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Bioremediation of Azo Dye Containing Textile Effluent using Adapted Bacterial Strains under Subsequent Microaerophilic -Aerobic Conditions

R Rajendran[1], S K Sundaram[1], P Prabhavathi[1]*, B V Sridevi[1],S D Kumar[2] and P Santhanam[2]

[1]PG & Research Department of Microbiology, PSG College of Arts & Science, Coimbatore – 641014, India.
[2]Department of Marine Science, School of Marine Sciences, Bharathidasan University, Tiruchirappalli-620024, India.

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Textile effluents are causing a wholesome of trouble in our vicinity, which needs to be taken good care of before being discharged in to the natural surroundings. In our present study, we have isolated about 5 different bacterial strains from azo dye containing textile effluent sample**.** The bacterial strains were allowed to act on the textile effluent under subsequent microaerophilic and aerobic conditions. In the treatment under microaerophilic condition the bacterial strains were allowed to form a biofilm on inert polyurethane foam as individual cultures and as a consortium, in a glass column. The effluent sample was passed through the column in a particular hydraulic retention time (8ml/hr). The samples were collected at the base after treatment in a conical flask, incubated in a metabolic shaker at 120 rpm for a period of 8 hours for aerobic remediation. The consortium was able to elicit an efficient remediation potential than the individual strains at an optimal retention time of 12 hours with 8 hours of aerobic agitation at a pH of 7 and at an optimal organic loading rate of 100% of the effluent load. Analyzing the effluent treated through GC/MS, which found that the toxic end products formed at the end of microaerophilic treatment was removed through the aerobic treatment modality, and the effluent was found to be devoid of any toxicity after clarification and disinfection processes.

Keywords: Decolorization, Textile effluent, Biofilm, Microbial remediation, Microbial biodegradation.

Introduction

Textile industry is one of the major revenue generating industries in the country. Coimbatore plays the southern hub providing a major share of income to the cities in India. Till a decade back the harmful effects of the synthetic azo dyes used in the dyeing units were not known scientifically to most of the people involved in the business. But the development of many sophisticated techniques has brought into limelight the environmental fatigues that the usage of azo dyes brings in. More than 30% of the dyes used in the dye bath ends in the effluent, which has to be treated before discharging into the natural water system; else it makes the water unsuitable for commercial use[1]. The organic content also contemplates to the reduction in the dissolved oxygen level in the natural water system when the effluent is thrown out. In order to make sure that both these factors were taken care of, an efficient cost effective methodology has to be deployed which should not only vanish the problems associated with the treatment of textile effluents but also should not infiltrate in to the expenses of textile industries, reducing their profits. Conventionally the treatment of textile wastes was done through physical and chemical methods and nowadays biological methods were also employed. But without the knowledge of after effects of the effluent there was a lethargic approach in the treatment systems prevailing in the city. The maximum the alkalinity of the effluent was increased using the calcium carbonate primarily for the removal of inorganic substances and to reduce the suspended solids in the effluent, it was passed on through the sand filters for the adsorptive removal of the colored dye compounds. This is one of the main reasons for the Government to enforce a strong rule which has put an end to the functionaries of these dyeing units at large as they were not able to cope up with the standards of the Tamil Nadu Pollution Control Board. With the use of biological systems the treatment of effluent is low cost, and there would be an efficient bioremediation capacity that would be incorporated as a result of the metabolic activities of the microbes[2]. The use of microbes which were already adapted to the harsh environment of the effluent would be the exact competitor which would serve the purpose of putting an end to the pollution

*Author for correspondence
E-mail: prabha_micro2007@yahoo.co.in

problem[3]. This is because under anaerobic condition the azo dyes reduces giving rise to more toxic and mutagenic, aromatic amines.

These aromatic amines cannot be reduced under anaerobic condition and hence would be reduced under aerobic status. This study includes isolation and investigation of bacteria capable of degrading azo dye containing textile effluent forming biofilm on inert polyurethane foam in a subsequent anaerobic-aerobic atmosphere. The process would be optimized under different conditions for improving the effectiveness of the treatment process. The toxicity of the treated effluent would be studied and a possible methodology for the efficient treatment of the effluent would be proposed for zero discharge of organic content to the surroundings.

Materials and methods

Sample Collection

Textile effluent was collected from a common effluent treatment plant in Karur District near Coimbatore, Tamil Nadu, India. The effluent was stored in refrigerator at 4^{o}C for further processing.

