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Original Research Article

Degradation and adsorption of industrial effluents by consortium of microbes isolated from agro forestry soil

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A B S T R A C T

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This research work was conducted to assess the physical, chemical (odor, temperature, pH, TS, TSS, TDS and BOD) and biological (Microbial count) properties of six different effluent samples (sewage, textile, coir, whey, leather and metal) of Madurai. Five bacterial isolates were isolated from vegetable cultivated soil of Kodaikanal and it was named as KM-1, KBE-1, KB-1 and KP1. Minimum pH (3.5), TDS (10mg/ml) in metal sample, TSS (9mg/ml) in whey sample, microbes (1cfu/ml) in dye sample, and BOD (49mg/ml) in whey sample, was observed after biological treatment. Maximum decomposition of waste was occurred, when we used microbial consortium (mixing of all the culture).Among the five isolates, KP-1 has high efficiency to degrade and decompose the waste material present in the samples.

Introduction

Water is one of the most important and abundant compounds of the ecosystem. All living organisms on the earth need water for their survival and growth. Only earth is the planet having about 70 % of water. But due to increased human population, industrialization, use of fertilizers in the agriculture and man-made activity it is highly polluted with different harmful contaminants. Therefore it is necessary that the quality of drinking water should be checked at regular time interval, because due to use of contaminated drinking water, human population suffers from varied of water borne diseases. It is difficult to understand the biological phenomenon fully because the chemistry of water revels much about the metabolism of the ecosystem and explain the general hydro - biological relationship (Simpi *et al*., 2011).

The increased use of metal-based fertilizer in agricultural revolution of the government could result in continued rise in concentration of metal pollutions in fresh water reservoir due to the water run-off. Also faucal pollution of drinking water causes water born disease which has led to the death of millions of people (Adefemi and Awokunmi, 2010). Having mainly excessive amounts of heavy metals such as Pb, Cr and Fe, as well as heavy metals from industrial processes are of special concern because they produce water or chronic poisoning in aquatic animals (Ellis, 1989). High levels of pollutants mainly organic matter in river water cause an increase in biological oxygen demand (Kulkarni, 1997), chemical oxygen demand, total dissolved solids, total suspended solids and faecal coli form. They make water unsuitable for drinking, irrigation or any other use (Hari, 1994).

Water is an essential compound for the survival and sustenance of life on the planet earth. The waste water or sewage water thrown out from industries is either used for irrigation purposes or it runs off to natural sources of water. If these effluents are not treated before their disposal they can be harmful for human consumption as well as for other uses too (Ahlawat and Kumar, 2009). The residual dyes from different sources e.g., textile industries, paper and pulp industries, dye and dye intermediates, tannery, and Kraft bleaching industries, etc. are contain wide variety of organic pollutants introduced into natural water resources or wastewater treatment systems.

One of the main sources with severe pollution problems worldwide is the textile industries and its dye-containing wastewaters. (10–25%) of textile dyes are lost during the dyeing process, and (2–20%) is discharged as aqueous effluents in different environmental components. In particular, the discharge of dye-containing effluents into the water environment is undesirable because of their colour, released directly and breakdown products are toxic, carcinogenic or mutagenic to life forms mainly because of carcinogenic, such as benzidine, naphthalene and other aromatic compounds (Suteu *et al*., 2009).

The textile industry consumes large amounts of potable and industrial water as processing water (90–94%) and a relatively low percentage as cooling water (6–10%). The recycling of treated wastewater has been recommended due to the high levels of contamination in dyeing and finishing processes (i.e. dyes and their breakdown products, pigments, dye, intermediate, auxiliary chemicals and heavy metals (Bertea and Bertea, 2008; Bisschops and Spanjers, 2003; Correia *et al*., 1994; Orhon *et al.*, 2001).

The coir industry is one of the major agro-based industries of the state contributing notable job opportunities to the rural communities. Coirpith, industry require a large amount of water and consequently generates an equally large quantity of waste water, which contains 27.8% of cellulose, 28.5% of lignin and 8.12% of soluble tannin like phenolic compounds (Vinodhini *et al.*, 2006). The effluent generated from the coir industry is acidic, also contains phenolic compounds and other toxic substances.

