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Research Article

Exploration of paper industry effluent for isolation of efficient starchy material degrader to promote bioremediation.

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ABSTRACT

Effluent samples were collected from a paper industry of Nanded district in Maharashtra, India. The composite water sample was subjected for its abiotic characterization. Total thirteen different physico-chemical parameters were tested. The values of TDS, TSS, BOD, COD and MPN of this water sample were found to be greater than maximum permissible limit set by different national and international organization. MPN index of collected water sample was determined 23. Total viable count of effluent sample was determined 21×10^2 cfu/mL. An efficient starch degrader was isolated from effluent sample and designated as EP1.

Keywords: BOD, COD, MPN index, Paper industry effluent.

INTRODUCTION

Among world India ranks 20th paper producing country[1]. The world demand for paper has grown rapidly and was around 5 to 6 % per year. The consumption of paper is increasing day-by-day in offices, institutions schools, colleges, packaging, writing and printing and also for the household activities. The natural raw materials used for the paper production are wood, cellulose, vegetables, bagasse, rice husk, fibers and also waste-paper. The wastewater released from many paper industries may have the values of their abiotic characters greater than the maximum permissible limit set by the different national and international organizations. This kind of waste water contain the excess quantity of chlorides, sulphates of sodium, fatty acids, tannins, resin acids, and chlorinated and sulphur containing compounds[2, 3]. Therefore the pulp and paper industries are facing today the disposal problem of waste water. Most of the paper industries discharge their insufficiently treated waste water into the rivers or streams that affect on aquatic flora and fauna. Thus, paper industries are disturbing the ecological balance of the environment due to release of a wide variety of wastewater[1]. Microorganisms surviving in such an effluent can be used in bioremediation for processing of waste materials in effluents.

The evaluation of physico-chemical properties of effluents from different paper industries provides data useful not only for hazard identification but also for comparative risk assessment[4]. Therefore the present study was aimed at abiotic characterization of effluent sample collected from a selected paper industry and isolation of efficient starchy material degrader to be used in bioremediation.

MATERIALS AND METHODS

Collection of effluent sample:

Effluent samples were collected from Om paper industry of Nanded district in Maharashtra. These samples were collected from five different outlets in

pre-sterilized sample bottles[5]. During sampling, pH and temperature of an effluent sample was recorded using a digital pH meter and thermometer respectively[6]. The effluent samples were transported in laboratory within 2 hours. The collected effluent samples were mixed in equal proportion to form a composite sample[4,7]. Physicochemical analyses of composite effluent sample were carried out using the standard methods[4-11].

Physicochemical analyses:

Physicochemical analyses of composite effluent sample were carried out to determine its abiotic characters. Different abiotic characters were determined viz. color, TDS, TS, TSS, DO, BOD, COD, total hardness, residual chlorine, methyl orange acidity and phenolphthalein alkalinity[4]. Color of water sample was determined by comparing with standard color chart.

Total dissolved solids (TDS) and total solids (TS) in the composite effluent sample were determined by evaporation method[4]. Total suspended solid (TSS) in the composite effluent sample was determined by subtracting predetermined values of TDS from TS[4]. Dissolved oxygen content in composite effluent sample was determined by Winkler's iodometric method[4]. Biological oxygen demand (BOD) was determined by subtracting the value of DO that was determined after incubation of 3 days from the value of initial DO of the composite effluent sample[4]. Chemical oxygen demand (COD) was determined by titration method using sodium thiosulphate as a titrant. Total harness of composite effluent sample was determined by using solochrome black T indicator in EDTA titration method[4]. Residual chlorine was determined by using starch indicator and 0.025 N sodium thiosulphate as titrant[4].

Acidity and alkalinity of composite effluent sample was determined by using methyl orange and phenolphthalein indicator respectively, in the titration method[4]. These experiments were performed in triplicate and mean burette readings were used for calculating values of respective parameters.

Determination of MPN index and CFU:

MPN index of composite effluent sample was determined using single and double strength lactose broth[12]. In a series of nine test tubes, first 3 test tubes containing single strength lactose broth were inoculated with 0.1 mL of the composite effluent sample. In the same series tube no. 4, 5 and 6 were inoculated with 1 mL of the composite effluent sample. Further, tube no. 7, 8 and 9 containing double strength lactose broth were inoculated with 10 mL of the composite effluent sample. All the tubes were incubated at 37 ^{o}C temperature for 48 h. Further, the value of MPN index was determined using the standard MPN index table[12].

CFU value of composite effluent sample was determined by incubating 0.1 mL composite effluent sample on tryptone-glucose-yeast extract agar plates in standard plate count method[13-16].