Isolation, screening and identification of adapted bacterial strains

The isolation of textile dye degrading facultative anaerobic bacterial stains was carried out through serial dilution method. Isolated bacterial strains were screened out by incubating them on nutrient broth medium containing various dyes. The selected efficient bacterial strains were identified based on the microscopic appearance and biochemical characters[6].

Characterization of the untreated effluent

The raw effluent was characterized by measuring the values of 11 different physico-chemical parameters. These parameters were chosen in accordance with the regulations of Tamilnadu Pollution Control Board. All the mentioned physico-chemical analyses were done immediately after the effluent sample was collected[4].

Bioremediation of azo dye containing textile effluent under subsequent microaerophilic and aerobic system

In this study, the effluent was treated using the bioreactor consisting of bacterial trickling filter packed with microbial biofilm for treating the effluent under oxygen limiting conditions followed by a continuous stirred reactor for treating the effluent sample under subsequent aerobic conditions[7].

Bacterial PU foam trickling filter

The bacterial trickling filter was built using borosil glass column with 40cm height and 7cm width. The glass column was filled with PUF pieces of 5×5 mm size which was used as immobilization support for the biofilm development[8]. The PUF were filled upto a height of 35 cm. The total volume of the reactor was 2 liters and has a working volume of 1.5 liters. Nutrient broth containing individual bacterial culture was poured in the column filled with sterilized PUF pieces and the column was left undisturbed for a period of 3 days for the development of biofilm with an average flow rate of 8ml/h with a hydraulic retention time of 12 hours and check their efficiency by physico-chemical parameters[7].

Subsequent aerobic treatment of azo dye containing textile effluent

A known quantity (100 ml) of effluent sample treated under anaerobic conditions was treated under aerobic condition by agitating it at 120 rpm in a mechanical shaker for a period of 8hrs. The sample treated under subsequent aerobic condition[7] was then clarified by the addition of 1% aluminium potassium sulphate, mixed for 5 minutes under rapid shaking condition of 200 rpm and left undisturbed under static condition for 60 minutes. The supernatant remaining after clarification was analyzed by various physico-chemical parameters[5].

Effect of incubation time on biofilm development

The optimum incubation time for biofilm development was determined by incubating the PUF along with consortium over a period of five different days (1, 2, 3, 4 and 5 days) and use the biofilm developed on each incubation period for the treatment of textile effluent. The effluent was collected simultaneously and subsequently treated aerobically in a metabolic shaker at 120 rpm for 8 hrs. The effect of dye degradation was studied by analyzing the various physico-chemical parameters.

Effect of Interactive variables on the Decolorization of Textile Effluent

The efficiency of decolorization was standardized under different conditions of pH (5, 6, 7, 8 and 9), hydraulic retention time (6 hrs, 9 hrs, 12 hrs 15 hrs and 18hrs) and organic loading rate (60, 80 and 100%).

Effect of Aeration time on detoxification of anaerobically treated effluent

Aeration time plays a vital role in the degradation of the toxic aromatic amines which might be formed during the anaerobic treatment. The effect of aeration time on bioremediation could be studied by agitating the anaerobically treated effluent at 120 rpm for different time periods (0hr, 4hrs, 8hrs, 12hrs and 24hrs). The degradation efficiency of the microbes under different aeration time could be analyzed by the physic chemical parameters.

Disinfection

About 0.1% of sodium hypochlorite was added to the effluent treated under simultaneous anaerobic-aerobic treatment and left undisturbed for a period of 1 hour. Before the addition of sodium hypochlorite the water sample was adjuster to pH 7. After the contact time the disinfected water was then plated on to the nutrient agar by spread plate technique and incubated at 37°C to find the effectiveness of the disinfectant in killing the microbes in the treated effluent. The plate was incubated along with the another plate inoculated with the raw effluent, anaerobically treated effluent and effluent sample treated under subsequent anaerobic-aerobic condition for the comparison of the colony count with that of the disinfected water[9].

Scanning electron microscopy (SEM) Analysis

Biofilm developed polyurethane foam was taken and air dried and SEM analysis (Jeol JSAL 6360) was done. SEM analysis was done to confirm the colonization of the bacterial biofilm and to detect whether the effluent has any toxic effect over the bacterial cultures and to check any morphological changes.