The present work focused on the treatment method of Sewage, Metal, Leather, Whey, Coir and dye waste were carried out using microbial consortium isolated from vegetative cultivated forest soil, to study the degradation and adsorption of organic and inorganic compounds of effluents. An attempt was made to find the physico-chemical properties of the waste water before and after treatment.

Materials and Methods

Physical, chemical and biological parameters were analyzed to evaluate wastewater pollution. For quantitative analysis of wastewater, pH, colour, odours, TS, TSS, TDS, bacterial viable count, BOD tests were considered.

Water sampling for analysis

Samples were collected from six different industrial wastes (Sewage water-1, Metal waste water-2, Coir waste-3, Whey waste-4 Dye waste water-5 and Tannery waste-6. Amount of each of the samples was 2 liters. For collection, bottles were cleaned with tap water, rinsed under the drain water, uncapped and water was collected from beneath the surface. Air bubbles were removed and the bottles were capped immediately (Besselievre and Schwartz, 1976). Each of the sample bottles was labelled with the necessary information.

Biological pollution

Master dilution was prepared by mixing 90 ml of distilled water and 10 ml of wastewater in a sterile conical flask. Then 10^{-2}, 10^{-3}, $l0^{-4}$, 10^{-5} and 10^{-6} times serial dilution were prepared from the master dilution by adding sterile distilled water. 0.1ml of water from 10^{-1} to 10^{-6} diluted solution were taken in six (each in three) sterile Petri plates by sterile pipette to nutrient agar (NA) media, After appropriate incubation period the plates were observed for the appearance of colonies and the numbers of colonies were counted.

Colour, odours and pH

Taking wastewater sample in a clean glass test tube, the color was evaluated visually. The odours of all three wastewater samples were smelled at 28^0 C and noted. pH was measured using pH paper.

Total solids in the effluent sample

Accurately weigh a clean dry silica crucible band record this weigh $W_{I.}$ Transfer unfiltered 100ml sample in the silica crucible. Evaporate the sample by placing it in a hot air oven at 105°C for 1 hr. Cool it in a desiccators. Take a crucible out of the desiccators and record its weight (W_F) (Repeat cycle of drying, cooling, desiccating, and weighing until a constant weight is obtained).Calculate the total solids of the given sample as follows

$$\text{Total solids (mg/L)} = \frac{(WI)-(WF)}{\text{sample volume,ml}} \times 1000$$

Where,

W_I – Initial weight of the crucible

W_F – Final weight of the crucible

TDS

Weigh clean dry silica crucible and transfer 100ml of the filtered sample in the silica crucible (W_I). Evaporate the crucible in a hot air one hour at 18°C. Cool it in a desiccators and record its weight Let be W_F (Repeat cycle of drying, cooling, desiccating and weighing until a constant weight is obtained) Now calculate the TDS of given using the formula.

Calculation

Total dissolved solids (mg/L) =

$$\frac{(W_1)-(WF)}{\text{Sample volume ,mL}} \times 1000$$

Where,

W_I-Initial weight of crucible

W_F-Final weight of crucible

TSS

Total suspended solids can be obtained by subtracting the total dissolved solids from total solids. Total suspended solids = total solids-total dissolved solids from total solids.

BOD

Collected sewage and add 50ml sewage sample to one liter of 8 mg/L O_2 containing water sample. Rinse BOD bottle clearly with water. Neutralize the pH of the water using acid or alkali. Fill two BOD bottle with water/sewage/Effluent sample. Avoid air bubble use one BOD bottle for DO estimation. 2 to 3 drops of sulphuric acid were added. Titrate 50ml of acidified sample against sodium thio sulphate solution .after the formation of pale yellow colour, add one or two drops of starch indicator to the sample. The sample was titrated again up to the disappearance of blue colour (Initial oxygen). Incubate remaining bottle at 20–27^{o}C for 3–5 days. Estimate oxygen concentration after 5 days of incubation (final oxygen).

