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Biological Removal of Pulp and Paper Mill TDS

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Pulp and paper mill is a major pollution generating industry. The aim of this research paper is to isolate autochthonous bacteria from the selected sites which can more effectively remove the TDS (Total Dissolved Solids) from the pulp and paper waste water. Treatment efficiency of combination of isolates was evaluated, followed by study of individual isolates comprising the consortium showing best result. Different consortia of isolated bacteria were able to reduce the TDS of pulp mill effluent in the range of 5.4% - 12%, after 5 days of incubation. The individual bacteria comprising of the three best consortia (C3, C4 & C5) were screened individually. Among these, the isolate B11 (MTCC accession number 5098) showed the best TDS reduction of 12.2% after 48h of incubation.

Key words: Bacteria, TDS (total dissolved solid), pulp and paper.

The pulp and paper industry is highly water intensive industry consuming enormous amount of water per ton of paper produced[1-4]. The worrying factor is that it discharges huge amount of the water consumed during processing. However, this water discharge is no longer the clean water that was used for processing. Instead, it contains high amounts of organic as well as inorganic compounds, which emanate from raw material as well as the chemicals used during processing and production of paper. Due to the above, there is a sharp increase in the overall pollutional load of the wastewater and subsequently, the receiving waters. The pulp and paper industry is one of the most polluting industries in the world[5]. The wastewater produced is a serious environmental hazard[6]. It is for these reasons; the pulp mill industrial wastewaters have attracted the attention of environmentalist and people, globally.

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Biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are indices of the biologically degradable and chemically oxidizable fractions of the wastewaters, respectively[7]. These are monitored regularly to give clearance to the discharge of the above wastewaters. A total dissolved solid (TDS) is a parameter, which gives us the index of dissolved compounds, both organic as well as inorganic present in the said wastewaters[8]. It is this dissolved strength of chemicals which imparts a toxic load to the effluents and hence overall pollutional load. However, this parameter has long been ignored because of lack of suitable technologies available for the same.

Total dissolved solids need to be monitored regularly in wastewaters since such load changes the quality and composition of the receiving waters as well, which would be deleterious in many ways, besides creating aesthetic problems. Dissolved solids have a direct impact on various parameters like hardness, heavy metal contentment and carcinogenicity of the receiving waters leading to imbalances in aquatic biota and reduced water quality for domestic use.

A survey conducted on the Nam Phong river in Thailand indicated that it was much affected by the contaminated waters which flowed from the nearby phoenix pulp and paper company and reportedly killed many fishes. Evaluation of these waters revealed that besides the BOD and COD load, the TDS content was very high[9]. Hence, even though this parameter may not seem important as far as the implementing agencies are concerned, yet the implications of its high loading are manifold.

Pulp and paper mills employ various raw materials and chemicals for processing and production. As a result, the above chemicals, in their varied forms, are present in the discharges emanated from these mills and contribute to the dissolved solids content. The organic dissolved compounds consist mainly of chlorinated compounds, which arise in the form of chlorinated resin acids like abietic and pimaric acids, etc. Organically bound halogens where the halogen is usually chlorine, imparts AOX (Adsorbable Organic Halides) in the effluent[10]. Besides the above chlorinated compounds, unsaturated fatty acids and degraded oxidation products of celluloses and carbohydrates are also responsible for the increase in the TDS load.

Among the inorganic dissolved components, free chlorine, sulphates, sulphides, carbonates, bicarbonates, predominate as the major anions; and calcium, magnesium, aluminum, iron and other heavy metal ions as the prevalent cations. Both inorganic and organic dissolved solids raise the TDS (mg/l) to a very high level. In India, the Bureau of Indian standards (BIS) has set up an upper limit of 2100 mg/l TDS for discharges into rivers and streams. However if evaluated properly, the pulp and paper effluents do not conform to the above standards, not withstanding the fact that a TDS level above 1200mg/l is considered to be toxic to the aquatic system (USEPA). However, since the available and currently used TDS reducing technologies are not able to practically reduce the TDS to a great extent, the governmental agencies have not lowered the upper limit.

