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A STUDY ON MICROBIAL DIVERSITY AND PHYSICO-CHEMICAL CHARACTERISATION OF DETERGENT INDUSTRY EFFLUENT

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ABSTRACT

An investigation was carried out to assess the detergent industry effluent on the microbial diversity viz. bacteria, fungi and cyanobacteria. Results of one year ecological study revealed that altogether 12 species of bacteria, 13 species of fungi and 29 species of cyanobacteria were isolated from the effluent stream. The species isolated, *Pseudomonas* with two species and others with single each were recorded. Among the fungi recorded the *Aspergillus* was found to be dominant with four species viz., *A. flavus, A. fumigatus, A. luchensis* and *A. niger*. The rest of the genera such as *Blastocladia* sp, *Candida* sp, *Penicillium javanicum, Saprolespgia* sp, *Trichoderma viride, Alternaria* sp, *Fusarium oxysporum* and *Rhodosporium* were recorded with single species each. Altogether 29 species of cyanobacteria belonging to eleven genera were collected from the effluent stream. Among the genera, *Oscillatoria* dominated the effluent with 10 species followed by *Phormidium, Lyngbya, Nostoc* and *Anabaena* with 3 each species, *Hapalosiphon* with 2 and *Microcystis, Plectonema, Fischerella, Chlorogloea* and *Synechococcus,* with single species each. *Chlorogloea fritschii* and *Fischerella ambigua* were not observed in summer season. *O. subbrevis, O. chlorine, Lyngbya majusculla* and *N. muscorum* were not observed in monsoon season. Higher amounts of phosphates and nitrates with sufficient amount of oxidizable organic matter, limited dissolved oxygen content and slightly alkaline pH were probably the factors favouring the growth of microbes especially cyanobacteria. The utilization of dominant species of cyanobacteria to monitor pollution in detergent effluent has been discussed.

KEYWORDS: detergent industry effluent, bacteria, fungi, cyanobacteria.

INTRODUCTION

Water is essential to all forms of life and makes up to 50-97% of the weight of all plants and animals and about 70% of human body. Water is also a vital resource for agriculture, manufacturing, transportation and many other human activities. Despite its importance, water is the most poorly managed resource in the world. The availability and quality of water always have played an important role in determining the quality of life. Water quality is closely linked to water use and to the state of economic development (Ramamurthy *et al*., 2014). Ground and surface waters can be contaminated by several sources. In urban areas, the careless disposal of industrial effluents and other wastes may contribute greatly to the poor quality of water (Ramamurthy *et al*., 2015). Most of the water bodies in the areas of the developing world are the end points of effluents discharged from industries.

Non-hazardous industrial wastes are those that do not meet the EPA's definition of hazardous waste - and are not municipal waste. The EPA estimated in 1980 that more than 70,000 different chemicals were being manufactured in the U.S., with some 1,000 new chemicals being added each year. Industrial waste has been a problem since the industrial revolution. Industrial waste may be toxic, ignitable, corrosive or reactive. If improperly managed, this waste can pose dangerous health and environmental consequences. In United States, the amount of hazardous waste generated by manufacturing industries in the country has increased from an estimated 4.5 million tons annually after World War II to some 57 million tons by 1975. In 1990, this total had shot up to approximately 265 million tons. This waste is generated at every stage in the production process, use and disposal of manufactured products. Thus, the introduction of many new products for the home and office - computers, drugs, textiles, paints and dyes, plastics - also introduced hazardous waste, including toxic chemicals, into the environment. These, too, must be managed with extreme care to avoid adverse environmental or human health impacts.

Natural soap was one of the earliest chemicals produced by man. Historically, its first use as a cleaning compound dates back to Ancient Egypt (Besselievre, 1969; Brownell, 1975; Callely, 1976). In modern times, the soap and detergent industry, although a major one, produces relatively small volumes of liquid wastes directly. However, it causes great public concern when its products are discharged after use in homes, service establishments, and factories (Carberry and Geyer, 1977).

A number of soap substitutes were developed for the first time during World War I, but the large-scale production of synthetic surface-active agents (surfactants) became commercially feasible only after World War II. Since the early 1950s, surfactants have replaced soap in cleaning and laundry formulations in virtually all countries with an industrialized society. Over the past 40 years, the total world production of synthetic detergents increased about 50-fold, but this expansion in use has not been paralleled by a significant increase in the detectable amounts of surfactants in soils or natural water bodies to which waste surfactants have been discharged (Callely, 1976). This is due to the fact that the biological degradation of these compounds has primarily been taking place in the environment or in treatment plants. Taking the above facts into consideration, a survey was undertaken in detergent effluent to explore the nature of microbial flora such as bacteria, fungi and cyanobacteria are screen in order to biodiversity index.

