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A techno-economic feasibility study on removal of persistent colour and COD from anaerobically digested distillery effluent: a case study from India

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Abstract The present study aims at evaluating the potential options for further reduction of persistent dark brown colour and COD from anaerobically digested distillery effluent from a distillery in West Bengal, India. Two alternatives viz. physico-chemical method and attached growth biological method were evaluated for their potential and cost effectiveness in removing the COD and colour from the effluent. Under the physico-chemical option, detailed coagulation study was carried out with a number of coagulants such as alum, potash alum, ferric chloride, ferrous sulphate and lime. Then, additional removal of COD as well as colour was tried by applying an oxidant such as hydrogen peroxide or bleaching powder. Finally, adsorption study was performed with adsorbents viz. powdered activated alumina, wood charcoal and activated bone char. Under the biological treatment option, the efficacy of two fungi namely *Aspergillus fumigatus* and *Coriolus versicolor* was studied in batch mode under sterile condition for different dilutions. Finally, the performance of *A. fumigatus* was evaluated in continuous mode under ambient condition with the aid of fabricated lab scale bio-filters for different flow rates. All experimental results clearly showed that the removal of colour and COD were inter-related. Physico-chemical treatment was efficient in overall 96% COD removal and 93% colour removal with a treatment cost of Rs. 3.21 per litre (1$ = Rs. 46), if double coagulation-flocculation followed by peroxide treatment followed by adsorption onto acid-treated bone char was adopted. Only double-flocculation followed by adsorption was also an alternative yielding an overall 84% COD removal and 74% colour removal at a very nominal cost of Rs. 0.41 per litre. A maximum of 70% colour could be removed by 10% *A. fumigatus* inoculum in raw wastewater sample in batch mode under sterile conditions. However, under ambient winter conditions, the maximum colour removal of 48% and COD removal of 32% could be achieved in continuous flow bio-filters. Treatments of the distillery wastewater by proposed physico-chemical method as well as by bio-filters employing attached growth of *A. fumigatus* have emerged out to be a promising and cost-effective option for the concerned industry as compared to conventional dilution approach.

Keywords Distillery wastewater · Effluent treatment · Physico-chemical treatment · Attached growth biological treatment · *Aspergillus fumigatus*

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Introduction

Distilleries have been classified as one of most polluting industries generating effluent with high COD/BOD_5 ratio. The effluent contains high percentage of organic and inorganic matter, in suspended as well as in dissolved form. The raw effluent has a very high organic content. BOD is generally of the order of 40,000–50,000 ppm and the COD is of the order of

80,000–1,00,000 ppm (Bhasin 1995). It necessarily needs treatment to bring these within permissible limits, which are 30 and 250 ppm for BOD and COD, respectively, before discharging into inland surface water (ISI 1986); otherwise it will consume a huge amount of dissolved oxygen and lead to the anaerobic condition of the receiving water stream.

In order to comply with the Indian environmental standards, there are several methods for pollution abatement in distilleries (Pandey and Carney 1989). The methods include both aerobic and anaerobic treatment processes as well as resource extraction, sewage farming, incineration, etc. A number of technologies for resource extraction for ammonia (BOD reduction 70–80%), potash and yeast (BOD reduction 40–50%) are available currently. Despite conventional primary and secondary treatment of the distillery spentwash, sometimes the final effluent quality does not conform to the effluent discharge standards mainly due to the high BOD as well as COD loading and hence further treatment (i.e. tertiary treatment) or dilution is needed prior to final disposal.

The wastewater of distillery has dark brown colour, mainly due to the presence of caramelised sugar, which is produced in the processes employed in extracting maximum quantity of sugar by the sugar mills. This process results in formation of brown Melanoidin pigment, the amino-derivative of aldose (sugar), generated as an end product of the complex Maillard reaction (Hodge 1953).

This colour, highly recalcitrant in nature, not only hampers the aesthetics but also blocks the passage of sunlight required for photosynthetic activities by the phytoplankton in receiving waterbody.

A number of studies had been undertaken in India and abroad to find suitable options for removal of colour present in distillery spentwash. Lehri and Viswanathan (1989) studied the effect of some polysaccharides, which were used as supplement to spentwash medium or as a carbon source on a semisynthetic medium for some bacterial strains used for distillery effluent treatment. On both the media pectin, insulin and dextrin supported considerable growth and brought 40–60% reduction in COD. It was suggested that those bacterial strains were useful for the treatment of industrial effluent not only for the distillery but also from other industries, without need for any aeration.

Performance of activated carbon and activated pyrochar to remove colour and COD from distillery spentwash was evaluated (Ramteke et al. 1989). They observed that activated carbon was most efficient as it could remove 92.5% colour at 2 g/L dose, as against 8 g/L for activated pyrochar. It was also observed that colour was caused due to only a part of organic matter in the waste as only 52% COD was removed by activated carbon and 64% COD was removed by activated pyrochar at 2 and 8 g/L doses, respectively.

Complete removal of BOD and colour from distillery effluent using electro-oxidation technique was reported (Berchmans and Vijayavally 1989). After treatment, pH of the water was found to be around 1.3 indicating an acid build-up. The reaction was taking place at an average cell voltage of 3.8 V at 1.5 A/m^2 in divided cell with a sintered PVC (60% porosity) diaphragm. The treated effluent was colourless with nil BOD.

Juwarkar (1987) conducted batch experiments and reported that approximately 50–60% of colour showing an absorbance maxima of 270 nm was removed within 10 days. The changes in group frequencies of control sample and experimental sample (inoculated with bacterial culture) indicated that due to bacterial treatment of wastewater, there was decrease in hydrogen bonding, methyl groups as well as ketonic group.

Decolourisation of melanoidin pigment present in spent wash by adopting chemical and biological methods was studied (Patil and Kapadnis 1995). Spent wash from an anaerobic digester was treated with H_2O_2, CaO and soil bacteria. At 144 h of incubation in presence of peroxide, the maximum decolourisation and COD reduction were 98.6 and 88.4%, respectively.

In another study (Gupta et al. 1996), bagasee (a solid waste from the sugar mills) activated with acid had been used as an adsorbent for removal of colour from an Indian distillery spentwash. Removal of colour had been determined by measuring its transmittance through Spectronic–20 spectrophotometer. Lime was also added to activated bagasse in another set of experiment to study its effect on colour removal. The colour removal of the order of 36%, which bagasee alone and 84% with lime, had been reported.