Currently available TDS reducing technologies are strictly physico-chemical in nature. The major technologies being reverse osmosis (RO)[11-16], electro dialysis reversal (EDR) and ion exchange[17-19].

All the above physic-chemical techniques have the major disadvantage of economics, reusability and treatability range. Disposal problems are also there since these methods do not eliminate TDS completely and accumulate such solids elsewhere, in some other forms.

Pulp mill effluents are markedly different in their nature and composition and hence the dissolved solid present therein are also different from those present in other effluents. Though biological treatment methods are always advantageous over the physico-chemical ones, TDS in pulp mill effluents is not effectively reduced by the conventional biological treatment. The overall pollution load, too phosphorous, cannot be taken care of by the above treatment. Since, these effluents are typically deficient in nitrogen and phosphorus therefore, it becomes imperative for ETP operators to add supplementary nutrients, such as urea and phosphoric acid during treatment of such effluents. An overdosing of such nutrients is always done to some degree to ensure sufficient nitrogen demand under all conditions. As a result, treated wastewater, usually contain excess amounts of both nutrients, contributing to the overall dissolved solid contents and potential impact on the receiving waters such as eutrophicaton.

It is because all of the above reasons, there is need for developing a biological treatment technique which would reduce the TDS levels in an inxpensive and environmental friendly manner. It was felt that bacteria isolated from natural environment, would be capable of reducing the level of TDS in wastewaters.

EXPERIMENTAL

Chemicals, Reagents, Glassware and Media

All chemicals and reagents used in the present study were of analytical grade and obtained from Hi-Media, India. The laboratory glass wares used were, washed with detergents and rinsed with distilled water then oven-baked at 200°C overnight, prior to use.

Isolation of bacteria

For isolation of bacteria, serial dilution technique was performed. Enrichment medium was prepared by adding 1ml of each inlet as well as outlet wastewater (separately) to a conical flask (250 ml) containing 100ml Nutrient Broth. Enrichment of media was carried out for a period of

24-48h at 37°C and 200 rpm. This enriched media was later used for serial dilution. Serial dilutions were prepared in Na_2HPO_4-NaH_2PO_4 (pH 6.8, 0.05M) in distilled water & 100µl of each dilution was spread on solid medium containing. Effluent - MSM plates. The plates were incubated for 24-48h at 37°C and bacterial colonies were isolated on the basis of morphological appearance.

Effluent - MSM plates: Filtered and autoclaved (15 psi for 15 min), wastewater was used as media for isolating autochthonous bacteria in different percentages viz., 100%, 80%, 50%, 30% and 10%. 2% agar was used for solidification.

Formulation & Screening of consortia

The nature of pulp and paper industry effluent is quite complex. For preparation of one consortium we have used three to four different isolates. Different consortia were formulated randomly primarily on the basis of their morphological appearance.

a) The inoculum was prepared by inoculating one loopful of all the 4 individual bacterial isolates separately in 25ml sterilized nutrient broth. The inoculated broths were incubated in an orbital shaker at 37°C for 16-18 hours, so as to obtain actively growing mother cultures. After achieving the desired growth (1.0 optical density at 650 nm), the cultures were centrifuged (at 6000 rpm for 20 min at 4°C). The 250ml of flasks containing 100ml of wastewater sample were inoculated with the pellets and incubated in shaker (at 120rpm at 37°C for 5 days). After 5days of incubation, sample was collected and TDS was analyzed. TDS was analyzed according to the standard procedure as mentioned in APHA[20].

b) The same bacterial consortia, in addition to seven more were used for checking their efficiency in TDS reduction of pulp mill effluent. The inoculum was prepared by inoculating one loopful of all the 4 individual bacterial isolates separately in 25ml sterilized nutrient broth. The inoculated broths were incubated in an orbital shaker at 37°C for 16-18 hours so as to obtain actively growing mother cultures. After achieving the desired growth (1.0 optical density at 650 nm), the cultures were centrifuged (at 6000rpm for 20min at 4°C). The 250ml of flasks containing 100ml of wastewater sample were inoculated with the pellets and incubated in shaker at 120rpm at 37°C for 3 days and 5 days. The reduction in TDS was analyzed by modifying the. TDS analysis method after 3days as well as after 5 days.