Dissolve oxygen of the given sample is calculated by the following:

$$0.2(\text{mg/L})\ \frac{\text{Titrant value} \times 0.025 \times 8 \times 100}{\text{Volume of the sample}}$$

0.025 – normality of the titrant

8 = Molecular weight of oxygen

BOD of the effluent was calculated by the formula: BOD=D1-D2

Treatment of waste waster by isolated microbes:

Collection and isolation of soil sample

Soil samples were collected from various cultivated area (Beans, carrot, Potato, Mullangi and Beet root) of Kodaikanal. Soil sample was serially diluted and plated on nutrient agar medium. Morphologically different colonies were selected from each soil sample. The selected microbes were identified by morphological and biochemical tests.

Preparation of culture for treatment

Prepared 100ml of five nutrient broths medium and inoculate five isolates separately. All the flasks were incubated at 37°C for 24 hrs. 50g of Mulluthanimetti powder was mixed with 25ml of culture to make a bead. This powder is used as carrier molecules for culture and adsorbent.

Biological treatment

Air activated sludge is an aerobic in which bacteria consume organic matter, nitrogen and oxygen from the waste water and grow new bacteria. The bacteria are suspended process in the aeration tank by the mixing action of the air blown into the wastewater. 100ml of sewage water was taken in 6 sterile bottles. (One for control and others are tests). 3 grams of bacterial beads were added separately in 5flasks except control. Sewage sample was incubated at 37°C for 7 days. After incubation, the pH, colour, odours, TS, TSS, TDS, bacterial viable count, BOD tests were analysed in both test and control. Same procedures were followed for dye, coir, whey, leather, metal and tannery effluents.

Preparation of bacterial consortium

Equal volumes of 5 different bacterial isolates were mixed together with Mulluthanimetti powder to make beads. 100ml of sewage water was taken in 2 sterile bottles. (One for control and others are tests). 3 grams of bacterial beads (consortium) were added in test sample except control. The sewage sample was incubated at 37°C for 7 days. After incubation, the pH, colour, Odours, TS, TSS, TDS, bacterial viable count, BOD tests were analysed in both test and control. Same procedures were followed for dye, coir, whey, leather, metal and tannery effluents.

Result and Discussion

The sewage, textile, coir, whey, leather and metal effluent were collected in different locations of Madurai was reported in Table 1. Physical, chemical and biological parameters were analyzed to evaluate wastewater pollution. For quantitative analysis of wastewater, pH, colour, odours, TS, TSS, TDS, bacterial viable count, BOD tests were considered. Table 2 shows that enumeration total microbes in the effluent samples before biological treatment. The isolated bacteria were identified by morphological and biochemical tests (Bergey's manual of systematic bacteriology, 1984). The identified bacterial strains were named as KM-1, KC-1, KBE-1KB-1 and KP-1. The physico-chemical parameters of sewage, textile, coir whey, leather and metal effluent were analysed. Considerable deviations are observed in the water quality parameters from the standard limits.

pH value of water is influenced by geology of catchments areas as well as agricultural runoff and buffering capacity of water and waste compounds. The limit of pH value for waste water is specified 6 to 9 by WHO (2006). In our study, we observed the pH value of different waste water sample were 6 (sewage), 5 (Metal), 7.8 (Leather), 6.8 (Whey), 6 (coir) and 6 (Dye) (Table 3–8). Savin and Butnaru, (2008) reported that the pH value of water sample under study fluctuates between 5.4 to10.8. This trend of pH value shows alkaline trend. Also the heavy metal toxicity gets enhanced at a particular pH. It is also taken as a measure of the concentration of organic matter present in any water. Jayalakshmi *et al*. (2011) found that substantial variations were recorded in the pH level. Always the average values of pH in all sites in all seasons were 5.3 to 9.2. If pH is above 7, this will indicate that water is probably hard and contains calcium and magnesium. The low pH (5.3) affected the bacterial growth. High alkaline pH (9.2) was recorded in water sample collected at site-V.

The toxicity of heavy metals also gets enhanced at particular pH. Thus, pH is having primary importance in deciding the quality of waste water effluents. Waters with pH value of about 10 are exceptional and reflect contamination by strong base as NaOH and $Ca(OH)_2$. The ranges of desirable pH value of ISI and WHO are 6–5 and 8–5 for drinking purpose. Irrigation with wastewater decreased soil pH. The reason is likely due to the decomposition of organic matter and production of organic acid in soils irrigated with wastewater.

