MICROBIOLOGICAL MONITORING IN BIODEGRADATION OF SEWAGE WASTE WATER

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Abstract

The performance of isolated designed consortia comprising for the treatment of sewage waste water in terms of reduction in COD (chemical oxygen demand), BOD (biochemical oxygen demand) and TSS (total suspended solids) was studied. Different parameters were optimized (inoculums size, agitation, and temperature) to achieve effective results in less period of time. The collection of soil sample from Koyambedu SPT plant and microbial activities in samples for isolation of bacteria, culture of bacteria, consortia of organisms for the degradation of sewage. Analysis based on strain and morphological characterization of organisms present in sewage. This process is to reduce the pollution load on sewage for the eco-friendly environment and energy consumption reduces cost for treatment. It is useful study on different types of organisms. Bacterial strains were isolated from the collected soil sample by serial dilution method. The brain heart agar (BHA) medium was used to isolate the bacterial strains from the samples. Serial dilution was performed by taking one gram of soil in 10 ml sterile distilled water and incubate

Keywords: *COD, TSS, SPT, BA, BOD, consortia, degradation, isolate, dilution method, pollution*

Introduction

Water pollution became a severe problem with the industrialization of nations, coupled with the rapid acceleration in population growth. Industrialization led to urbanization, with people leaving the land to work in the new factories. Domestic wastes from the rapidly expanding towns and wastes from industrial processes were all poured untreated into the nearest rivers. Effluent waters, which should be removed from settlements and industrial enterprises, are known as sewage. Effluents are classified by their origin as domestic or public sewage, industrial effluents, and atmospheric (rain) run off. The sanitary requirements for the composition and properties of water bodies appreciably limit the discharge of sewage into water bodies. The term **"sewage sludge"** or **"bio solids"** represents the insoluble residue produced during wastewater treatment and subsequent sludge stabilization procedures, such as aerobic or anaerobic digestion. The treatment of sewage wastewater in terms of reduction in COD (chemical oxygen demand), BOD (biochemical oxygen demand) MLSS (mixed liquor suspended solids), and TSS (total suspended solids) was studied. Different parameters were optimized (inoculum size, agitation, and temperature) to achieve effective results in less period of time. Bio technological approach has been utilized to treat the sewage wastewater using specific bacteria having bio degradative potential for sewage wastewater.

Need for the Experiment

Biological treatment is necessary if organic matter is to be removed from water. Nonetheless, biological treatment offers an economical alternative to physical and chemical treatment methods. It is the most widely used method for removal as well as partial or complete stabilization of biologically degradable substances present in waste waters. The mechanism underlying biological treatment is the decomposition of finely dispersed matter, colloidal and dissolved substances by metabolism of aerobic microorganisms. The susceptibility of organic substances contained in sewage, to biochemical oxidation coupled with the presence of specific bio oxidation agents, that is, microorganisms, is a prerequisite for efficient biodegradation.

Scope of the Experiment

Most industrial effluents contain hazardous chemicals that may have direct or indirect impacts on aquatic biota by bioaccumulation along the food chain and which may later become biomagnified. Many heavy metals that are found in these effluents have been shown by previous studies to be toxic to both aquatic fauna and flora and therefore stricter regulation of these industries is needed. This present study confirmed the capability of different microbes to break down the pollutants in three effluents, pharmaceutical, textile and local dye, to a less toxic form. These microbes should be enhanced in their natural ecosystem in other to be able to degrade more of these pollutants. This method should be embraced because of its advantage over other methods; it is environmentally friendly, lower cost, equally effective, and able to bring about a cleaner and more sustainable ecosystem.

Objectives of the Experiment

The selected formulated bacterial consortium comprising of the isolated bacterial strains acts in a synergistic way and is capable of degrading the easily assimilable organic compounds present in sewage wastewater. Consortium is capable of effectively reducing the pollutional load of the sewage waste water. Utilize microbial agents such as bacteria to reduce pollutant loads such as heavy metals in effluent samples.