Modified TDS analysis method

The samples were withdrawn in appropriate aliquots and centrifuged at 7000rpm for a period of 20min. The supernatant was then passed through a 0.45µ (Millipore) filter. The filtrate was then measured through a clean preweighed beaker. The beaker has been rinsed with triple distilled water, before transferring the sample. The beakers containing the sample were then placed at 180°C in a hot air oven for over night drying. The beakers were then desiccated to cool to room temperature and weighed to calculate the weight of the residue.

TDS (mg/l) is calculated using the following formula:

TDS (mg/l) = (A-B) / sample volume

A = final weight of the beaker with dried filtrate

B = initial weight of the beaker without sample

Isolation of bacteria

The consortia formulated from above isolates were able to reduce the TDS after 5 days. In order to achieve reduction in lesser time fresh isolation from suitable site was done. Bacteria were isolated from a ten-year old site where saw dust continually accumulated over the period.

Enrichment medium was prepared by adding 5g of soil to a conical flask containing 75ml of distilled water. 75 ml of soil extract in 150ml of nutrient Broth. To this 5ml each of 0.1% (v/v) of lignin and cellulose were added along with 100µl of candid B. Enrichment of media was carried out for a period of 48h at 37°C and 120 rpm. This enriched media was later used for serial dilution. Serial dilutions were prepared in Na_2HPO_4-NaH_2PO_4 (pH 6.8, 0.05M) in distilled water and 100µl of each dilution was spread on solid medium containing soil extract, 0.3% lignin, 100µl of Candid C and 2% agar. The plates were incubated at 35°C for 24-48h at 37°C and bacterial colonies were isolated on the basis of morphological appearance.

Soil Extract was prepared from the soil collected from above mentioned site. 1 kg of the soil was dried at 50°C for 2hrs. 400gm of the dried soil was autoclaved with 960ml single distilled water for 1hr at 15 lbs. after autoclaving; the sample was centrifuged at 5000 rpm for 10min at 5°C. The supernatant (soil extract) was collected and used for preparation of medium for isolation.

Formulation & Screening consortia

Different bacterial isolates were screened for their ability to remove TDS from pulp & paper effluent. For preparation of one consortium we have used four – five different isolates. The inoculum (mother culture) was prepared by inoculating one loopful of individual bacterial isolates in 25ml of sterilized nutrient broth. The inoculated broths were incubated in an orbital shaker at 37°C for 16- 24h so as to obtain actively growing mother cultures. The above mentioned actively growing cultures were inoculated separately in 100ml of sterilized nutrient broth and incubated at 37°C, 120rpm for 16- 18h. All the isolates were taken in 50ml graduated centrifuge tube and centrifuged (at 6000rpm, for 20minutes at 4°C). The pellet obtained was washed twice using sterile phosphate buffer (pH 6.8, 0.05M) and resuspended in small volume of the same. The 250ml of flasks containing 100ml of wastewater sample were inoculated with the pellets and incubated in shaker (at 120rpm at 37°C for 48h). The reduction in TDS was analyzed by modified TDS method.