Isolation and screening for starch hydrolyzing activity:

The volume of 0.1 mL from the composite effluent sample was inoculated and spread on nutrient agar plates. These plates were incubated at 30 ^{o}C temperature for 48 h. Selected isolates were spot inoculated on starch agar plates and incubated at 30 ^{o}C temperature for 24. After incubation, Grams iodine solution was flooded onto the same plates to observe zone of clearance against blue-black background[12,17,18,19].

RESULTS AND DISCUSSION

Determination of pH and temperature of effluent sample:

The pH and temperature values of effluent water samples have been given in Table 1. All effluent samples were having a bad odor and turbid colloidal appearance. New Zealand organization (2008) has fixed maximum admissible limit value of pH, 7 to 8.5 of drinking water[17].

Determination of TDS, TSS and TS of effluent sample:

Total dissolved solids are measurement of inorganic salts, organic matter and other dissolved materials in water[24]. Total dissolved solids (TDS) of the composite effluent sample was calculated as 1115 mg/L. Total solids (TS) and total suspended solids (TSS) of the composite effluent sample were determined 2115 mg/L and 1000 mg/L respectively. Central Pollution Control Board, New Delhi (1975) has given tolerance limit of TDS and TSS for irrigation as 1000 and 200 mg/L, respectively[31]. The values of TDS and TSS determined by us are greater than these standard limit values. TDS level in water less than 500 mg/L is generally considered to be good for drinking purpose[17]. WHO (World Health Organization) has fixed the standard for TDS as 2000 mg/L for the discharge of wastewater into surface water[32]. The United States Environmental Protection Agency recommends for necessary treatment of effluents when TDS concentrations exceed 500 mg/L or 500 ppm[24]. Consumption of water with high concentrations of total dissolved solids has been reported to cause disorders of alimentary canal,

respiratory system, nervous system, coronary system besides, causing miscarriage and cancer[29].

Determination of DO, BOD and COD of effluent sample:

Dissolved oxygen (DO) is the measure of the degree of pollution by organic matter, the destruction of organic substances as well as the self purification capacity of the water body[29]. The DO of the composite effluent sample was determined 62 mg/L. Moreover, BOD and COD values were determined as 55 mg/L and 122 mg/L, respectively. Biological oxygen demand (BOD) measures amount of oxygen required by microorganisms for breaking down organic matter, whereas chemical oxygen demand (COD) measures amount of oxygen required to breakdown both organic and inorganic matters[29]. As per the environment protection rule (1986), the BOD value of surface inland water is permissible up to 30 mg/L[17]. BOD value of effluent sample from Om paper industry, Nanded was well within ISI limit (100 mg/L) but not within the permissible limit of Minimum National Standard (MINAS) (30 mg/L) set by Central Pollution Control Board, India to discharge for irrigation[20,21,23].

Determination of total hardness, residual chlorine, acidity, and alkalinity of effluent sample:

Total hardness and residual chlorine of the composite effluent sample was recorded 166 mg/L and 10.25 mg/L. Methyl orange acidity was calculated 177.5 mg/L. Phenolphthalein alkalinity of collected effluent sample was found to be zero since no change in color was observed after addition of phenolphthalein indicator in the sample.

Comparative study:

Physicochemical characteristics of effluent samples collected from Om paper industry, Nanded were compared with few other previously reported paper industries in India (Table 2). TDS in effluent sample from South India paper mill, Chikkayana Chatra, Karnataka (2950 mg/L) was found to be highest among the selected effluent samples from various paper and pulp industries of India[25] (Figure 1). TSS in effluent sample reported by us from Om paper industry, Nanded (1000 mg/L) was found to be highest among the effluent samples from selected paper and pulp industries (Figure 2). Total solids in effluent sample from the paper mill, Uttar Pradesh was found to be highest among the selected effluent samples[22] (Figure 3). DO of the effluent sample from the same paper meal was found to be lowest (2 mg/L) among the reported values of DO of selected effluent samples[22] (Figure 4). BOD and COD (5999 and 7112 mg/L, respectively) of the effluent sample from pulp and paper mill, Haryana was found to be highest among the selected effluent samples involved in this comparative study[27] (Figure 5 and Figure 6). Among the effluent samples from selected paper mills, the total hardness of effluent sample from the paper industry in Tamilnadu was found to be highest (640 mg/L)[29] (Figure 7).

Determination of MPN index:

No change in color was observed in test tube no. 1 to 6. The color of lactose broth in test tube number 7 to 9 was turned into yellow and gas formation was also observed. Therefore according to standard MPN index table, the MPN index of the composite effluent sample was determined 23. The permissible value of MPN has fixed less than 1 by different national and international organizations[18].