Fresh sewage is typically gray in color. However, as the travel time in the collection time increases, and more anaerobic conditions develop, the color of the wastewater changes sequentially from gray to dark gray and ultimately to black. In our work, the raw sewage sample was black in colour but after bacterial treatment, it becomes colourless due to the decomposition of organic matter by the isolates and odour also acceptable one (Table 3). The metal effluent sample changes from dark grey colour to light grey, Leather effluent (Red to light red), Whey effluent (white to colourless), coir effluent (Brownish black to pale brown), dye effluent (Dark yellow to Pale yellow) (Table 3–8).

Odours in domestic waste water usually are caused by gases produced by the decomposition of organic matter or by substances added to the wastewater. Fresh, aerobic, domestic wastewater has been said to have the odour of kerosene or freshly turned earth, which is less objectionable than the odour of wastewater that has undergone anaerobic decomposition. Aged, septic sewage is considerably more offensive. The characteristic rotten-egg odour of hydrogen sulfide and the mercaptants is indicative of septic sewage, which is produced by anaerobic microorganisms that reduce sulfate to sulfide. Industrial wastewater may contain either odourous compounds or compounds that produce odours during the process of wastewater treatment. The importance of odours at low concentrations in human terms is related to the psychological stress they produce rather than to the harm they do to the body. Offensive odours can cause poor appetite for food, lowered water consumption, impaired respiration, nausea and vomiting, and mental perturbation.

Physico-chemical characters of various effluent samples were shown. In untreated effluent, the concentration of BOD was found in 290 mg/l in sewage sample. This indicates that there could be low oxygen available for living organisms in the sewage wastewater (Table 3–8). The BOD level of the metal effluent was 150 mg/L, in leather (220mg/l), in whey (95 mg/l), in coir (115mg/) and in dye (123 mg/l). But in treated sewage effluent, the concentration of BOD was found in 150mg/l by the culture KC-1, in metal effluent was 78 mg/L by KP-1, in leather (155mg/l) by KP-1, in whey (49 mg/l) KP-1, in coir (63mg/) by KP-1and in dye (84 mg/l) by KP-1.

The BOD and COD level of the effluent were high as compared to IS Standard, their levels were not so much high. There was no carbonate in the effluent but fairly high level of free CO2 and bicarbonate was recorded as 128 (mg/L) and 150 (mg/L), respectively (Kasthuri *et al*., 2011). The DO in untreated effluent and DO in the downstream was observed to deplete faster than upper-stream could be attributed to the presence of degradable organic matter which resulted in a tendency to be more oxygen demand. The effluent waste discharge to surface water source is largely determined by oxygen balance of the system and its presence is essential in maintaining life within a system (Rao, 2005).

Dissolved oxygen concentration in unpolluted water normally range between 8 to 10 (mg/l) and concentration below 5 (mg/l) adversely affect aquatic life (Lokhande *et al*., 2001). The concentration of BOD was found in the range of 221(mg/l) to 699(mg/1). (Goyal varsh *et al*., 2013). The greater the decomposable matter present, the greater the oxygen demand and the greater the BOD values (Savin and Butnaru, 2008).
The total solid content of different waste water was shown in Table 3–8. The high TS may be attributed to use of salts during dyeing process. The TS ranged from 1475.6 to 13499.2(mg/l)., were study on physico-chemical parameters of wastewater from Taloja Industrial area, Mumbai, India (Lokhande *et al*., 2001). High content of dissolved solids affects the density of water, influences osmo regulation of freshwater organisms, reduces solubility of gases (like oxygen), and utility of water for drinking, irrigation purpose (Lokhande *et al*., 2001). The high TSS values were observed at site-3 and minimum values were also observed at site-1. Same results of TSS 200-6000 mg/l were worked out the analysis of industrial effluents and its comparison with other effluents from residential and commercial areas in Solan, H.P. **(**Ahlawat and Kumar, 2009).