Screening of single isolates selected from the screened consortia

The inoculum (mother culture) was prepared by inoculating one loopful of all individual bacterial isolates in 25ml of sterilized nutrient broth. The inoculated broths were incubated in an orbital shaker at 37°C for 16- 18h so as to obtain actively growing mother cultures. The above mentioned actively growing cultures were inoculated separately in 100ml of sterilized nutrient broth and incubated at 37°C, 120rpm for 16- 18h. All the isolates were taken in 50ml graduated centrifuge tube and centrifuged (at 6000rpm, for 20minutes at 4°C). After centrifugation, supernatant was discarded and the pellet was washed twice with 50mM sodium phosphate buffer.

The 250ml of flasks containing 100ml of wastewater sample were inoculated with the pellets and incubated in shaker (at 120rpm at 37°C for 48h). The reduction in TDS was analyzed by modified TDS method.

Optimization of effluent biomass ratio

Different effluent biomass ratios (1:0.25, 1:0.50, 1:0.75, 1:1) were standardized in order to get efficient reduction in TDS.

The inoculum was prepared by inoculating one loopful of selected isolate in a 25ml of sterilized nutrient broths having 0.01% tween 80. The inoculated broths were incubated in an orbital shaker at 37°C for 16-18h so as to obtain actively growing mother cultures. This actively growing culture was used to inoculate 25ml, 50ml, 75ml & 100ml of sterilized nutrient broths separately and incubated at 37°C, 120rpm for 16-18h. After the growth was achieved the cultures were centrifuged on 6000rpm at 4°C for 20min. The pellet of cultures were washed with phosphate buffer (pH 6.8) and used to inoculate four 100ml of wastewater samples individually in ratios (wastewater: pellet); (1:0.25), (1:0.50), (1:0.75) (1:1). These inoculated wastewater samples were incubated in shaker at 120rpm at 37°C for 48h. After 48h of incubation, the samples were analyzed for TDS reduction

RESULTS AND DISCUSSION

Isolation

Twenty bacterial isolates were purified from the above mentioned isolation procedure. It was hypothesized that bacteria isolated from their natural habitat have capability of surviving in harsh conditions by developing some catabolic enzymes systems, specific for particular components present in the natural habitat. The isolated colonies were diverse in their morphologies, ranging from small pin-pointed to large sized; smooth margined to

Table 1. Percentage reduction in TDS of pulp mill effluent samples by mixed bacterial consortia

Consortia	Percentage Reduction in TDS	
	3rd day	5th day
C1	5.2	10.2
C2	4.4	12.7
C3	4.1	11.2
C4	6.4	10.6
C5	6.8	9.6
C6	10.2	9.6
C7	5.6	10.3
C8	9.5	8.3
C9	7.2	7.8
C10	5.2	10.0
C11	3.8	9.2
C12	1.0	9.0
C13	5.2	10.3
C14	2.4	5.4
L1	3.8	8.9
L2	0.97	8.4

wrinkled periphery; shining to dry and so on.

Screening of consortia

a) The TDS reduction was observed after 5 days of incubation. An increase in the TDS levels were observed on addition of biomass, which could be due to the passage of bacteria passing through GFC filter (pore size 1.2µ), thus contributing to the weight

Table 2. Percentage TDS reduction by consortia formulated from soil bacteria over a period of 48hours

Consortia	Percentage Reduction in TDS	
	24 hrs	48 hrs
X1	2.9	5.3
X 2	0.0	5.3
X 3	5.1	8.4
X 4	4.4	6.2
X 5	1.3	6.6
X 7	1.3	2.2

Table 3. Percentage TDS reduction by individual soil bacteria

Bacteria	Percentage Reduction in TDS	
	24 hrs	48hrs
B1	4.9	7.96
B2	9.3	9.5
B3	3.8	9.4
B4	0.5	6.4
B5	4.4	4.4
B6	6.7	9.1
B7	2.3	6.2
B8	4.2	6.4
B9	2.8	4.6
B10	6.1	6.4
B11	10.1	12.2
B12	2.6	4.6
B13	5.1	5.1
B 14	2.0	6.2
B15	3.1	3.1