Isolation and screening:

In 100 µL of the composite effluent sample, 210 CFU were recorded. Total viable count in composite effluent sample was calculated as 21×10^2 cfu/mL. Of the 12 morphologically distinct isolates, 5 isolates have showed zone of clearance. These isolates were designated as EP1 to EP5. Isolate EP1, EP2, EP3 EP4 and EP5 have showed 4.1, 3.5, 3.2, 3.0 and 2.5 cm zone of clearance on starch agar plates. Isolate EP1 was found to be an efficient amylase producer. Identification of EP1 to EP5 isolates is in the progress. Isolation of different biotechnologically important bacterial species viz. *Bacillus acidiceler* strain BPE1 (KF560545), *Bacillus amyloliquefaciens* (KF204579), *Bacillus subtilis* strain AVS1 (KM110978), *Bacillus cereus* strain RW (HG421740), *Bacillus oceanisediminis* (KF204587), *Bacillus megaterium* (KF204586), *Bacillus mycoides* (KF204585), *Bacillus thuringiensis* (KF204583), *Bacillus pumilus* (KF204582), *Bacillus licheniformis* (KF204580), *Brevibacterium stationis* strain E9-2 (FJ573172), *Staphylococcus sciuri* strain E9-4 (FJ573174), *Aeromonas* sp. pp1 (JX913806) and *Thauera* sp. pp3 (JX913808) have been previously reported from soil irrigated with effluents of paper and pulp industry or the premises of these industries, as per the genebank records viewed on 20th August, 2015. Isolation of two bacterial species viz. *Serratia marcescens* strain ISTDF2 (EU834944) and *Pseudomonas aeruginosa* strain VG1 (JN674083) was previously reported from effluent samples of paper industries (Genebank accession numbers of these strains have been given in brackets). Microorganisms surviving in such an effluent can be used in bioremediation for processing of waste materials in effluents. Moreover, most of these isolates were reported as cellulase producers.

Table 1
pH, Temperature and color of effluent water samples from the paper industry.

Characters	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
pH	7.2	7.1	6.6	5.9	8.3
Temperature	30 ºC	34 ºC	29 ºC	31 ºC	32 ºC
Color	Dark brown	Light brown	Dark brown	Dark brown	Dark brown

Table 2
Comparison of physicochemical characteristics of effluent samples collected from Om paper industry, Nanded with few other previously reported paper industries in India

Physico-chemical parameters	A	B	C	D	E	F	G	H	I	J
TDS (mg/L)	1115	1760	NR	NR	381.2	2950	1994	1244	1792	1803-1830
TSS (mg/L)	1000	820	399.2	409.2	27.5	NR	491	476	60	76-80
TS (mg/L)	2115	3000	NR	NR	NR	NR	2486	NR	1852	NR
DO (mg/L)	62	2	NR	NR	NR	7.43	3.61	NR	NR	NR
BOD (mg/L)	55	124	232.7	340.8	45.2	380	5999	NR	14	NR
COD (mg/L)	122	NR	835.6	930	316.2	1830	7112	NR	205	195-205
Total hardness (mg/L)	166	NR	NR	NR	NR	NR	NR	NR	640	65-102

A= Om paper industry, Nanded, B= Recycled paper mill, Uttar Pradesh[22], C= pulp and paper mill, Uttarakhand[24], D= pulp and paper mill, Uttar Pradesh[24], E= South India paper mill, Chikkayana Chatra, Karnataka[25], F= paper mill, situated at Nilakottai[26], G= Pulp and paper mill, Haryana[27], H= Paper industry effluent, Jammu and Kashmir[28], I= Paper industry in Tamilnadu[29], J= Treated effluent of paper mill, Uadhamsingh Nagar[30], NR: Not reported

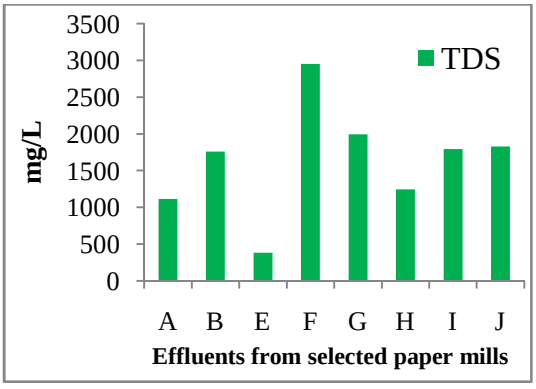

Fig. 1
Comparative study of TDS of effluents from previously reported paper industries

