Qualitative and Microbial analysis of raw and soil biotechnology treated domestic sewage water from Palghar, Maharashtra, India

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Abstract: ***The study was carried out to determine the quality of sewage water before and after treating water by soil biotechnology method. Various parameters on which quality was determined were pH, chemical oxygen demand, Biological oxygen demand, TSS, TDS and Total Kjeldhal Nitrogen. Both the samples were also analyzed using Atomic Absorption Spectroscopy (AAS) to detect the concentration of Sodium, Potassium and heavy metals like Nickel, Copper and Aluminum. Microbial analysis was carried out to find out the presence of bacteria, fungi, yeast etc. The results obtained from this study reveals that SBT treated sewage water is harmless for human handling and is beneficial for agricultural purpose.***

Keywords - Sewage water, SBT treated water, Bacteria, Agriculture, BOD, COD, Psychrophiles, Mesophiles, thermophiles, Azotobacter, Irrigation, MPN, Coliforms, Standard plate count

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I. INTRODUCTION

One of the major wastes disposed every day is domestic sewage. It is 99.9% pure water by weight, rest of the 0.1% contains variety of suspended and dissolved impurities which causes significant problems and also contains disease causing microbes [1]. Most of the communicable diseases are due to unsafe water; most of which is contaminated either by sewage or agricultural run-off. At Govardhan Eco Village (GEV), the domestic sewage waste is treated by Soil Biotechnology Method.

SBT consists of resistant control and includes soil, formulated coarse filter media and a select culture of organisms such as plants and earthworms. It involves a combination of both biological and physical processes for processing of waste water or used water and it derives its fundamental principle from the operation of a terrestrial ecosystem. It is a natural bio-geochemical cycle of nature and hence proves to be most effective eco-friendly technology for waste water treatment. The SBT plant at Govardhan Eco Village recycles 30,000 liters of sewage water every day [2].

Chemical and Microbial analysis of domestic sewage water is done before and after treatment to prove the benefits of this eco-friendly technology.

II. PARAMETERS ANALYZED

Following water quality parameters were analysed to find out the water pollution status: (1) pH (2) Total dissolved oxygen (DO) (5) Chemical oxygen demand (COD) (6) Biochemical oxygen demand (BOD) (7) Total Kjeldhal Nitrogen (8) Oil and Grease (9) Detection of sodium, potassium, Nickel, Copper and Aluminium by AAS (10) Enumeration of total viable bacteria, coliforms, psychrophiles and thermophiles (11) Detection of actinomycetes and fungi.

III. WATER QUALITY PARAMETERS AND THEIR EFFECTS ON AGRICULTURE

The water was collected from GEV, Palghar district in order to study physico-chemical and microbial characteristic of domestic sewage water pre- and post-treatment. Various samples were collected following the standard methods described for sampling. The procedures and standard methods were used for qualitative and quantitative assessment of water quality parameters.

3.1 pH

pH is the measure of the acidity or alkalinity of a solution. Water pH between 6.5 and 8.5 is considered to be advantageous for irrigation. pH values above and below the normal range, indicates that action needs to be taken to get better crop performance [3].

3.2 COD

Chemical oxygen demand is a measure of the necessary oxygen for the chemical oxidation of the organic matter. It can be high without any negative effects for the plants and the soil. In some cases, high organic matter content is considered positive as it improves the water holding capacity of the soil on the long term.

3.3 BOD

Biological oxygen requirement is a measure of the quantity of oxygen required for the biological oxidation of organic waste. It can be used as a measure to find out the efficiency of waste water treatment plants.

3.4 TSS

Total suspended solids are the dry-weight of particles blocked by a filter [4]. TSS, measured in mg/l, can be calculated as

$$\text{TSS}(\text{mg/l}) = \frac{(\text{final wt} - \text{initial wt}) \times 1000}{\text{Amount of sample taken}}$$

3.5 TDS

"Total dissolved solids" refer to any minerals, salts, metals, cations or anions dissolved in water. It comprises of inorganic salts and some minute amounts of organic matter that are dissolved in water [5], [6].

3.6 Total kjeldhal nitrogen

The nitrogen cycle is the measure by which atmospheric nitrogen is made obtainable in different forms to living organisms. Starting from the basic molecules of ammonia, nitrate and nitrite up to the more complex proteins and amino acids. nitrogen is very essential for living organisms to function. This is a very important function in the smooth operation of many wastewater treatment plants in India. [7], [8].

3.7 Oil and Grease

Oil and grease is organic toxic waste which causes ecology damages for aquatic organisms, plant, animal, and is mutagenic and carcinogenic for human being. They form a layer on water surface that decreases dissolved oxygen [9].