Bioremediation and decolourisation of anaerobically digested distillery spent wash by a facultative anaerobic pure bacterial culture was reported (Kumar et al. 1997). A facultative anaerobic pure bacterial culture capable of growth on 12.5% (v/v) diluted digested spent wash supplemented with glucose (10 g/L) was isolated from an Indian distillery. It achieved 31% decolourisation and 57% COD reduction after 7-day incubation. The advantages of using such a culture for digested spent wash bioremediation are apparent in providing a realistic approach for decreasing its pollution potential prior to disposal.

In the bioremediation process of anaerobically digested dark coloured molasses spentwash, 49–52% decrease in effluent's colour and reduction in COD level up to 54–57% were achieved using bacterial cells in free and immobilised system, respectively, in 5 day batch cultivation (Shibu et al. 1999). A bacterial isolate identified as *Lactobacillus casei* was cultivated in a fermentation medium containing biogas plant effluent. This effluent was generated after anaerobic digestion of molasses spentwash in biogas fermenters. During this process of bioremediation, a metabolite lactic acid was produced by this bacterial isolate with the yield of 10.9 and 11.3 mg/mL, in free and immobilised cells fermentation, respectively.

Batch decolourisation of molasses by suspended and immobilised fungus *Geotrichum candidum* Dec1 was studied and reported (Kim and Shoda 1999). This fungus, which exhibits a broad dye-decolourising spectrum, was used to decolourise molasses during semi-batch cultivation. An 80% decolourisation of molasses and a stable peroxidase activity were maintained for approximately 4 weeks, after which, both activities deteriorated significantly. Subsequently, repeated batch cultivation of fungal cells immobilised on polyurethane foam was employed to solve the aforementioned problems and stable decolourisation of molasses as well as stable peroxidase activity was realised for more than 8 weeks.

Enzymatic decolourisation potential of the same fungus were studied in depth. A novel decolourising peroxidase gene (DyP) was cloned from a cDNA library of the newly isolated strain of fungus *G. candidum* Dec1 (Sugano et al. 1999).

The production of dye-decolourising peroxidase (DyP) was investigated by cultivating *G. candidum* Dec1 using molasses as a carbon source (Lee et al. 2000). Molasses at concentrations greater than 10 g/L was found to increase the decolourisation activity of the culture broth because the amount of enzyme produced was enhanced. However, complete inhibition of DyP activity by molasses was observed at the concentration of 20 g/L, indicating that the inhibitory effect of molasses on the culture broth activity to decolourise the dye was involved. When the culture broth was diluted 25 times, the dye-decolourising activity was seven times as much as that of non-diluted culture broth.

In another study by Kim et al. (2001), aryl alcohol oxidase (AAO) produced by *G. candidum* Dec1 was purified. H_2O_2 produced by concomitant AAO oxidation of veratryl alcohol (VA) to veratraldehyde was consumed by a peroxidase (DyP) purified from Dec1 culture, leading to the in vitro decolourisation of a dye. In the liquid culture of Dec1, the existence of H_2O_2 and veratraldehyde was confirmed during cultivation, when dye-decolourisation and AAO activities were maintained. This indicates that VA produced by Dec1 was oxidised by AAO to veratraldehyde, generating H_2O_2, which supported dye-decolourising activity of Dec l in vivo.

Study on production of decolourising enzyme from *G. candidum* Dec1, on solid-state culture of *Aspergillus oryzae* RD005 was performed and reported (Sugano et al. 2001). The productivity of a peroxidase (DyP) originating from *G. candidum* Dec 1 was enhanced in the solid-state culture using *Aspergillus oryzae* RD005. When the humidity, water content, and temperature were adjusted to 60%, 50% and 27°C, respectively, the productivity of DyP reached 5.3 g/kg of wheat bran, which was used as the solid medium. The yield of 5.3 g/kg wheat bran corresponded to the yield of a 56 kg submerged culture.

Microbial decolourisation of Melanoidin-containing wastewaters by combined use of activated sludge and the fungus *Coriolus hirsutus* was achieved (Miyata et al. 2000). A white rot fungus, *C. hirsutus*, exhibited a strong ability to decolourise melanoidin in cultures not supplemented with nitrogenous nutrients. The study suggested an inhibitory effect of organic N on melanoidin decolourisation. Therefore, for enhancing the decolourisation of melanoidin in wastewaters by the fungus, activated sludge pretreatment of the wastewaters was expected to be effective, i.e. activated sludge is capable of converting available organic N into inorganic N. To confirm this, waste sludge heat treatment liquor (HTL), wastewater from a sewage treatment plant, was pretreated with activated sludge. In practice, pretreatment of HTL under appropriate conditions accelerated the fungal decolourisation of HTL. In the pretreated HTL, the fungus was shown to produce a high level of manganese-independent peroxidase (MIP). Addition of Mn(II) to the pretreated HTL caused a further increase in the decolourisation efficiency of the fungus and a marked increase in the manganese peroxidase (MnP) activity. Consequently, the increases in MIP and MnP activities were considered to play an important role in the enhanced ability of *C. hirsutus* to decolourise HTL.

The present study aims at evaluating the potential options for further reduction of colour and COD from treated distillery effluent generated by a distillery situated in West Bengal, India. It is a molasses based distillery producing both beverage and industrial alcohol. The plant is situated on the east bank of a river and is surrounded by vast stretch of agricultural fields. The study primarily focussed on removal of COD and colour. The aspects of sludge management were not within the scope of feasibility study.

The distillery has a well-operated integrated effluent treatment plant to treat the wastewater generated from the fermentation-distillation process. The effluent volume ranges from 0.6 to 0.65 MLD (million litres per day). The raw effluent, popularly termed as *slop*, containing about 1,00,000 mg/L of COD and 40,000 mg/L of BOD primarily is stored in a couple of slop pits. This slop is directly fed to the anaerobic contact digester that reduces COD and BOD to nearly 40,000 and 7,000 mg/L, respectively. Biogas, the digestion product, is used as fuel to the boilers and the effluent, further treated by the clarifiers, is fed to the assembly of six bio-filters. The bio-filter output is then diluted twice with fresh groundwater, upstream and downstream to the final clarifier, and finally discharged into the river. In the final effluent, BOD and COD concentrations are 30 and 150 mg/L, respectively. During the whole treatment process, though the effluent colour changes from dark brown to greenish brown, the colour intensity remains unchanged and consequently creates serious aesthetic problem and disrupts the natural ecosystem of the downstream stretch of the river. The aim of the present study is to reduce the persistent colour and the COD of the effluent treated by the ETP up to maximum possible extent without any dilution in order to prevent depletion of invaluable groundwater resource.