Characterization of the treated textile effluent for analyzing the toxicity of the effluent

GC-MS was used to identify the metabolites formed after degradation of azo effluent by the bacterial biofilm. The sample was taken and mixed with equal volume of diethyl ether and allowed to evaporate to dryness. The remaining residue was collected and mixed with acetonitrile and GC/MS analysis of untreated and treated effluent was carried out using a THERMO GC - TRACE ULTRA VER: 5.0, THERMO MS DSQ II. The ionization voltage was 70 ev. Gas chromatography was conducted in temperature programming mode with a 100 - 250°C, RATE: 8/Min, HOLDING TIME: 10 Min @250. The initial column temperature was 40°C for 4 minutes, then increased linearly at 10°C per minute to 270 °C and held at 4 minutes. The temperature of injection port was 275°C and GC/MS interface was maintained at 300°C (version 1.10 beta Shimadzu).

Results and Discussion

Isolation and screening and identification of the bacterial isolates

The agar plates amended with mixture of dyes incubated under anaerobic condition after 24 hours of incubation shows zone of clearance of dyes around the bacterial colonies. Fifteen predominant facultative bacterial strains which showed a zone of clearance of dyes were isolated from the plate and pure cultures, which were maintained and used for further quantitative decolorization assay. Among fifteen strains isolated, five strains (isolate 1, 3, 6, 8, and 15) exhibited significant color removal of more than 60%. The work went in hand with the work of He Fang

Table 1—Bioremediation efficiency of the bacterial isolates (individually) and as consortium - treated under anaerobic and subsequent aerobic condition

Parameters	% Reduction													
	Anaerobic condition							Subsequent aerobic condition						
	Initial	1	2	3	4	5	6	Initial	1	2	3	4	5	6
pH	7.1	9.86	11.2	4.51	7.04	14.08	9.86	7.1	7.04	7.04	1.41	4.23	8.45	5.63
Color (490 nm)	0.37	32.4	29.73	21.62	27.03	29.73	62.65	0.37	54.05	54.05	32.43	40.54	37.84	83.78
Turbidity(620 nm)	0.38	76.3	73.68	52.63	63.16	60.53	60.53	0.38	81.58	78.95	60.53	73.38	65.79	86.84
TSS (mg/L)	7100	26.7	21.13	39.44	4.23	12.68	32.39	7100	54.93	45.07	29.58	38.03	38.03	94.37
TS (mg/L)	9600	22.9	25	39.58	31.25	25	35.52	9600	59.38	54.17	47.92	56.65	52.08	91.67
TDS (mg/L)	12000	16.6	20	6.67	26.67	23.33	40	12000	75	72.5	45	65	60	95
COD(mg/L)	7680	72.9	75	82.03	62.5	77.08	84.38	7680	89.58	86.98	79.17	81.77	79.95	95.83
Hardness (mg/L)	1500	74.6	73.33	70.67	72	76	78.6	1500	78.67	66.67	58.67	70.67	68	92
Salinity	7.89	6.08	7.22	3.42	2.28	4.69	8.75	7.89	10.01	8.37	5.07	7.22	8.62	21.42
Resistivity	46.12	25.6	20.58	23.14	27.6	26.86	31.61	46.12	28.45	28.88	24.11	26.5	24.87	29.64
Conductivity	13.35	2.17	2.70	0.22	0.3	3.75	8.61	13.35	2.17	2.62	0.22	4.12	5.62	7.12

Note : In- Initial; 1- *Bacillus* sp.; 2- *Actinomycetes* sp.; 3 - *Micrococcus* sp., 4 - *Pseudomonas* sp.; 5 - *Staphylococcus* sp.; 6 - Consortium *et al.,*[10] where the cultures were isolated based on the zone of clearance of the microbes on solid plates which were further used in the remediation of textile effluents containing a mixture of azo dyes.Five predominant bacterial strains were selected from fifteen strains exhibiting the maximum decolorization efficiency were identified as *Bacillus* sp., *Actinomyces* sp., *Micrococcus* sp., *Pseudomonas* sp., and *Staphylococcus* sp.,. In the work of Olukanni *et al.,*[11] they used *Micrococcus* sp., *Bacillus* sp., *Pseudomonas* sp., and *Staphylococcus* sp., for decolorization of azo dyes which showed 97% decolorization.

Characterization of the untreated effluent

The eleven parameters were measured according to the standard procedure and all the parameters were found to be higher than the TNPCB limits. Since the values were found to be high when compared with the permissible limits, when released as such to the environment it pollutes the water body as it contains heavy organic load and toxic substances. Similar results were observed for Wisaam *et al*.,[12] reported that the raw effluent contain large number of pollutants and the parameters that were analyzed were also found to be high and ensuring that the effluent has to be treated before its disposal to environment.

