.25 ↓ | 59.07 ↓ | 63.96 ↓ | 65.22 ↓ | 65.21 ↓ | 67.93 ↓ |
| 10 | Biochemical oxygen demand | 56.12 ↓ | 61.55 ↓ | 63.8 ↓ | 64.44 ↓ | 65.13 ↓ | 66.16 ↓ |
| 11 | Total nitrogen | 40.66 ↓ | 41.17 ↓ | 52.46 ↓ | 53.9 ↓ | 54.26 ↓ | 56.64 ↓ |
| | Ammonical nitrogen | 47.05 ↓ | 50.48 ↓ | 51.87 ↓ | 54.08 ↓ | 52.49 ↓ | 54.69 ↓ |
| | Nitrite-nitrogen ($NO_2^-$) | 100.0 ↑ | - | 100.0 ↑ | 11.11 ↓ | 66.67 ↑ | 100.0 ↑ |
| | Nitrate-nitrogen ($NO_3^-$) | 8.33 ↓ | 7.14 ↑ | 60.0 ↓ | 11.11 ↑ | 23.33 ↓ | 35.42 ↓ |
| | Kjeldhal nitrogen | 19.39 ↓ | 20.87 ↓ | 54.53 ↓ | 59.02 ↓ | 62.86 ↓ | 66.67 ↓ |
| 12 | Phosphorus (as P) | 48.15 ↓ | 51.04 ↓ | 58.33 ↓ | 56.8 ↓ | 59.69 ↓ | 61.22 ↓ |
| 13 | Oil & grease | 2.44 ↓ | 2.63 ↓ | 4.04 ↓ | 4.39 ↓ | 3.13 ↓ | 5.56 ↓ |
| 14 | Hydrogen sulphide | 38.71 ↓ | 44.83 ↓ | 59.38 ↓ | 60.34 ↓ | 64.20 ↓ | 67.82 ↓ |
| 15 | Sludge volume index | 21.74 ↓ | 23.33 ↓ | 20.87 ↓ | 23.85 ↓ | 22.66 ↓ | 22.58 ↓ |

*(All parameters are expressed in mg/litre (ppm) except pH, electric conductivity and temperature)

**Table 2: Effect of Hydraulic Retention Time for Domestic Sewage Treatment**

| S.No. | Physico-Chemical Parameters* | % Removal of Pollutant Compared to Un Inoculated Reactor | | | | | |
|---|---|---|---|---|---|---|---|
| | | 4 hours | 8 hours | 12hours | 16 hours | 20 hours | 24 hours |
| 1 | pH | - | 0.1 ↑ | 0.1 ↑ | 0.1 ↑ | 0.1 ↑ | 0.1 ↑ |
| 2 | Electric conductivity | 10.04 ↓ | 19.54 ↓ | 8.96 ↓ | 10.08 ↓ | 12.12 ↓ | 11.55 ↓ |
| 3 | Temperature | - | 0.5°C ↓ | - | - | 0.5°C ↑ | - |
| 4 | Total suspended solids | 30.55 ↓ | 48.23 ↓ | 58.90 ↓ | 60.93 ↓ | 61.81 ↓ | 61.97 ↓ |
| 5 | Volatile suspended solids | 24.89 ↓ | 42.01 ↓ | 50.0 ↓ | 55.73 ↓ | 58.59 ↓ | 59.20 ↓ |
| 6 | Chlorides | 22.22 ↓ | 38.07 ↓ | 54.0 ↓ | 58.02 ↓ | 59.16 ↓ | 58.14 ↓ |
| 7 | Hardness | 18.72 ↓ | 21.73 ↓ | 30.09 ↓ | 35.02 ↓ | 40.03 ↓ | 40.98 ↓ |
| 8 | Alkalinity | 17.75 ↓ | 24.65 ↓ | 40.18 ↓ | 44.58 ↓ | 51.01 ↓ | 50.21 ↓ |
| 9 | Chemical oxygen demand | 35.03 ↓ | 47.91 ↓ | 62.47 ↓ | 63.46 ↓ | 63.65 ↓ | 64.01 ↓ |
| 10 | Biochemical oxygen demand | 32.11 ↓ | 46.42 ↓ | 60.87 ↓ | 62.25 ↓ | 63.87 ↓ | 63.9 ↓ |
| 11 | Total nitrogen | 24.82 ↓ | 30.35 ↓ | 47.13 ↓ | 52.06 ↓ | 55.39 ↓ | 55.64 ↓ |
| | Ammonical nitrogen | 25.51 ↓ | 30.28 ↓ | 48.93 ↓ | 53.07 ↓ | 56.11 ↓ | 56.97 ↓ |
| | Nitrite-nitrogen ($NO_2^-$) | - | 33.33 ↑ | - | 66.67 ↑ | - | 73.33 ↑ |
| | Nitrate-nitrogen ($NO_3^-$) | 9.09 ↓ | 7.69 ↑ | 14.29 ↑ | 9.09 ↑ | 73.33 ↑ | 15.15 ↑ |
| | Kjeldhal nitrogen | 24.81 ↓ | 36.11 ↓ | 49.22 ↓ | 55.18 ↓ | 59.86 ↓ | 59.63 ↓ |
| 12 | Phosphorus (as P) | 29.35 ↓ | 36.18 ↓ | 52.08 ↓ | 55.62 ↓ | 59.8 ↓ | 61.01 ↓ |
| 13 | Oil & grease | - | - | 5.26 ↓ | 6.45 ↓ | 6.25 ↓ | 7.89 ↓ |
| 14 | Hydrogen sulphide | 25 ↓ | 32.18 ↓ | 55.17 ↓ | 56.67 ↓ | 62.07 ↓ | 64.29 ↓ |
| 15 | Sludge volume index | 22.06 ↓ | 20.0 ↓ | 21.54 ↓ | 22.13 ↓ | 27.5 ↓ | 32.31 ↓ |