Methodology

The prime target of the research work was to find out the most suitable option among a number of different approaches to remove the colour and COD from already treated distillery effluent. The methodology prioritised the least costly as well as the easiest methods possible. The whole research was divided into two separate parts namely physico-chemical treatment and biological treatment.

Before all the experiments were performed, the anaerobically treated effluent, taken as raw effluent for the present study, was characterised for pH, COD and colour. Throughout the research work, COD was measured by close reflux method (APHA 1989) and colour was measured spectrophotometrically at 420 nm by diluting the samples 200 times with distilled water.

Physico-chemical treatment

The treatment methodology was framed as coagulation followed by chemical oxidation followed by adsorption. The batch studies were performed in order to evaluate the performances of different coagulants, oxidants as well as adsorbents.

The three stages of the treatment methodology adopted were as follows.

First stage: selection of suitable coagulant

Studies were performed for each of the coagulants viz. Alum [$Al_2(SO_4)_3 \cdot 18H_2O$], potash alum [$K_2SO_4 \cdot Al_2(SO_4)_3 \cdot 24H_2O$], ferrous sulphate [$FeSO_4 \cdot 7H_2O$], ferric chloride [$FeCl_3 \cdot 6H_2O$] and lime [$Ca(OH)_2$] to find the optimum pH as well as dose. For each of the above coagulants tried, 100 mL sample of the anaerobically treated spent wash was taken and a jar test (Garg 1996) was carried out to find the optimum coagulant doses. By maintaining the optimum dose conditions, optimum pH was found by jar test for each of the coagulants. Flash mixing was done for 1 min followed by slow mixing for 20 min. The COD and colour were measured from the supernatant after allowing the sample to settle for 30 min.

The performance of alum with respect to COD and colour removal was studied after single flocculation as well as after double flocculation (i.e. after single flocculation, the same experiment was carried out with the supernatant), pH being adjusted with the help of lime. For potash alum, colour and COD were measured after single flocculation while pH was adjusted with the help of lime. $FeSO_4$ was added in dry powdered form and during the experiments, pH was adjusted with 1 N HCl and 1 N NaOH. $FeCl_3$ was also tried in powdered form. The dose and the pH study were performed in the same manner as done with $FeSO_4$. Hard lime was procured from local market and the dose and pH study were performed in the same manner.

Second stage: action of strong oxidising agent

Commercially available bleaching powder [$Ca(OCl)_2 \cdot H_2O$] and hydrogen peroxide [50% H_2O_2] were tried as oxidants to remove the COD and colour from the first-stage effluent, i.e. the effluent after coagulation. Mixing of oxidants with wastewater and measurement of the target parameters were done in the same way as performed during coagulation study. The effluent from oxidation stage was used for adsorption study.

Third stage: selection of suitable adsorbent

Detailed dose study, pH study and equilibrium study were performed for each of the following adsorbents in order to find out the optimum adsorbent dose, optimum pH and the equilibrium time (i.e. the time of

contact required for the adsorbent and the adsorbate to approach a steady-state adsorption equilibrium), respectively.

Powdered activated alumina was procured from the market (75–250 mesh size; SISCO Laboratory; chemical activity grade 1). The dose varied from 1 to 10 g/L. By varying the pH and maintaining the optimum dose, optimum pH was found. COD and colour were measured by maintaining the optimum dose and pH at an interval of 15 min. The equilibrium study was continued for a total duration of 135 min.

Wood charcoal was bought from local market and converted into powdered form (75–250 mesh size) by crushing and sieving. The dose was varied from 10 to 60 g/L. Varying the pH and maintaining the optimum dose condition, colour and COD reduction were measured. Equilibrium study was done for 360 min while the samples were drawn out for measurement at an interval of 45 min.

Powdered bone char of same size as that of the wood charcoal was made from crushed bones procured from a fertilizer shop. Preparation of activated bone char was done by dehydration at 150°C, carbonisation at 700°C and activation by H_2SO_4 and NaOH (Bobade 1996).

Three types of bone char were investigated:

1. *Unactivated bone char:* The dose varied from 5 to 30 g/L, by maintaining a shaking time of 15 min to find out the optimum dose. The pH was varied from 2 to 12 by maintaining the optimum dose condition and optimum pH was found for maximum reduction of COD and colour. The study was done for 150 min, maintaining the optimum pH and dose condition. At that optimum pH-time-dose condition, COD and colour removal were measured.
2. *Bone char activated with 1 N NaOH:* The experimental method was same as those of powdered bone char without any activation.
3. *Bone char activated with 1 N H_2SO_4:* The optimum pH was first found out by jar test. Maintaining the optimum pH condition, the dose varied from 2 to 14 g/L, while shaking time was maintained to 15 min. Total duration of the study was 120 min, where the equilibrium time at optimum condition was found. At that optimum pH-time-dose condition, COD and colour removal were measured.

Biological treatment

The methodology of biological treatment was based upon the performances of two filamentous fungi, *Aspergillus fumigatus* and *Coriolus versicolor*. These genera have been reported to be efficient in decolourisation of the brown-coloured melanoidin pigment present in the distillery effluent by Institute of Fermentation, Osaka, Japan (http://www.ifo.or.jp).

Freeze-dried pure cultures of the two filamentous fungi were bought from Microbial Type Culture Collection, Institute of Microbial Technology (IMTECH), Chandigarh, India and the feasibility study was done in the laboratory.

Batch mode

The batch study was carried out in three steps. In the first step, the cultures were revived from their freeze-dried form into normal growing condition. In the next step, fungal growth was studied in liquid media. Finally, the growth as well as the colour removal was studied in wastewater media. Instantaneous COD could not be monitored for batch study due to time constraint. The details of each of the above-mentioned steps are presented below.

Step 1: revival of fungal culture

Revival of the cultures was performed in sterile condition (inside laminar hood) as per the specifications stipulated by IMTECH, Chandigarh, India. Microbiological specifications of the cultures are presented in Table 1.

Preparation of growth medium

The following procedure was followed in order to prepare 1 L of each medium.

To prepare medium no. 117 (for *A. fumigatus*), 10 mL of Czapek concentrate, 1 g of K_2HPO_4, 5 g of yeast extract, 30 g of sucrose and 15 g of agar were dissolved into 1 L of distilled water. Ten millilitres of Czapek concentrate was prepared by dissolving 3 g of $NaNO_3$, 0.5 g of KCl, 0.5 g of $MgSO_4 \cdot 7H_2O$ and 0.01 g of $FeSO_4 \cdot 7H_2O$ into 10 mL of distilled water. Czapek concentrate can be stored without sterilisation.