Effluent treated in the trickling filter under anaerobic condition

Effluent treated with biofilm containing the consortium under anaerobic condition showed efficient reduction in parameters when compared to the individual cultures with a reduction percentage of 62% decolorization. The percentage reductions of other parameters were found to turbidity-60.53%, COD-90%, TS-35.52%, TSS-33%, TDS-40%, hardness-78.6%, resistivity-31.61, conductivity-8.61%, salinity-8.75%. The decolorization in the effluent was due to the breakdown of the azo bond under anaerobic condition by the enzymes produced by the bacterial consortium. The effluent treated with the *Bacillus* sp., showed greater reduction percentage in all the parameters among the five tested organisms. The *Bacillus* sp., exhibited highest bioremediation efficiency followed by *Actinomyces* sp., *Pseudomonas* sp., *Staphylococcus* sp., with the least activity exerted by *Micrococcus* sp., with the decolorization percentage of 21.6%. In the work of Selene *et al.,*[13] they used the biofilm for the treatment of liquid pollutants in a fluidized bed reactor with the biofilm. Similarly previous workers[14] were used a consortium of microorganisms with the development of biofilm for the efficient remediation of the textile effluent.

Effluent treated under subsequent microaerophilic and aerobic condition

The anaerobically treated effluent was collected from bacterial trickling filter and aerated in mechanical shaker at 120rpm. The washed out organisms from the trickling filter collected in the effluent acts as the inoculum for aerobic treatment. Mainly color removal was in the anaerobic condition although small reduction also occurs in subsequent aerobic treatment with the removal of potent toxic aromatic amines. Percentage of color removal in aerobic condition by the consortium clarified by aluminium potassium sulphate increased from 62% in anaerobic treatment to 83.78% during subsequent aerobic treatment. Reduction percentage of other parameters also increased considerably. Other parameters include TS-91.67%, TSS-94.37%, TDS-95%, COD-95.83%, turbidity-86.84% and hardness-92%, Salinity-21.42%, conductivity-7.12%, and resistivity-18.47% (Table 4). The coagulation efficiency depended on the coagulant dosage. About 1% coagulant dosage of potash alum was used as standard. Thus simultaneous anaerobic and aerobic treatment of azo effluent was carried out to decolorize and to detoxify the dyes and the pollutants. The detoxification of the aromatic amines was done under aerobic condition with the auto oxidation of aromatic amines on aeration. The formation of aromatic amines in the anaerobic treatment was found out by the slight tinge of green color which on further aerobic treatment reduced the color, indicates the breakdown of aromatic amines. Thus after the subsequent anaerobic-aerobic treatment of the effluent, there was a reduction in the color and other parameters. Aluminium potassium sulphate neutralizes the repulsive electrical charges of the surrounding particles and hastens aggregation of the coagulated particles thus forming larger flocs which on gravitation force settle down thus leaving clear supernatant. Several studies reveal evidence for the partial or complete removal of aromatic amines in the aerobic stage[15]. Many of the studies reporting aromatic amine removal, is not clear whether the removal is due to degradation, adsorption or chemical reactions. Although autoxidation of aromatic amines during aerobic treatment, with a slight decrease in color[16].

Optimization of incubation time for biofilm development

An optimal reduction in physico-chemical parameters was observed in the incubation period of 3rd day biofilm