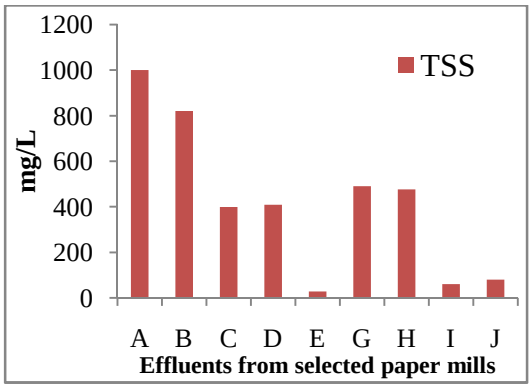

Fig. 2

Comparative study of TSS of effluents from previously reported paper industries

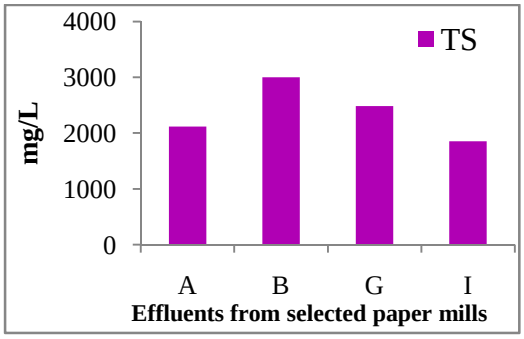

Fig. 3

Comparative study of TS of effluents from previously reported paper industries

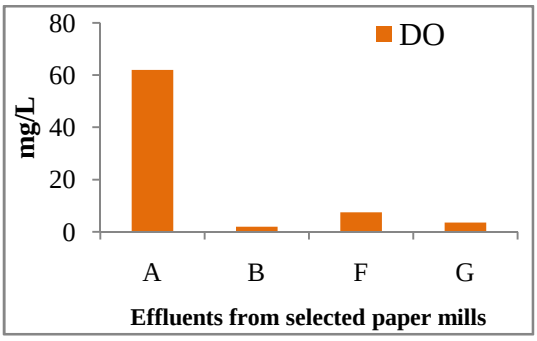

Fig. 4

Comparative study of DO of effluents from previously reported paper industries

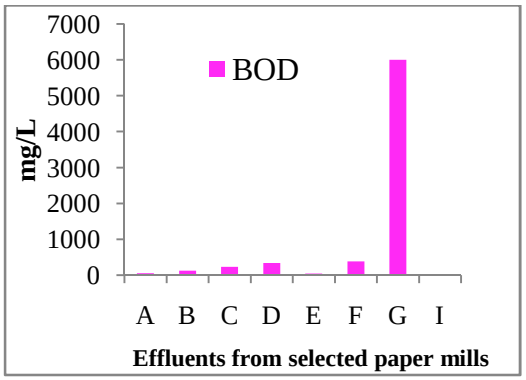

Fig. 5
Comparative study of BOD of effluents from previously reported paper industries

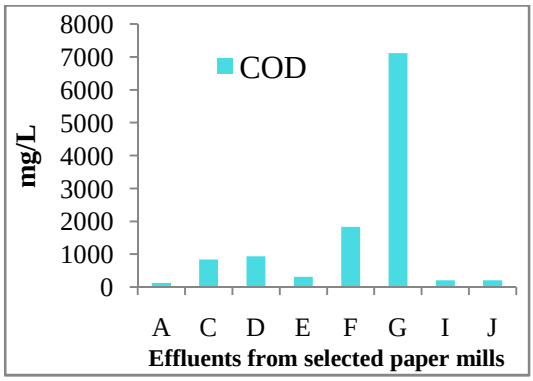

Fig. 6
Comparative study of COD of effluents from previously reported paper industries

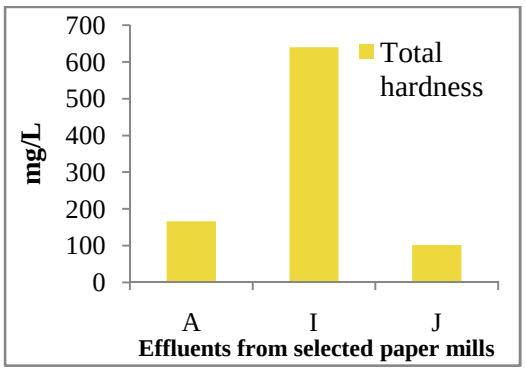

Fig. 7
Comparative study of total hardness of effluents from previously reported paper industries

CONCLUSION

Thirteen different abiotic characters of the composite effluent sample from Om paper industry were determined. The values of TDS, TSS, BOD, COD and MPN of this water sample were found to be greater than maximum permissible limit set by set by Central Pollution Control Board, India. This is an indication of pollution hazard caused by weak waste water treatment practices performed in this paper industry prior to release the waste water in the outlets. This study has provided the current status of effluent sample quality of one of the paper industry from Nanded district of Maharashtra.

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