Table 1 Microbiological specification of cultures

Parameter	Culture 1	Culture 2
Genus species	*Aspergillus fumigatus*	*Coriolus versicolor*
MTCC no.	870	138
Growth medium no.	117	8
Incubation temperature	30°C	25°C
Growth condition	Aerobic	Aerobic
Incubation time	3 days	10 days

The precipitation of $Fe(OH)_3$ can be resuspended by shaking well before use.

Medium no. 8 (for *C. versicolor*) was prepared by dissolving 5 g of yeast extract, 10 g of glucose, 15 g of agar into 1 L of distilled water while pH was adjusted to 5.8 by acetic acid.

Inoculation of cultures

Fifty millilitres of each medium were taken in two different conical flasks. The flasks were then plugged by cotton caps and the caps were again covered by non-absorbing paper with rubber band. The flasks were labelled accordingly and were sterilised by autoclaving at 110°C for 20 min. After autoclaving, the flasks were allowed to cool down at room temperature and were, then, stored in cool and dark place.

Next day, when the media became solidified, a fraction of each culture was transferred into the solid surface of the medium specified for each culture. The transfer process was done inside laminar hood in order to avoid contamination.

Finally the flasks were kept in different BOD incubators at favourable temperature conditions specified for each fungal culture as mentioned in Table 1.

Step 2: study in specified liquid media (broth)

After 15 days when sufficient fungal growth became visible on solid culture medium for each organisms, the growth study was performed with the liquid fungal suspension. The whole work was performed inside laminar hood to ensure sterile environment. Two types of specified liquid broths were prepared with the same constituents and following the same protocols as per the specifications for medium no. 117 for *A. fumigatus* and medium no. 8 for *C. versicolor*, respectively, without adding agar in order to prevent solidification.

Sixty numbers of 30-mL test tubes were taken and divided into two sets. Five millilitres of broth for *Aspergillus* was poured into each of the 30 test tubes of one set. Similarly 5 mL of broth for *Coriolus* was poured into each of the remaining 30 tubes of the other set. The transfer of broths into the tubes was done inside the hood. Now, all the 60 test tubes were tightly plugged by cotton caps and then sterilised in autoclave. Two different suspensions of fungal spores were, then, prepared inside the hood by adding one loopful of each of the two fungi into 20 mL of sterilised distilled water, respectively. The suspensions were made homogeneous by using vibrating shakers.

Now, inside the laminar hood, all the 30 test tubes each containing 5 mL of specific broth for *Aspergillus* were inoculated by 0.5 mL *Aspergillus* inoculum and each of the remaining set of 30 test tubes were inoculated by same volume of *Coriolus* inoculum. Inoculum size for each of the two organisms was 10^6 CFU (colony forming unit) per mL of broth. One test tube, which was not inoculated, acted as control.

All the 60 test tubes were, then, plugged with cotton caps and put inside a shaker at a temperature of 30 ± 5°C. This time was noted as zero time base, from which the growth of fungal biomass was monitored at different time intervals. The growth was measured by taking fresh weight of fungal biomass after filtering out the liquid medium at every 6 h for *Aspergillus* and at every 12 h for *Coriolus*.

Step 3: study in wastewater media

This study was performed with *A. fumigatus* only. *C. versicolor* had been discarded for its long generation time (at 30 ± 5°C) and pH dependence. Four sets of test tubes were taken (each set contained 15 test tubes). Two sets, i.e. 30 tubes amongst which each tube, contained 5 mL of raw wastewater and the other two sets, where each tube contained 5 mL of diluted wastewater (1:1 dilution with tap water), were first plugged with cotton and autoclaved.

The aqueous suspension of fungal spores was homogenised by vibrating shaker. Inside the laminar hood, all tubes, 15 containing raw wastewater and 15 tubes containing diluted wastewater, were inoculated with 0.5 mL inoculum (i.e. 10%) and the remaining two sets were inoculated with 0.25 mL (i.e. 5%) inoculum. Finally, all the 60 tubes were plugged with cotton caps and were put inside shaker at 30 ± 5°C. Colour and biomass were monitored at 24-h interval.

Continuous mode

In this phase of study, the colour as well as COD removal efficiency of *A. fumigatus*, grown onto solid surface of bio-filter media, were studied in ambient condition for different flow rates.

For this study, two lab-scale bio-filters were fabricated with perplex sheet. Two-millimetre-thick clay pipes of 25 mm diameter and 120 mm length, procured from a local pottery, were used as solid mediums. The schematic details of the bio-filters are presented in Fig. 1.

At the beginning of continuous study, the clay pipes were moistened by the wastewater. Then in open atmosphere at an ambient temperature of around 15–20°C, fungal spores were inoculated onto the

surfaces of the pipes of both the filters, which were operated in series.

Nearly after 25 days, clearly visible fungal growth was observed on the surfaces. It was verified and confirmed microscopically by comparing with its pure culture that *A. fumigatus* was present in abundance on surfaces along with a mixed population of different microorganisms. Now, wastewater was applied in the trickling filters. The colour and COD removal was studied at two different flow rates—0.065 $m^3/m^2/h$ (1 L/h) and 0.13 $m^3/m^2/h$ (2 L/h), respectively.

Results and discussion

Physico-chemical treatment

The anaerobically treated effluent, taken as raw effluent for the present study, was first characterised. Initial COD and pH were found to be 34,800 mg/L and 8.2, respectively. The colour was dark greenish brown.

Selection of suitable coagulant

From experiment, the optimum pH and dose for commercial alum was found to be 10.5 and 10 g/L, respectively. The COD and colour reduction was found to be 38.3 and 45%, respectively, after single flocculation. It was also observed that the performance of alum depends upon pH adjustment. For potash alum, tried as an alternative to alum, the optimum pH and dose were found to be 8 and 10 g/L, respectively. The COD and colour reduction were found to be 40 and 44.5%, respectively, after single flocculation. The optimum pH and dose were found to be 7.5 and 8 g/L, respectively, for $FeSO_4$. The reduction of COD was 25.8% and colour removal was 29.5%. For $FeCl_3$ the optimum pH after single flocculation was found to be 10. The percentage COD and colour removal calculated were 31.1 and 30, respectively, at an optimum dose of 10 g/L. The colour turned to a little more greenish. For lime used as coagulant, the optimum pH and dose were found to be 10 and 12 g/L, respectively, in which the removal of COD was 37% and colour removal was 35.6%. Comparative efficacy of the coagulants in conjugal removal of colour and COD are presented in Fig. 2a, b, respectively.