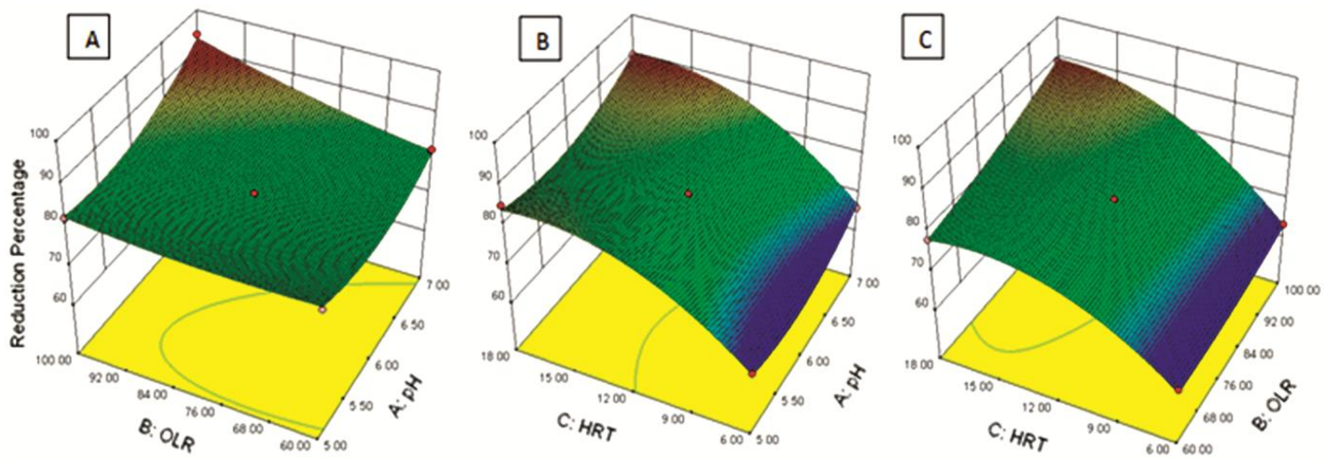

Fig. 1- Effect of Interactive variables on the Decolorization of Textile Effluent

development. After a period of 3 days the reduction efficiency decreases since 3 days was found to be optimal for growth and development of bacterial cultures and production of enzymes. After 3 days the bacterial cultures enter a death phase where they could not produce enzymes necessary for degradation. The enzymes that were produced already acts on the effluent but the efficiency of few proteins decreases as time increases. So the degradation efficiency decreases beyond 3 days[17].

Effect of aeration time on bioremediation

Aromatic amines formed in anaerobic condition degrade on aeration. Maximum reduction in parameters was observed after 8hrs aeration. Beyond this aeration time there was no significant reduction in the parameters (pH-11.27%, Color-86.76%, turbidity-81.58%, TS-94.58%, TSS-91.55%, TDS-94.83%, COD-96.35%, hardness-92%, resistivity-30.62%, conductivity-15% and salinity-29.02%). The bioremediation efficiency increases on aeration for a period of 8 hrs. As the aeration time increases beyond 8 hrs the reduction percentage decreases since on aeration the organism in the effluent multiplies and the turbidity increases with increase in organic load and the total solid content. The much lower removal of COD and color in aerobic condition when compared to anaerobic condition could be because of insufficient amount of biodegradable substances remained after anaerobic treatment.

Effect of Interactive variables on the Decolorization of Textile Effluent

Maximum reduction was observed in the optimal pH 7 with a reduction percentage of more than 93% (Fig. 1a & 1b). At optimal pH the enzymes present in the system participates actively and degrades the effluent. The effect of pH on degradation by microbes related to the transport of dye molecules across the cell membrane necessary for bioremediation. According to Sharma *et al.*[18] the optimal pH for decolorization of textile effluent was found to 7. As the HRT increases the biodecolorization efficiency increased above 90% at a retention time of 18 hrs (Fig. 1b & 1c). An increase in HRT reduces the volume to be treated per unit time and so effectively degraded. These results were correlated with the previous workers[14] in the treatment of textile wastewater by the fungal consortium with the pH range of 6-7 and the HRT was maintained at 12 hrs. A significant reduction in color observed in the 100% organic loading rate with a decolorization efficiency of nearly 90%. As the organic load increases the bioremediation efficiency increases due to the fact that increased organic load has increased amount of nutrients which the organisms utilize for its growth and produce enzymes. As per the analysis done with the plots observed it was understood that the consortium was efficient at a pH 7, retention time 18 hrs and 100% organic loading rate. Optimization of the cultural conditions was basically done in terms of the decolorization efficiency and the same cultural conditions stand for the reduction of other physico-chemical parameters as well.

Disinfection

The effluent treated under anaerobic and subsequent aerobic condition was further treated with the sodium hypochlorite and the treated water was plated on nutrient agar by spread plate technique. The plate inoculated with the raw effluent sample showed clump of colonies and swarming growth where the colonies were too numerous to count. The plate inoculated with the anaerobically treated effluent showed colony count of more than 300. The