*(All parameters are expressed in mg/litre (ppm) except pH, electric conductivity and temperature)

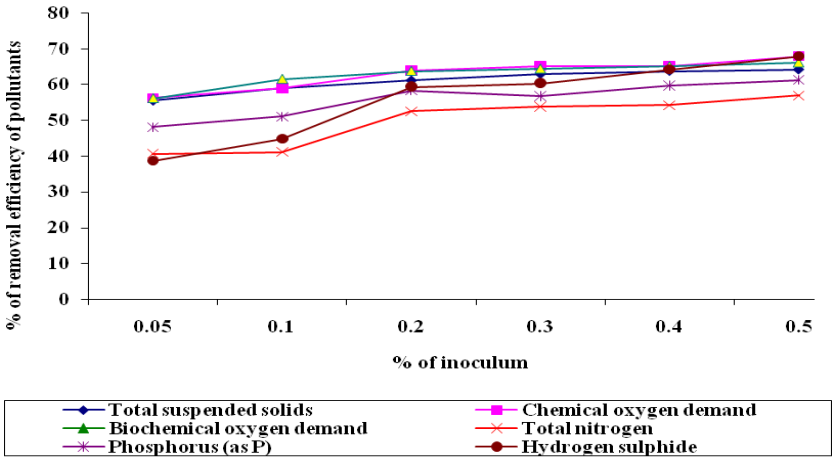


**Figure 1: Effect of Microbial Consortium Concentration on Pollutant Removal Efficiency**

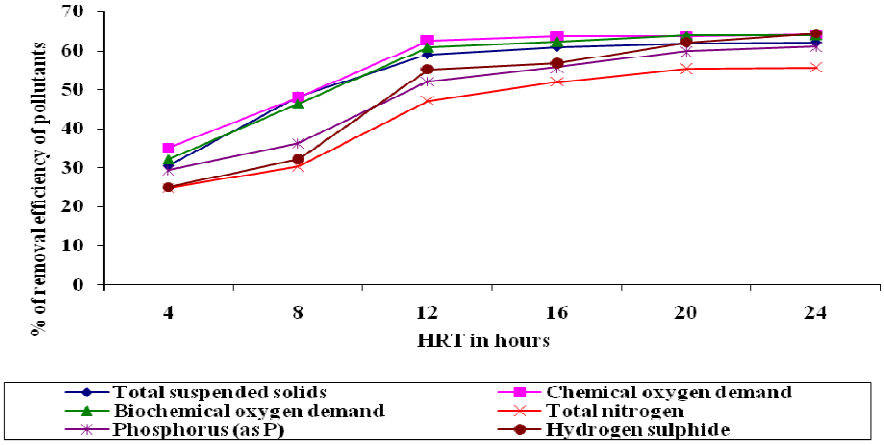


**Figure 2: Effect of Hydraulic Retention Time (HRT) on Pollutant Removal Efficiency**

## CONCLUSIONS

In present study, domestic wastewater sample (sewage) was tested and treated with a set of consortium. After treatment, a reduction was observed in all the physico-chemical parameters. The bacterial cultures treated wastewater and showed a sharp reduction in BOD i.e. 56 -66% in the presence of 0.05 – 0.5ppm of microbial culture. The study concludes that the microbial consortium was effective in the reduction of pollutants. The bacterial consortium showed more reduction in the parameters in comparison to single bacterial culture. Through the results presented here it was shown that if good stable and effective wastewater treatment is achieved, then waste water reused become increasingly safe.

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