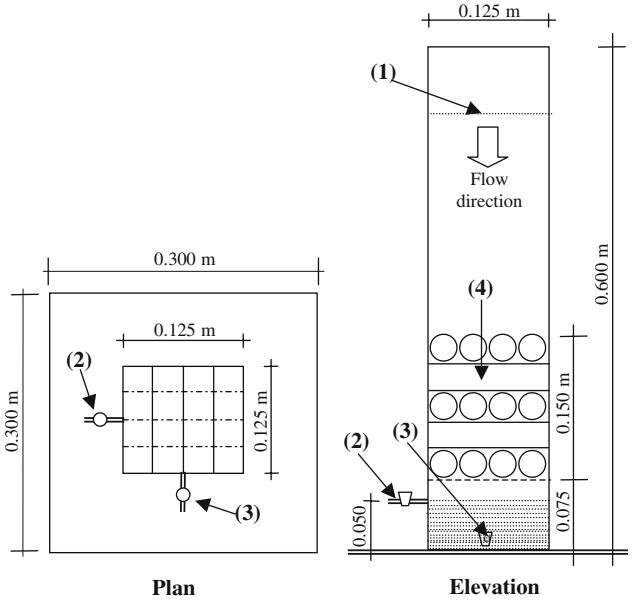
It can be observed from the figure that the removal of COD and colour is simultaneous and hence it may be concluded that the colour imparted to the spent wash is due to the presence of fine and organic matters in dissolved state.

From all the coagulant studies as above, the most effective removal had been found with alum when lime was used for pH adjustments. This option can be selected for the primary treatment of the spent wash. To increase the percentage removal of COD and colour, the supernatant had been again flocculated after adding the optimum alum dose (10 g/L) and maintaining the optimum pH of 10.5 with the help of lime. After 30 min settling time, the percentage removal of COD and colour were found to be 53.2 and 55%, respectively, after double flocculation.

Action of strong oxidising agent (after double flocculation)

As shown in Fig. 3a, for bleaching powder, the optimum dose and pH were found to be 1.5 g/L and 6, respectively, resulting in 42.4% reduction in COD and 51.2% colour removal. Whereas, for H_2O_2, the optimum dose was 10 mL/L and optimum pH was 10. COD removal was observed to be 78.3% whereas colour removal was 73.4%, as shown in Fig. 3b.

Since H_2O_2 showed much more efficacy in conjugal removal of COD and colour, compared to that of bleaching powder, it emerged as the automatic choice despite higher cost involvement, because our prime target was to remove COD and colour to the maximum



(1) = Flow distributor,

(2) = Treated effluent collection outlet with stopper,

(3) = Bottom sludge collection outlet with stopper,

(4) = Solid medium for attached growth

Fig. 1 Details of lab-scale bio-filters

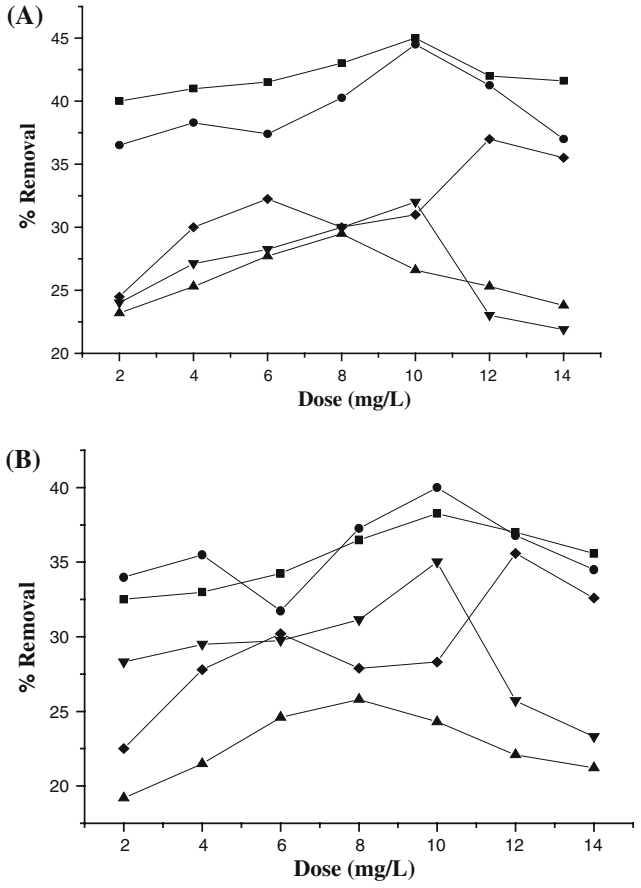


Fig. 2 Comparative efficacy of the coagulants. Alum at pH 10.5 (*filled square*), potash alum at pH 8 (*filled circle*), ferrous sulphate at pH 7.5 (*filled triangle*), ferric chloride at pH 10 (*filled inverted triangle*), lime at pH 10 (*filled diamond*), in removal of **a** colour and **b** COD

possible extent. Even after peroxide treatment, considerable amount of COD and colour remained in the wastewater. To remove the soluble organics, adsorption by means of suitable adsorbents was studied as the next treatment method.

Selection of suitable adsorbent (after peroxide treatment)

For powdered activated alumina, the optimum dose was found to be 4 g/L and the optimum pH was found to be 6. At the optimum dose and pH, the equilibrium time came out to be 40 min for which, the removal of residual COD and colour (after peroxide treatment) were 65.3 and 63%, respectively.

In case of powdered wood charcoal, the optimum dose was found to be 30 g/L whereas the optimum pH was noted to be 6.5. Equilibrium time was found to be 135 min. Additional removal of COD and colour at the optimum conditions were found to be 45.2 and 31.5%, respectively.

Results for three types of powdered bone char, which were thoroughly investigated, are presented below:

1. *Unactivated bone char:* The optimum dose was found to be 15 g/L. The pH at the optimum dose was found to be 8 for maximum reduction of COD and colour. Maintaining the optimum pH and dose condition, the equilibrium time was found to be 60 min. At that optimum pH-time-dose condition, residual COD and colour removal came out to be 46.2 and 28.3%, respectively.
2. *Bone char activated with 1 N NaOH:* The results found were almost same as those of unactivated powdered bone char in additional removal of COD and colour.
3. *Bone char activated with 1 N H_2SO_4:* The optimum pH was found to be 5. At the optimum pH condition, optimum dose was found as 8 g/L and finally the equilibrium time at optimum pH-dose condition was found to be 60 min. At that optimum pH-time-dose condition, removal of residual COD and colour, remaining after the peroxide treatment, were found to be 66.2 and 42.5%, respectively.