MATERIALS AND METHODS

Effluent was collected from detergent industry waste, Orathanadu, Thanjavur district, Tamil Nadu, India. Samples were collected in large sterilized container and brought to the laboratory. Physico-chemical characteristics were done on the same day when the samples were brought to the laboratory. The effluent samples were filtered through cotton to remove suspended coarse particles before use. Population of microbes was isolated from the effluent samples by serial dilution technique. Bacteria were identified based on colony characteristics, Gram staining methods and by various biochemical studies as given by Bergey's (1984) Manual of Determinative Bacteriology. Fungi were identified by using standard manuals, such as Mannual of Soil Fungi (Gillman, 1957), Dematiaceous Hyphomycetes (Ellis, 1971). Effluent samples were collected in duplicate from station in pre-sterilized bottles. Samples were collected from the places along with effluents in polythene bags. Standard microbiological methods were followed for the isolation and identification of cyanobacteria (Desikachary, 1959). Physico-chemical characteristics of effluent were done according to the Standard Methods (APHA, 1995). Temperature and pH of the effluent were measured at the station itself.

Table 1. Characteristics of effluent observed in four seasons.

S.No.	Parameters	Summer	Premonsoon	Monsoon	Post monsoon
1.	Temperature ^{0}C	23.75 ± 1.05	21.17 ± 0.65	19.85 ± 0.17	21.45 ± 0.16
2.	pH	8.97 ± 0.26	8.78 ± 0.28	8.54 ± 0.37	8.81 ± 0.22
3.	Total suspended solids	1992 ± 15.5	1978 ± 28.7	1953 ± 58.2	1971 ± 22.5
4.	Total dissolved solids	1432 ± 15.2	1387 ± 14.5	1376 ± 18.3	1395 ± 11.7
5.	Free carbon-di-oxide	27.3 ± 1.06	24.9 ± 1.07	19.8 ± 1.12	23.7 ± 1.16
6.	Carbonate	2.40 ± 0.15	2.18 ± 0.18	3.70 ± 0.14	2.60 ± 0.16
7.	Bicarbonate	56 ± 1.81	57 ± 2.13	59 ± 1.19	58 ± 1.26
8.	BOD	296 ± 5.27	252 ± 6.51	241 ± 9.15	263 ± 7.26
9.	COD	518 ± 8.12	441 ± 4.84	390 ± 6.71	445 ± 5.18
10.	Dissolved oxygen	3.21 ± 0.12	4.18 ± 0.17	4.93 ± 0.24	4.09 ± 0.17
11.	Nitrate	92.7 ± 2.44	93.9 ± 2.81	96.9 ± 2.29	94.1 ± 2.52
12.	Nitrite	66.1 ± 2.27	69.9 ± 2.17	78.2 ± 2.12	71.5 ± 2.16
13.	Ammonia	41.3 ± 3.24	44.6 ± 3.08	46.2 ± 3.17	45.4 ± 3.42
14.	Total phosphate	70.02 ± 4.18	72.05 ± 4.13	80.25 ± 4.19	71.45 ± 4.71
15.	Inorganic phosphate	36.52 ± 2.19	38.93 ± 2.17	39.25 ± 2.18	38.25 ± 2.32
16.	Organic phosphate	35.50 ± 3.15	38.06 ± 3.18	40.50 ± 3.42	39.24 ± 3.72
17.	Calcium	196 ± 2.10	183 ± 2.42	174 ± 2.17	185 ± 2.18
18.	Magnesium	154 ± 2.17	147 ± 2.54	135 ± 2.52	146 ± 2.17
19.	Chloride	69.5 ± 4.62	63.4 ± 4.57	62.6 ± 4.43	65.7 ± 4.35

(Each value represents mean ± SD of three observation).

Monsoon (Oct-Dec); Postmonsoon (Jan-Mar); Summer (Apr-June); Premonsoon (July-Sep)

* Except pH and temperature all values expressed in mg^{-1}.

Table 2. Bacterial flora observed from detergent effluent of four different seasons.

S. No.	Name of bacteria	Summer	Premonsoon	Monsoon	Post monsoon
1.	Escherichia coli	+	+	+	+
2.	Enterobacter aerogens	+	+	+	+
3.	Klebsiella pneumoniae	+	+	+	+
4.	Proteus vulgaris	+	+	+	+
5.	Pseudomonas sp.	+	+	+	+
6.	Serratia sp.	+	+	+	+
7.	Salmonella sp.	+	+	+	+
8.	Shigella sonnei	+	+	+	+
9.	Streptococcus faecalis	+	+	+	+
10	Micrococcus sp.	+	+	+	+
11	Bacillus subtilis	+	+	+	+
12	Staphylococcus faecium	+	+	+	+

+ : Observed in all the months.