Collection of Samples and Materials

Collection of sample Sewage sludge soaked soil samples were collected from a Sewage Treatment Plant - Koyambedu in Chennai. Soil sediments were collected at 20 cm depth using plastic borer and transferred to sterile plastic zip lock covers in field and were transported immediately to the laboratory and stored for further study.

- Area coverage : 82%
- Total sewer consumers: 8, 76,891
- Length of sewer mains: 5200 Km
- No. of pumping station: 265
- Treatment plant: 12 nos

Sample Sewage Water

The samples collected and stored in clean polythene bottles fitted with screw caps and brought to the laboratory in the sampling for detailed Physico-chemical analysis.

Figure 1 Sample collection of waste water

Soil Sample

Collection of Soil Samples

Soil samples were collected in sterile plastic containers from niche areas near the KOYEMBEDU sewage treatment plant Chennai, for the isolation of bacterial isolates.

Figure 2 Sample collection of soil sample

Properties of the Soil Samples

Moisture was determined according to Nascimento 2018. For this, sludge samples of 10 g were oven dried at 65°C, for 48 h. pH was measured using 2 g of moist sample and 20 ml of deionized water, which was stirred for 5 min at 220 rpm and rested for 30 min

Physical Properties of Materials

Figure 3 PH Value and PH meter

Table 1 Physical properties of before treatment water

Physical Parameter	Before Treatment Water (Control)
Appearance	Off white
Odour	Pungent
Turbidity NTU	2
Electrical conductivity (micro mho/cm)	3502
pH	5

Table 2 After treatment water

After treatment water									
SW 1	**SW 2**	**SW 3**	**SW 4**	**SW 5**	**SW 6**	**SW 7**	**SW 8**	**SW 9**	**SW 10**
Off white	Off white	Off white	Off white	Off white	**Cloudy**	Off white	Off white	Off white	Off white
Pungent	Pungent	Pungent	Pungent	Pungent	**Pungent**	Pungent	Pungent	Pungent	Pungent
1	1	1	1	1	**0.5**	1	1	1	1
3200	3176	3430	3390	3471	**3043**	3207	3146	3289	3278
6	6.2	5.7	6.1	6.0	**7.2**	6.3	5.4	5.7	5.3

Experimental Work

Isolation of Bacterial Isolates

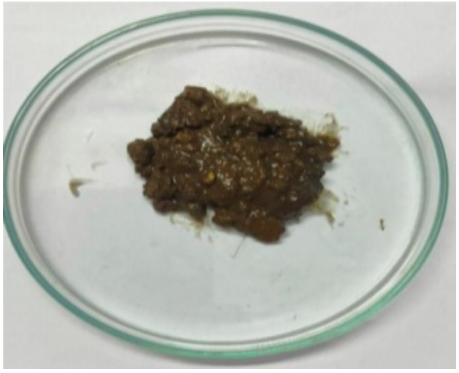

Figure 4 Bacterial isolates

Sewage sludge soil sample used for bacterial isolation study

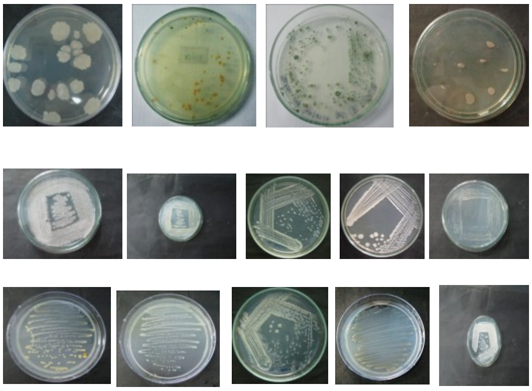

Figure 4. Isolated and pure bacterial isolates

Table 3. BOD and COD and TSS Estimation

Sample name	BOD (mg/L)	COD (mg/L)	TSS (mg/L)
Control (Untreated sewage water)			
SW 1	78	196	546
SW 2	68	172	624
SW 3	62.8	152	569
SW 4	48	150	545
SW 5	59.6	168	600
SW 6	**55**	**62**	**244**
SW 7	61	131	325
SW 8	52	110	374
SW 9	60	70	578
SW 10	70	155	269