Thus, the acid-treated bone char came out as the best adsorbent resulting in an overall 96% COD removal and 93% colour removal. The results of pH, dose and equilibrium time study for acid-treated bone char are presented in Fig. 4a–c, respectively.

Biological treatment

The findings of the biological study that was carried out to evaluate the decolourisation potential of two fungi are discussed in this section.

Batch mode

The results of three stages of the batch study are presented here.

Revival of fungal culture

The fungal spores were inoculated onto solid agar media in sterile condition. After 1 week, green spores of *A. fumigatus* were visible. *C. versicolor* took about 12 days to show visible growth. After 15 days, the next steps of the batch study were carried out.

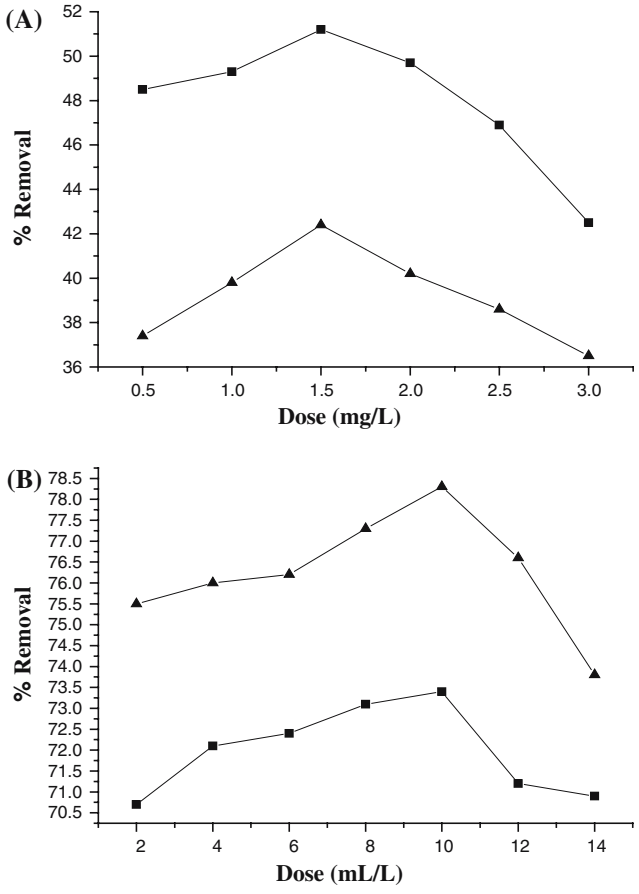


Fig. 3 Optimum dose of **a** bleaching powder at pH 6, **b** hydrogen peroxide at pH 10, for conjugal removal of colour (*filled square*) and COD (*filled triangle*)

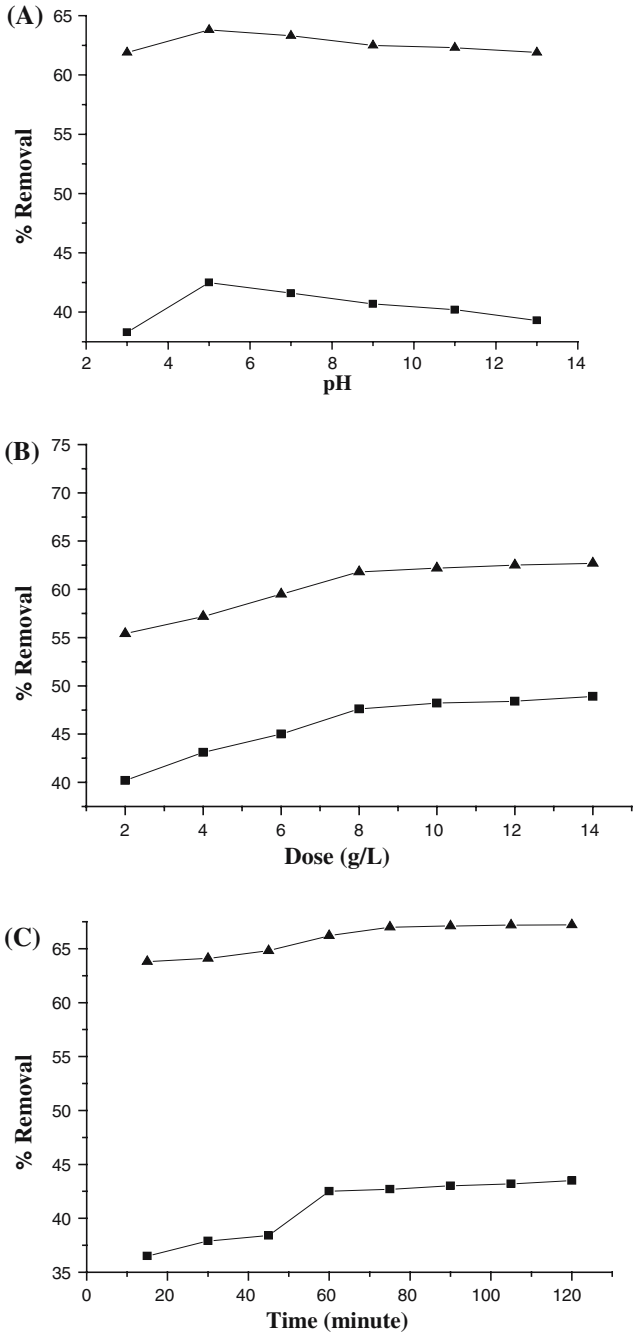


Fig. 4 **a** pH study, **b** dose study at pH 5, **c** equilibrium time study at pH 5 and dose of 8 g/L, with acid-treated bone char used as adsorbent for removal of colour (*filled square*) and COD (*filled triangle*)

Study in specified liquid media

The results of growth study for fungal suspensions in liquid environment are presented here. Though same amount of spores was inoculated for each culture, *Aspergillus* showed better growth and the characteristic growth curve was more satisfactory for *Aspergillus* (Fig. 5a, b). For the control, no fungal growth was observed.

Though *C. versicolor* took more time to grow due to a prolonged lag phase of around 175 h. The reason might be that the temperature, i.e. 30 ± 5°C at which the growth study was performed was higher than its optimum growth temperature, i.e. 25°C. However, later it showed exponential growth.