Table 3. Fungal flora observed from detergent effluent of four different seasons.

S.No.	Name of fungi	Summer	Premonsoon	Monsoon	Post monsoon
1.	Aspergillus flavus	++++	+++	+++	+++
2.	A. fumigatus	++	+	+	+
3.	A. luchensis	++	++	++	+
4.	A. niger	+++	++++	+++	++++
5.	Blastocladia sp.	+	-	-	-
6.	Candida sp.	+	+	-	+
7.	Penicillium javanicum	+++	++	++	++
8.	Saprolespgia sp.	++	+	+	+
9.	Trichoderma viride	+++	+++	++	+++
10.	Alternaria sp.	+	-	-	-
11.	Fusarium oxysporum	++++	+++	+++	+++
12.	Rhodosporium sp.	+++	+++	++	+++
13.	Curvularia sp.	+	+	+	+

++++ : Observed in all the months

+++ : Observed in above five months

++ : Observed in two to four months only

+ : Observed in one month only

- : Not observed

Table 4. Cyanobacterial flora observed from detergent effluent of four different seasons.

S.No.	Nameof cyanobacteria	Summer	Premonsoon	Monsoon	Post monsoon
1.	Microcystis aeruginosa	++	+++	++	+++
2.	Oscillatoria rosea	-	+++	+++	+++
3.	O. chlorine	+++	+++	-	+++
4.	O. tenuis	+++	++++	+++	+++
5.	O. brevis	+++	+++	+	+++
6.	O. guttulata	+	++	++	++
7.	O. subbrevis	+++	+	-	+
8.	O. salina	+++	+++	+++	+++
9.	O. earlei	+++	+++	++	+++
10.	O. clarisentrosa	+++	-	++	-
11.	O. princeps	++++	++++	++++	++++
12.	Phormidium tenue	++	++++	++	+++
13.	P. mucosum	++++	++	++	++
14.	P. valderianum	++	+	++	+
15.	Lyngbya majusculla	+++	+	-	+
16.	L. spiralis	+	-	+++	-
17.	L. martensiana	+	-	+++	-
18.	Plectonema radiosum	+++	+++	+++	++
19.	Nostoc communae	+++	+++	++	+++
20.	N. calcicola	++	++	+	++
21.	N. muscorum	+++	+	-	++

22.	Anabaena fertilissima	+++	++	++	++
23.	A. flosaquae	++	+	+	+
24.	A. sphaerica	++	-	++	-
25.	Fischerella ambigua	-	++	++	+
26.	Chlorogloea fritschii	-	++	+++	+
27.	Synechococcus elongatus	+++	++	++	++
28.	Hapalosiphon fontinalis	++	-	++	-
29.	H. welwitchii	+++	+	++	++

++++ : Observed in all the months

+++ : Observed in above five months

++ : Observed in two to four months only

+ : Observed in one month only

- : Not observed

RESULTS AND DISCUSSION

The results of physico-chemical characterization of detergent effluent are analyzed. The effluent was slightly alkaline and contained high amounts of nitrate, nitrite and ammonia, total phosphate, inorganic and organic phosphate and calcium in all the seasons examined. Very low level of dissolved oxygen and high levels of BOD and COD were recorded during the study period. High amount of total suspended solids were recorded in summer and pre-monsoon seasons (Table 1). Total dissolved solids were high in summer followed by post monsoon and pre monsoon seasons. Bicarbonate was observed only during monsoon seasons. Nutrients such as nitrate, nitrite and inorganic phosphates were high in monsoon, whereas total and organic phosphates were maximum during monsoon season. BOD and COD were very high during summer on the other hand high level of DO was recorded during monsoon. Dissolved oxygen level was very low during summer and high in monsoon. Most of the parameters tested were slightly higher in summer than monsoon, post monsoon and pre monsoon seasons.

The physicochemical analysis of the effluent revealed that it is slightly alkaline in nature and also the presence of high quantity of both organic as well as inorganic nutrients in all the seasons examined (Table 1). Values of DO were very low indicating highly obnoxious conditions. Though BOD and COD levels in the present study were high as per WHO standards, their levels were not so much high as compared to other types of effluents such as paper (Somashekar and Ramasamy, 1983; Manoharan and Subramanian, 1992b), distillery (Jain *et al.*, 2001, Veerasamy *et al.*, 2011) and dye effluent (Sulaiman *et al.*, 2002). Most of the parameters tested were slightly higher in summer than in other seasons. Somashekar and

Ramaswamy (1983) reported similar results in paper mill effluent in different seasons. They recorded objectionable amounts of BOD and COD, oil and grease, total dissolved solids, and algal nutrients such as ammonia nitrogen, nitrate nitrogen, silicates, phosphates and calcium. Such a trend was observed in the detergent effluent also. Sahai *et al.* (1985) analyzed pollution load of four different effluents such as fertilizer, sugar, distillery and domestic sewage. Among these, highly objectionable amounts of various pollutants including BOD and COD were recorded in distillery followed by sugar, fertilizer and domestic sewage.