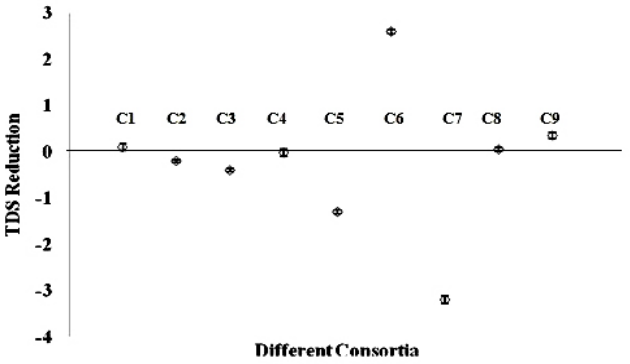

Fig. 1. Percentage reduction in TDS by mixed bacterial consortia

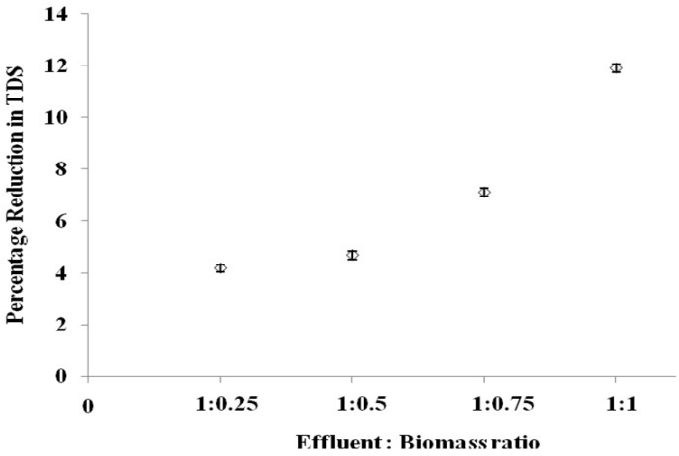

Fig. 2. Percentage reduction in TDS using different biomass loadings of isolate B11

of the residue obtained (Fig. 1)

b) The same bacterial consortia, in addition to seven more were used for checking their efficiency in TDS reduction of pulp mill effluent by modifying in TDS analysis method. A TDS reduction of up to 12% was observed in the pulp mill effluent by C2 consortia, after 5 days incubation. While observing the result it was seen that after three days of incubation the TDS reduction was up to 10.2% by consortia C6. Increasing the incubation time from 3 days to 5days results revealed, the increase in TDS reduction by C2 i.e. 12% whereas the reduction value was reduced to 9.6% in case of C 6 (Table 1).

Screening of consortia

The time in TDS reduction was more i.e., 5 days. In order to achieve reduction in lesser time, it was thought that fresh bacterial isolation from suitable site was done. Different consortia were randomly formulated and screened for their TDS reducing capability. The treatability assay was conducted and analyzed over a period of 48 hours. Three consortia were found to be giving TDS reduction between 6 – 8% after 48hours (Table 2).

Screening of single isolates

The individual bacteria comprising of the three best consortia were screened individually for their TDS reducing ability. Out of all individual bacteria, it was found that bacterial isolate number B11 was giving a percentage reduction of up to 10.1 after 24 hours and 12.2 after period of 48 hours (Table 3).

Optimization of bacterial density

Percentage reduction in TDS using different biomass loadings was monitored. Effluents: biomass loadings ratios 1:0.25, 1:0.50, 1:75, 1:1 were used for studying TDS reduction from pulp mill effluents. Samples were analyzed for reduction in TDS levels. It was found that effluent: a biomass loading of 1:1 was giving the best results among all the tested ratios (Fig. 2).

CONCLUSION

The isolated bacterium is capable of reducing the TDS levels of the pulp mill effluents in a reproducible manner. This kind of bacterial isolates of TDS reduction from pulp mill effluents is novel. The isolate B11 (MTCC accession number 5098) showed the best TDS reduction of 12.2% after 48h of incubation

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