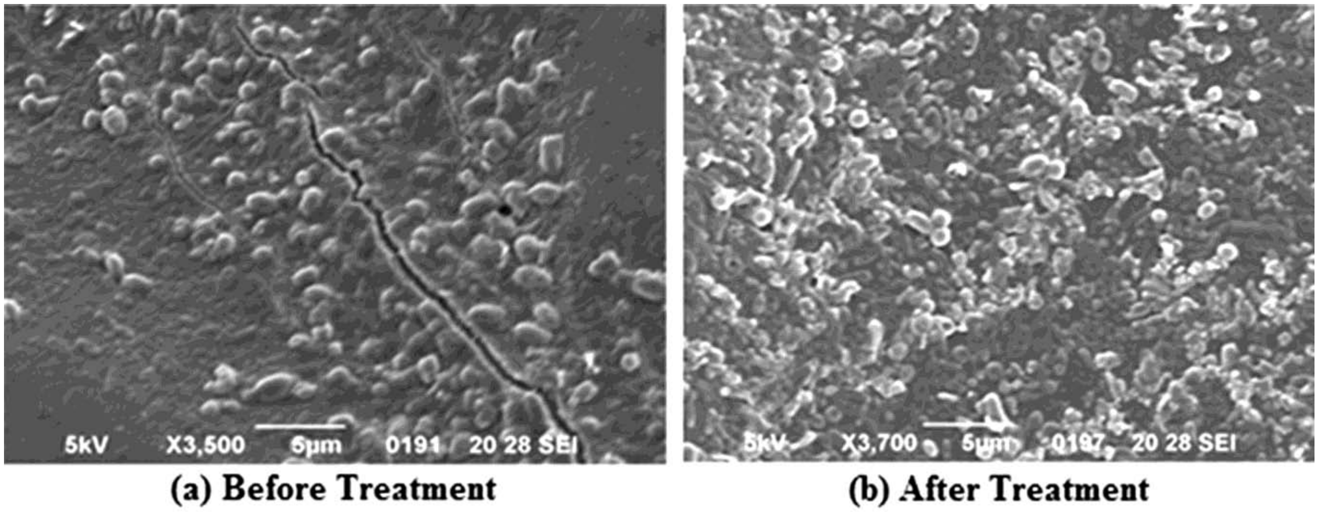

Fig. 2- SEM fixed on polyurethane foam before and after treatment

subsequent anaerobic-aerobic treated effluent with the addition of aluminium potassium sulphate showed minimum number of colonies. Thus the simultaneous anaerobic-aerobic treated effluent showed reduction in number of colonies which on further treating with sodium hypochlorite on plating did not show any colonies on incubation which indicated that all the organisms were killed by the disinfectant.In the work of Lee and Gunten[9], they compared the use of various disinfectants to treat the waste water and found efficient in the removal of micro pollutants of municipal waste water.

Scanning electron microscopy (SEM) Analysis

It was evident from the scanning electron microscope that the biofilm developed on PUF comprises of both the rods and cocci. The bacterial morphology before and after the effluent treatment represents there was no morphological damage for the bacteria which shows the effluent does not have any adverse effect over the bacterial cultures. Some worker[19] have reported that the *T.versicolor* immobilized on poly urethane foam confirmed by SEM analysis showed efficient reduction in the treatment of effluent (Fig. 2).

Characterization of the treated textile effluent for analyzing the toxicity of the effluent

In GC-MS analysis, the untreated raw textile effluent showed the presence of Furan, 2-(methoxymethyl), n-cetyl thiocyanate and heptadecanoic acid-methyl ester which were found to be the toxic product present in the untreated raw effluent sample. The sample treated under anaerobic condition showed reduction in number of peaks which indicated that the dyes present in the effluent had been broken down by the bacterial consortium. In this chromatogram four major peaks were observed which correspond to 2H-1,4-Benzodiazepin-2-one, 7-chloro-1, 3, 2 H-1,4-BENZODIAZEPIN-2-ONE, Benzeneethan amine and pentanoic acid. Out of this four compounds three compound were found to be toxic which might be formed during anaerobic treatment. The chromatogram corresponding to the simultaneous microaerophilic-aerobic treated effluent showed a major reduction in all the organic contents and the number of peaks that were observed was reduced to significant extent when compared to untreated sample. This indicated that the effluent treated under subsequent anaerobic-aerobic treatment was found to be effective in the degradation of effluent. About two peaks which were observed in the chromatogram of the treated effluent were analyzed and found to be 5-hexenal and hexanedioic acid bis (2-ethylhexyl ether). Lai *et al*.,[20] reported that most of the pollutants present in the cooking effluent were degraded by the biological process coupled with coagulation process.

Conclusion

The usage of biological systems for the treatment of textile effluent has indeed gained more attention as a safe and a cheaper treatment methodology. With the advent of biofilms and columns the time for treating the effluent load reduces significantly than the conventional biological treatment technique. Also this work was done till the disinfection stage thus proving that even when effluent is treated using this methodology there is no probability of the microbes reaching the natural water system. Such methodologies being cost effective and at the same time efficient in the treatment process could be employed successfully at a large scale in industries replacing the existing methods.

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