The total dissolved solid of the various effluent samples was shown in Table 3–8. Total Dissolved Solid (TDS) and Total Suspended Solid (TSS) detected could be attributed to the high color. The concentration of TDS and TSS were found in the range of 3260mg/l–17700mg/l and 124(mg/l)–930(mg/l). TDS concentration was very high at UN 8, 10, 13, 15 and the highest concentration of TSS was found at UN 3, 8, 9, 12, 15, 16 .The solids present in the water, besides effecting the growth of the plants directly, also affects the soil structure, permeability and aeration thus affecting the plant growth. The effluent waste discharge to surface water source is largely determined by oxygen balance of the system and its presence is essential in maintaining life within a system. Dissolved oxygen concentration in unpolluted water normally range between 8 to 10 mg/l and concentration below 5 (mg/l) adversely affect aquatic life (Rao, 2005).

The maximum values of TDS were obtained from site-2, and minimum values were obtained at site-1 (Table 1). High content of dissolved solids affects the density of water, influences osmo regulation of freshwater organisms, reduces solubility of gases (like oxygen), and utility of water for drinking, irrigation purpose (Lokhande *et al*., 2001). The high TSS values were observed at site-3 and minimum values were also observed at site-1. Same results of TSS 200-6000mg/l were worked out the analysis of industrial effluents and its comparison with other effluents from residential and commercial areas in Solan, H.P. (Ahlawat and kumar, 2009).

Similar results were obtained in studying the effect of industrial effluents and wastes on physicochemical parameters of river Rapti (Chaurasia and Tiwari, 2011; Islam *et al*., 2011 and WHO, 1999). The physico-chemical analysis of the effluent showed its slightly acidic nature and also the presence of high quantity of both organic as well as inorganic nutrients (Table 1). The value of Dissolved Oxygen (DO) was low which indicated that the highly obnoxious condition. The BOD and COD level of the effluent were 240 and 482 mg/L. Though BOD and COD level of the effluent were high as compared to IS Standard, their levels were not so much high. There was no carbonate in the effluent but fairly high level of free CO_2 and bicarbonate was recorded as 128 mg/L and 150 mg/L, respectively.

Microbial population in effluent

Table 2 and 9 shows the different types of microorganisms that are present in the waste water (before and after treatment) namely bacteria and fungi. It was observed that Bacillus sp members dominated in the effluent stream. This is attributed to favourable conditions of oxidizable organic matter, less DO and high calcium contents an observation which supports Rao (1955). The cyanophyceae grow luxuriously with great variety and abundance in ponds rich in calcium (Munawar, 1970).

The total number of fungal isolates distributed in 3 different genera were isolated and identified among the genera, *Aspergillus* with 8 species, was found to be dominant genus. Kousar *et al.* (2000) isolated 23 species from dye effluent, polluted habit with *Aspergillus* as the dominant genus. The 8 different species of bacteria were isolated. Further, effluent are rich in nutrients due to the loading of organic wastes, they afford ideal habitats for different microorganisms including algae, fungi and bacteria.

Coir pith is biologically active. In addition to providing an environment for plant roots, they also support a diverse population of microorganisms. These organisms obtain energy from cellulose and other carbon based compounds in the mix and competes with the plant roots for nutrients, moisture and oxygen. The vast majority of these organisms are not pathogenic and their presence near the roots can be beneficial in a number of ways such as in suppressing of the development and proliferation of soil borne diseases. This is achieved by competing for food and space. In most instances, the pathogen is restrained by the sheer force of numbers of the friendly or beneficial organisms.

Table.1 Effluent samples collected in different location

S.No	Sample	Collected location
1	Sewage	Teppakulam
2	Coir	Vadipetti
3	Leather	Dindugal
4	Dye	Anupanadi
5	Whey	Anna Nager
6	Metal	Kelivazhal

Table.2 Microbial population in the industrial effluent before treatment

S.NO	SAMPLE	BACTREIA			FUNGI		
		10^{-3}	10^{-4}	10^{-4}	10^{-3}	10^{-4}	10^{-5}
1.	SEWAGE	182	25	1	18	3	-
2.	METAL	14	2	-	4	-	-
3.	LEATHER	2	-	-	1	-	-
4.	WHEY	175	31	5	31	2	-
5.	COIR	158	29	8	43	4	2
6.	DYE	31	2	-	12	4	-

Table.3 Analysis of Physico-chemical characteristics of sewage water before and after treatment