A. fumigatus showed a short lag phase of 20 h and its optimum growth temperature (30°C) is almost same as the ambient temperature of tropical Indian climate. Therefore, *Aspergillus* was chosen for the next phases of the study as it can be implemented in the field conditions at the ambient temperature. *C. versicolor* had been discarded for its long generation time (at 30 ± 5°C) and pH dependence.

Study in wastewater media

In this phase, colour removal efficiency of *A. fumigatus* was studied in sterile condition. Wastewater samples

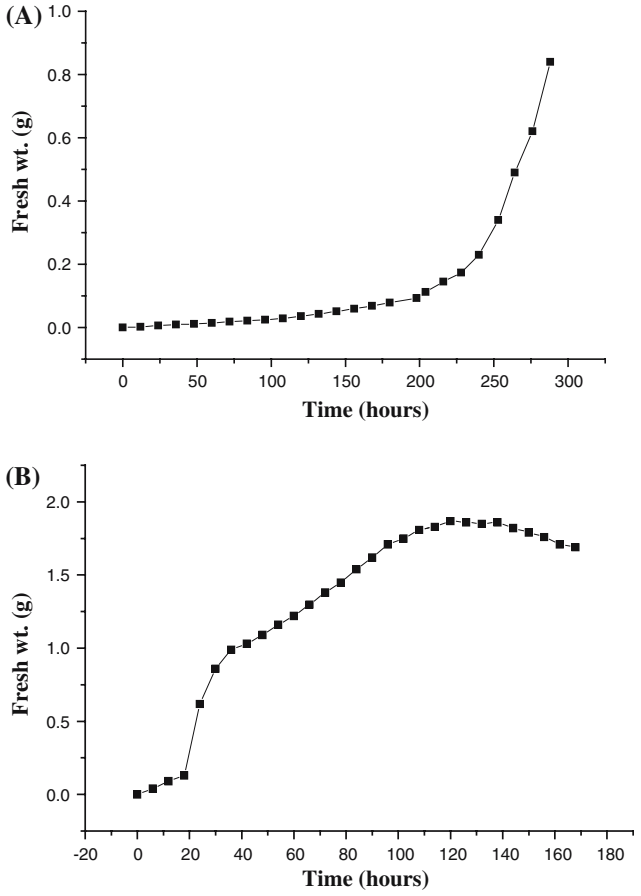


Fig. 5 Characteristic growth curve of **a** *Coriolus versicolor* and **b** *Aspergillus fumigatus*

were autoclaved prior to inoculation in order to ensure that there was no other microorganism present in the wastewater. This study was performed for different dilutions of sample (i.e. for different substrate concentrations) and for different inoculum concentrations.

Figure 6a describes the results for 10% inoculum in raw wastewater. On the 15th day, maximum colour removal was found to be 70%. Since the raw effluent contains higher substrate as compared to diluted effluent, 10% inoculum representing a higher fungal population resulted in a better colour removal efficiency.

For 5% inoculum in raw sample, maximum colour removal was 37% as shown in Fig. 6b. Lower removal efficiency, compared to the previous one, might be resulted from less biomass production.

Figure 6c, d represents the result of 10% inoculums in 1:1 diluted effluent (25% colour removal) and that of 5% inoculums in 1:1 diluted sample (35% colour removal), respectively. Here, the possible reason of less efficiency might be the lower substrate concentration since the effluent was diluted.

The colour removal study was performed for a total duration of 15 days for each of the above four cases. From the results, absorbance (directly proportional to the colour concentration of the supernatant) was found to decrease with an increase in biomass production for all cases. Therefore, it can be inferred that the colour removal is a function of fungal growth, which is in turn a function of initial microbial population, i.e. initial concentration of the inoculums and substrate concentration, i.e. concentration of sample. Since no declining phase was reached, higher percentage of colour removal may be expected for durations more than 15 days.

Continuous mode

This study was performed in ambient condition to simulate field condition in order to understand the effect of different flow rates on simultaneous removal of colour and COD from raw effluent.

Figure 7a shows the efficiency of two bio-filters operated in series at a flow rate of 0.065 $m^3/m^2/h$ (1 L/h). After 7 days of run, colour and COD removal achieved were 48.4 and 32.1%, respectively.

Removal efficiency was found to reduce when the flow rate was doubled to 0.13 $m^3/m^2/h$ (2 L/h), other conditions remaining the same. Colour and COD removal were 26.4 and 20.8%, respectively (Fig. 7b).

Reduced efficiency might be caused due to less contact time and enhanced sloughing rate due to increase in flow rate. Ambient temperature also plays an important role for the proper growth of the fungus. For *A. fumigatus*, the temperature favourable for growth was 30°C. Since the experiments were carried out in winter with an average ambient temperature of 15–20°C, higher efficiency could not be achieved.

Treatment economics

Economy is the keyword for industries. Any process suitable for industrial application must be cost-effective. The aim of this project was to find out a solution which is not only technologically feasible but also cost-effective. This section assesses the economic viability of the treatment processes, which emerged out to be effective in removal of colour and COD from distillery effluent. Initial investment as well as the operation and maintenance cost is analysed as given in Tables 2 and 3. It is assumed that the sludge generated in various proposed processes can be accommodated in the existing sludge drying beds of the distillery.