Bacteria isolated from the detergent effluent were identified based on colony morphology, Gram staining, and various biochemical characteristics. The characteristics of isolated bacteria are given in the table 2. Totally twelve different bacteria were isolated from the effluent sample. The species isolated were *Escherichia coli, Enterobacter aerogens, Klebsiella pneumonia, Proteus vulgaris, Pseudomonas* sp, *Serratia* sp, *Salmonella* sp, *Shigella sonnei, Streptococcus faecalis, Micrococcus* sp, *Bacillus subtilis* and *Staphylococcus faecium*. All the species were recorded in all the seasons. All the species were recorded in all the seasons. There was not been much work regarding the isolation and identification of bacteria from chemical industry and other related effluent samples. Jain *et al.* (2001) isolated three different bacterial strains from the distillery sludge to treat predigested distillery wastewater. In the present study totally thirteen different species of fungi belonging to nine genera were isolated from the effluent (Table 3). Among the fungi recorded the *Aspergillus* was found to be dominant with four species viz., *A. flavus, A. fumigatus, A. luchensis* and *A. niger*. The rest of the genera such as *Blastocladia* sp, *Candida* sp, *Penicillium javanicum, Saprolespgia* sp, *Trichoderma viride, Alternaria* sp, *Fusarium oxysporum* and *Rhodosporium* were recorded with single species each. Similarly *Neurospora crussa* was not observed during rainy season. Kousar *et al.* (2000) isolated 23 species of fungi from dye effluent polluted habitat with *Aspergillus* as the dominant genus.

Altogether 29 species of cyanobacteria belonging to eleven genera were collected from the effluent stream (Table 4). Among the genera, *Oscillatoria* dominated the effluent with 10 species followed by *Phormidium, Lyngbya, Nostoc* and *Anabaena* with 3 each species, *Hapalosiphon* with 2 and *Microcystis, Plectonema, Fischerella, Chlorogloea* and *Synechococcus,* with single species each. *Chlorogloea fritschii* and *Fischerella ambigua* were not observed in summer season. *O. subbrevis, O. chlorine, Lyngbya majusculla* and *N. muscorum* were not observed in monsoon season. Similarly *Oscillatoria clarisentrosa,* *Lyngbya spiralis, L. martensiana, Anabaena sphaerica* and *Hapalosiphon fontinalis* were not observed in post monsoon and pre monsoon season. Many publications emphasize the importance of light, temperature, pH, carbon dioxide, organic matter, alkalinity, nitrates and phosphates as factors important in determining the distribution of cyanobacteria (Singh, 1960; Philipose, 1960; Venkateswaralu, 1969, Munawar, 1970). In the present study, as a whole, conditions in the effluent appeared to be favourable for the cyanophycean members. The effluent had high oxidizable organic matter, nutrients such as nitrates and phosphates with high calcium content. They noted that oxygen deficiency favoured cyanobacterial growth. Dominant and persistent occurrence of most of the species of *Oscillatoria* and *Phormidium* indicate their capacity to thrive in the type of man-made habitat. Moreover these findings show that there are certain species of cyanobacteria which are tolerant to organic pollution and resist environmental stress caused by the pollutant.

Rich blooms of some cyanobacteria such as *O. animalis, O. salina, O. brevis, O. curviceps, O. subbrevis, P. anomala, P. incrustatum, P. submembranaceum* and *Aphanocapsa pulchra* have been observed throughout the year. This abundance is attributed to favourable conditions of oxidizable organic matter, less dissolved oxygen and high calcium content an observation which supports Rao (1955) and Venkateswarlu (1969). Observations of Munawar (1970a, b) suggest that Cyanophyceae grow luxuriously with great variety and abundance in ponds rich in calcium. Venkateswarlu (1969) observed that high orthophosphate levels favoured the development of cyanobacterial bloom. Sarojini (1996) observed positive correlation between phosphate and cyanobacteria. The luxuriant growth of cyanobacteria at low concentration of oxygen and in the presence of high concentration of nitrogen and phosphate has also been reported by Rai and Kumar (1977). Similar observations were also made in the present study with reference to various nutrients. Rai and Kumar (1976a) observed non heterocystous forms in the polluted waters rich in nitrogen. Present investigation also showed dominance of non heterocystous forms and not single species of heterocystous cyanobacteria.

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