S.NO	Culture	pH	Colour	Odour	TS mg/l	TSS mg/l	TDS mg/l	BOD mg/l
1	Control	6.0	Black	Non acceptable	643	200	446	290
2	KM1	6.5	Black	Non acceptable	1196	150	1046	210
	KC1	7.0	Whight	Acceptable	411	322	89	150
4	KBE1	6.5	Whight	Acceptable	392	284	76	205
5	KB1	4.8	Black	Non acceptable	382	277	63	170
6	KP1	5.0	Black	Non acceptable	315	224	56	190

Table.4 Analysis of Physico-chemical characteristics of metal effluent before and after treatment

S.NO	Culture	pH	Colour	Odour	TS mg/l	TSS mg/l	TDS mg/l	BOD mg/l
1	CONTROL	5	Grey	Non acceptable	450	350	150	150
2	KM1	4.8	Light grey	Acceptable	50	60	10	125
3	KC1	4.5	Light grey	Acceptable	670	560	110	114
4	KBE1	4	Light grey	Acceptable	650	450	200	97
5	KB1	3.8	Grey	Non acceptable	540	420	120	83
6	KP1	3.5	Light grey	Acceptable	430	225	205	78

Table.5 Analysis of Physico-chemical characteristics of leather effluent before and after treatment

S.No	Culture	pH	Colour	Odour	TS mg/l	TDS mg/l	TSS mg/l	BOD mg/l
1	Control	7.8	Red	non acceptable	436	426	5	220
2	KM1	6	Light red	Acceptable	126	31	95	197
3	KC1	6.5	Light red	Acceptable	774	185	589	189
4	KBE1	7	Light red	Acceptable	534	31	403	178
5	KB1	7.5	Light red	Acceptable	74	32	42	163
6	KP1	8	Light red	Acceptable	638	469	169	155

Table.6 Analysis of Physico-chemical characteristics of whey effluent before and after treatment

S.No	Culture	pH	Colour	Odour	TS mg/l	TDS mg/l	TSS mg/l	BOD mg/l
1	Control	6.8	White	Non acceptable	705	431	274	95
2	KM1	6.5	White	Non acceptable	176	145	31	86
3	KC1	6	Pale white	Acceptable	185	170	55	77
4	KBE1	5.8	Pale white	Acceptable	230	130	100	65
5	KB1	5.5	White	Non acceptable	654	662	32	52
6	KP1	5	White	Non acceptable	47	38	9	49

Table.7 analysis of physico-chemical characteristics of coir effluent before and after treatment

S.No	**Culture**	**pH**	**Colour**	**Odour**	**TS mg/l**	**TDS mg/l**	**TSS mg/l**	**BOD mg/l**
1	Control	6	Brownish black	Non acceptable	53	28	25	115
2	KM1	5.8	Pale brown	Acceptable	122	28	94	108
3	KC1	5	Pale brown	Acceptable	473	34	439	98
4	KBE1	4.5	Pale brown	Acceptable	68	13	55	85
5	KB1	5	Pale brown	Acceptable	166	35	131	77
6	KP1	5.8	Pale brown	Acceptable	192	24	168	63

Table.8 Analysis of physico-chemical characteristics of dye effluent before and after treatment

S.No	**Culture**	**pH**	**Colour**	**Odour**	**TS mg/l**	**TDS mg/l**	**TSS mg/l**	**BOD mg/l**
1	Control	6	Dark yellow	Non acceptable	550	480	70	123
2	KM1	5.8	Pale yellow	Acceptable	430	380	50	117
3	KC1	5	Pale yellow	Acceptable	240	150	90	112
4	KBE1	4.6	Pale yellow	Acceptable	150	40	110	108
5	KB1	5	Dark yellow	Non acceptable	60	30	30	99
6	KP1	5.8	Dark yellow	Non acceptable	180	90	90	84

Table.9 Microbial population in the industrial effluents after treatment

S.No	**Sample**	**Bacteria**			**Fungi**		
		10-3	10-4	10-5	10-3	10-4	10-5.
1.	Sewage	163	17	2	17	4	1
2.	Metal	12	-	-	4	-	-
3.	Leather	4	-	-	-	-	-
4.	Whey	181	35	2	21	1	-
5.	Coir	143	31	7	37	2	-
6.	Dye	22	1	-	7	-	-

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