3.8 Aluminum

Can cause non-productivity in acid soils (pH < 5.5), but more alkaline soils at pH > 7.0 will precipitate the ion and eliminate any form of toxicity
[10], [11].

3.9 Nickel

Toxic to most of the plants ranging 0.5 mg/l - 1.0 mg/l. Toxicity can be reduced at alkaline pH or neutral [10].

3.10 Copper

Toxic to most of the plants ranging 0.1 to 1.0 mg/l in nutrient solutions. Availability of high pH (alkaline soil) or Copper can cause leaf chlorosis as well as the suppression of root growth [10], [11].

3.11 Sodium

Sodium exists in almost all water used for irrigation and is not essentially a cause for concern unless high concentrations are present. High concentrations (> 70 mg/L) can be dangerous to both plants and soils. Sodium in water used for irrigation can be absorbed by roots and flora, and foliar burning can occur if excess amounts accumulate in leaf tissue [12].

3.12 Potassium

The potassium (K^+) cation performs similar to sodium in the soil and is very commonly found in natural waters in small amounts.

3.13 Microbiological analysis

Since the composition of wastewater varies, the types and numbers of organisms will fluctuate. Fungi, protozoa, algae, bacteria and viruses are present. Raw sewage water may contain millions of bacteria per millilitre including the coliforms, streptococci, anaerobic spore-forming bacilli, the proteus group and other types originating in the intestinal tract of humans. The causative agents of poliomyelitis, hepatitis, typhoid, dysentery and cholera may occur in sewage. Certain bacteriophages are easily isolated from sewage water.

The potential methane producers such as *Methanococcus, Methanobacterium, Methano-sarcina* contribute to the production of anaerobic and temperature elevated conditions in sewage [13]. Bacterial growth is very receptive to temperature because high temperature can give rise to the fluidity of the phospholipid bilayer which leads to cell lysis. However, bacteria are known to have increased enzymatic activity at higher temperature because of increased thermal energy. For example, when thermophilic sludge treatment is compared to mesophilic treatment, the sludge biodegradability is higher with thermophilic degradation [14].

3.13.1 Total viable count

In routine analysis the total number of bacteria present in 1 ml of sewage is determined by standard plate count method. One set of plates is incubated at 37^0 C for 48 h (mesophilic bacteria). Another set of plates is incubated at 22^0 C for 72 h (psychrophilic bacteria) and yet another set of plate is incubated at 55^0C for 72 h (thermophilic bacteria). After incubation the colonies are counted and the amount of cfu/ml (colony forming units) can be calculated [15]. Plate count technique is useful in finding out the efficiency of operation for destroying or removing the organisms. A microbial count can be made before and after a specific treatment and results obtained indicate the degree to which the bacterial population has been reduced. A sample of water containing less than 100 bacteria per ml is considered to be safe [5].

The total number of psychrophilic bacteria

Non pathogenic bacteria have the ability to grow mainly at lower temperatures. It is important that Gram-negative bacteria in water produce lipopolysaccharides in their cell wall which can be toxic – like endotoxins of pathogenic bacteria. Because of this, the growth of their numbers in water should be constantly monitored. A large increase in their numbers is an evidence of the presence of easily available organic compounds in the water. Theoretically, the presence of 0.1 mg organic carbon in water can show the result in an increase of bacteria up to 10^8 cfu in 1 ml. Phosphorus is also a factor which stimulates the growth of psychrophilic microorganisms. Adding even small amounts of this element (i.e.,50mg/l) causes 10 times the acceleration of bacterial growth in a water treatment plant [15].

The total number of mesophilic bacteria

High numbers of bacteria growing at 37^0C are more dangerous because among this high population of bacteria, pathogenic forms may be found which can be dangerous for human health. High number of bacteria available in samples of water can prove a point that water treatment process has proceeded badly or that polluted water is siphoned [15].

The total number of thermophilic bacteria

It is an index of production of gases such as methane that elevate the temperature of sewage and allow the proliferation of thermophilic bacteria [14].

3.13.2 Total coliform and fecal coliform (MPN - Most probable number)

It is statistical method based on the probability theory. In this technique, the sample is diluted serially till the numbers of organisms reach the point of expansion. From each and every dilution several multiple tubes of a specific medium are immunized. Presence of organism is indicated by acid and gas in the medium. Pattern of positive and negative test results are then used to calculate the number of coliforms in the original sample. Since the test gives the most likely number of organisms present in the sample. it is also known as MPN test [16].

3.13.3 Study of biological nitrogen fixers

Azotobacter is a free living, non-symbiotic nitrogen fixing bacterium that brings about biological nitrogen fixation [17]. Since the sewage is SBT treated it is possible that these valuable microorganisms may enter the treated sewage by diffusion from the soil. Enrichment technique is used to create an increase the number of this organism as its number is very few in soil samples and hence in the treated sewage.