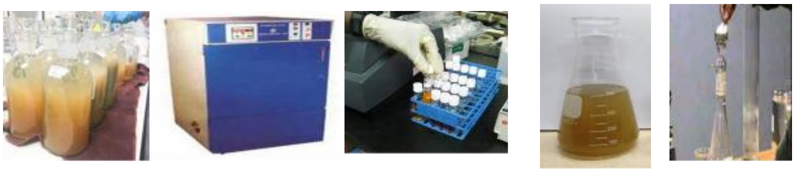

Figure 4 Bod bottles and incubator samples

Table 4 Morphological characteristics of the bacterial strain SW6

Test	Isolated strain – SW6 (*Bacillus* sp.)
	Colony Morphology
Margin	Irregular
Elevation	Convex
Surface	Dull
Opacity	Opaque
Gram's reaction	+ ve
Cell shape	Rods
Endospore	+
Shape	Oval
Motility	+

Table 5 Physiological characteristics of the isolated strain SW6

Tests		Isolated strain – SW6 (*Bacillus* sp.)
pH	pH 4.0	-
	pH 5.0	-
	pH 6.8	+
	pH 8.0	+
	pH 9.0	-
	pH 11.0	-
Sodium Chloride (%)	2.0	+
	4.0	+
	7.0	+
	8.0	+
	10.0	-

Temperature	4 °C	-
	10 °C	-
	25 °C	+
	30 °C	+
	37 °C	+
	42 °C	+
	45 °C	-
	55 °C	-
	65 °C	-

Table 6 Morphological characteristics of the isolated strain SW6

Test	Isolated strain – SW6 (*Bacillus* sp.)
	Colony Morphology
Margin	Irregular
Elevation	Convex
Surface	Dull
Opacity	Opaque
Gram's reaction	+ ve
Cell shape	Rods
Endospore	+
Shape	Oval
Motility	+

Table 6 Bio chemical tests

S.no	Bio chemical tests	Sewage isolate SW6
1	Indole test	negative
2	Methyl red test	positive
3	Voges Proskauer test	positive
4	Triple sugar iron agar test	positive
5	Motility Indole Lysine (MIL)	negative
6	Citrate test	positive
7	Urease test	negative
8	Catalase test	positive
9	Oxidase test	negative
10	Nitrate reduction test	positive
11	**Acid production from carbohydrates**	
12	D-glucose	positive
13	L-arabinose	negative
14	Sucrose	positive
15	D-fructose	positive
16	D – xylose	negative
17	I-inositol	negative
18	Raffinose	negative
19	D-mannitol	negative
20	Cellulose	negative
	Identified as	***Bacillus sp.***

Table 7 BOD/ COD/ TSS

Sample name	BOD (mg/L)	COD (mg/L)	TSS (mg/L)
Control (Untreated sewage water)			
SW 3 alone	63	154	568
SW 6 alone	54	60	243
SW 6 + SW 3 consortia	**40**	**58**	**220**

Conclusion

The SW6 bacterial strain shows best result in degradation from others strain of sewage samples. This SW6 is capable of effectively reducing the pollutional load of the sewage in terms of COD, BOD, and TSS within desired limits that is 55mg/l, 62mg/l, 244mg/l. The sewage isolate of SW6 is identified as bacillus sp.The selected formulated bacterial consortium comprising of isolated bacterial strain acts in a synergetic way and is capable of degrading the easily assimible organic compound present in sewage waste water. This consortium is capable of effectively reducing the pollutional load of the sewage in terms of COD, BOD, TSS within desired limits, that is 40mg/l, 58mg/l, 220mg/l. this use of such specific consortia can over inefficiencies of the conventional biological treatment facilities currently operational in sewage treatment plants. The isolated bacterial strains were sub cultured in BHA medium to achieve purity and pure mother cultures were flooded with 20% glycerol and stored in -20° C.

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