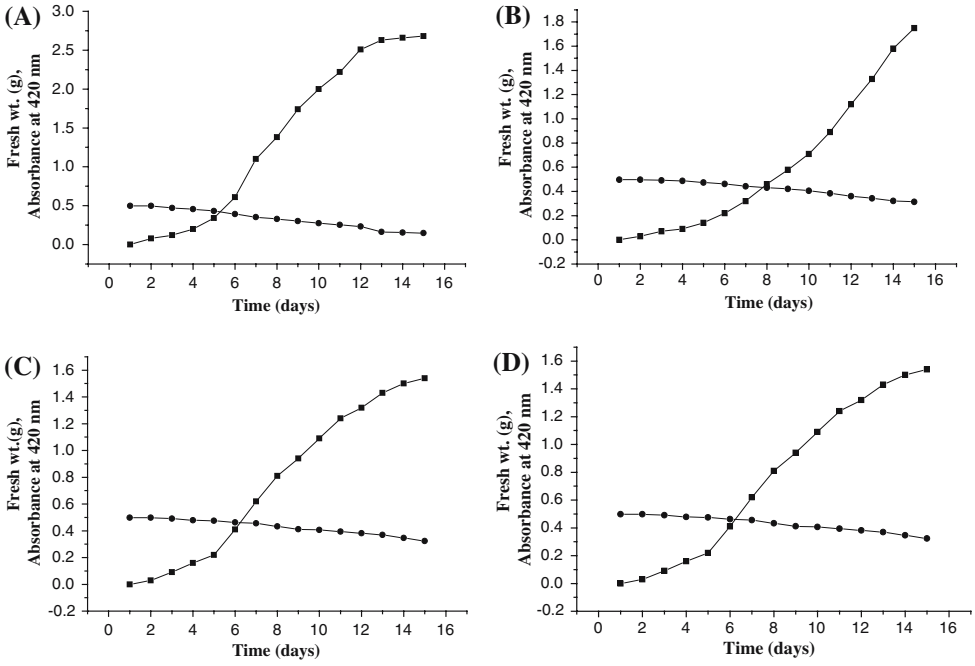


Fig. 6 Fungal growth (*filled square*) and colour removal (*filled circle*) for **a** 10% inoculum in raw effluent (removal 70%), **b** 5% inoculum in raw effluent (removal 37%), **c** 10% inoculum in 1:1 diluted effluent (removal 25%), **d** 5% inoculum in 1:1 diluted effluent (removal 35%)

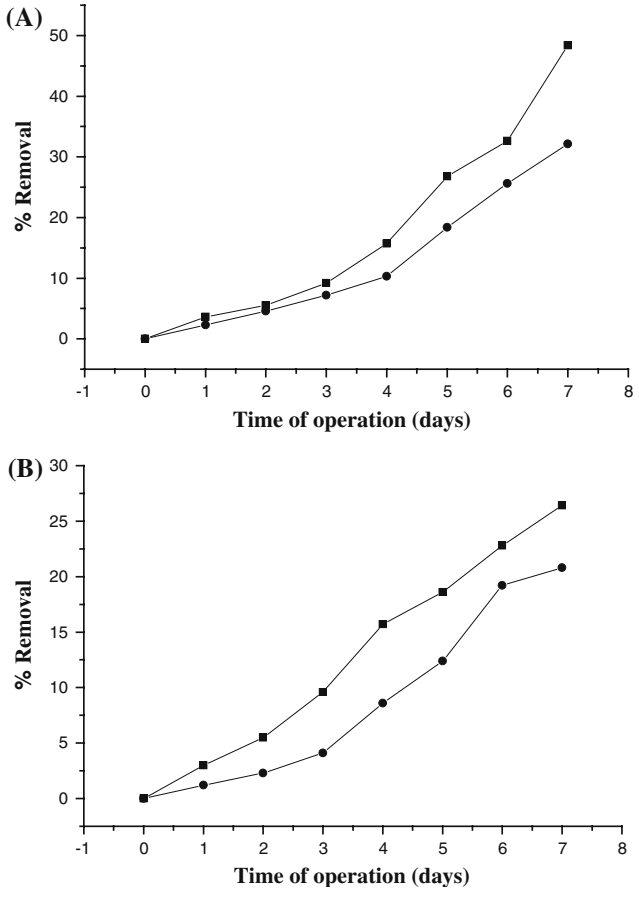


Fig. 7 Performance of two lab scale bio-filters used in series for **a** low flow rate—0.065 $m^3/m^2/h$ (1 L/h) and, **b** high flow rate—0.13 $m^3/m^2/h$ (2 L/h), in conjugal removal of colour (*filled square*) and COD (*filled circle*)

From Tables 2 and 3, it may be inferred that physico-chemical treatment may be adopted without chemical oxidation stage. With this option, overall 84% COD removal and 74% colour removal can be achieved at the cost of Rs. 0.41 per litre of wastewater (1$ = Rs. 46).

Even the above option with the chemical oxidation at the cost of Rs. 3.21 per litre of wastewater is also not very expensive, compared to present-day practice of diluting the effluent with fresh water at the cost of Rs. 2–3 per litre. In this case, the removal efficiency achievable is excellent, i.e. overall 96% COD removal and 93% colour removal.

For biological treatment, initial investment vanishes as the distillery already has six bio-filters with sophisticated growth media. Only cost involved initially is that of inoculation of the cultures onto the media surface. Operation and maintenance cost is also negligible. Biological treatment option appears to be promising because of its almost negligible cost involvement. Only initial inoculation would be required to be done, and then the system would become self-sustainable.

Conclusions

All experimental results clearly show that the removal of colour and COD is inter-related. Physico-chemical treatment is efficient in overall 96% COD removal and 93% colour removal with a treatment cost of Rs. 3.21

Table 2 Economics of physico-chemical treatment process

Process	Initial cost	Operation and maintenance cost				
		Dose	Volume treated (MLD)	Material requirement (per day)	Rate (Rs. per unit)[a]	Daily cost (Rs.)[a]
Coagulation by alum with double flocculation	Negligible	10 g/L	0.65	6.5×10^3 kg	25	1,62,500.00
Chemical oxidation by 50% hydrogen peroxide	Negligible	10 mL/L	0.65	6,500 L	280	18,20,000.00
Adsorption by acid-treated bone char	As applicable	8 g/L	0.65	5.2×10^3 kg	20	1,04,000.00
Total treatment cost: Rs. 3.21 per litre (with chemical oxidation) and Rs. 0.41 per litre (without chemical oxidation)						

MLD million litres per day

[a] 1$ = Rs. 46

Table 3 Economics of biological treatment process

Process	Initial cost	Operation and maintenance cost
Existing bio-filters with attached growth of *Aspergillus fumigatus*	Only inoculation cost	Negligible
Total cost: only inoculation cost		

per litre of wastewater, if double coagulation–flocculation followed by peroxide treatment followed by adsorption onto acid-treated bone char is adopted.

Only double-flocculation followed by adsorption is also an alternative, capable of yielding an overall 84% COD removal and 74% colour removal at a very nominal cost of Rs. 0.41 per litre of wastewater.

A maximum of 70% colour could be removed by 10% *A. fumigatus* inoculum in raw wastewater sample in batch mode under sterile conditions. However, under ambient winter conditions, the maximum colour removal of 48% and COD removal of 32% could be achieved in continuous flow bio-filters. Treatment of the distillery wastewater by bio-towers employing attached growth of *A. fumigatus* emerged out to be a promising option. Moreover, it may result in much better efficiency in conjugal removal of colour and COD if this pilot study is repeated in ambient summer conditions.

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