IV. WATER QUALITY PARAMETERS AND THEIR EFFECTS ON AGRICULTURE

4.1 pH: pH was determined by pH metry using a pH meter [18].

4.2 COD: [5]0.1N Potassium dichromate, 0.1M sodium thiosulphate, 2M sulphuric acid, 1% starch solution. COD was determined by titrimetry by using procedure using Aneja.

4.3 BOD: [5]0.5% Allyl thiourea, 1N sulphuric acid, sodium hydroxide. BOD was determined by titrimetry by using procedure using Aneja.

4.4 TSS: APHA 2540 D Total Suspended Solids Dried at 103–105°C [19].

4.5 Oil and grease: American Public Health Association (APHA) 5520 B. Partition-Gravimetric Method [20].

4.6 Microbiological analysis:

Raw and treated sewage waste matter samples were incubated overnight at 37^0C, before analysis. The samples were sampled to enumerate total possible counts and total coliform counts. Also, the fungi and actinomycetes present in the collected samples were studied. The sample was also checked for the availability of nitrogen fixing bacteria, *Azotobacter spp*.

The samples were 10-fold serially diluted and the total viable count was carried out using appropriate dilutions, sterile molten nutrient agar medium and incubation conditions by pour plate method [15]. Determination of total coliform count was carried out by MPN (three tube method) using single and double strength Lauryl tryptose broth [16]. For the study of fungi and actinomycetes, a loopful of each of the samples were streak isolated on sterile Sabouraud's dextrose agar and sterile Kenkunight and munnair's agar plates respectively [5]. *Azotobacter spp* was isolated by enrichment in Sterile Ashby's mannitol broth medium and subsequent streaking on sterile Ashby's mannitol agar plates [5].

V. RESULT AND DISCUSSION

5.1 Results of chemical analysis:

Parameters	**Desirable limit for irrigation**	**Untreated sewage water**	**SBT treated sewage water**
pH	6.5-8.5	6.97	7.58
COD (mg/l)	-	474	119
BOD (mg/l)	100	169	39
TSS (mg/l)	200	134	38
TDS (mg/l)	< 500 - No potential problem; 500-2000 slight to moderate; >2000 severe	716	650
Total Kjeldhal Nitrogen (mg/l)	-	7.48	4.02
Oil and Grease (mg/l)	10	3	0.1

Parameters	Desirable limit for irrigation	Untreated sewage water	SBT treated sewage water
Aluminium (as Al) mg/l	5	0	0
Nickel mg/l	0.2	0	0
Copper mg/l	0.2	0	0
Sodium mg/l	0-50	5.1	5.05
Potassium mg/l	5-10	7.13	7.01

5.2 Results of microbiological analysis:

Parameters	Untreated sewage water	SBT treated sewage water
Total Viable count		
Mesophilic count	$6*10^6$ cells/ml	93 cells/ml
Psychrophilic count	$5.3*10^4$ cells/ml	32 cells/ml
Thermophilic count	69 cells/ml	27 cells/ml
Coliform count	$2.1*10^4$ cells/ml	Absent
Yeast	*Candida Species*	-
Fungi	-	*Aspergillus Species*
		Penicillium Species
		Slime moulds
Actinomycetes	*Nocardia Species*	-
Biological nitrogen fixers	-	*Azotobacter species*

DISCUSSIONS

This study completely focused on the effect of SBT treatment on domestic sewage and determination of the possibility of treated sewage for human handling and irrigation.

In the result sections we noticed considerable decreases in almost all parameters from "untreated sewage water" to "SBT treated sewage water".

In chemical analysis, pH of treated water is within the desirable limit for irrigation. COD and BOD reduce considerably after treatment making itself suitable for human handling. With respect to total dissolved solids (TDS) the potential risk is reduced after treatment, though not completely risk free. The reduction in the total kjeldhal nitrogen content can be due to the fact that organic matters are eliminated after treatment. Elements like Aluminum, Copper and Nickel are absent. Sodium and potassium are within the limits.

A considerable reduction in the total number of viable organisms is observed in treated sewage suggesting that the potential risk to the health of humans can be almost eliminated by SBT treatment of sewage. Moreover, coliforms are completely eliminated reducing the risk of dissemination of these organisms in the environment which pose a serious threat of gastro-intestinal tract infections in humans. *Penicilium species* and *Aspergillus species* are potential antibiotic producing fungi that increase the competition for nutrients thus reducing the load of bacterial pathogens. *Azotobacter species* able to fix up atmospheric nitrogen into soil by biological nitrogen fixation, making it available to the plants.

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