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BIOLOGICAL AOX REMOVAL OF PULP MILL PLANT EFFLUENT BY *PSEUDOMONAS AERUGINOSA* – BENCH STUDY

Anil Kumar[a], Purnima Dhall[b], Rita Kumar[b]

[a]*National Institute of Immunology, Aruna Asaf Ali Marg, a10067 New Delhi, India*
[b]*Institute of Genomics and Integrative Biology, Mall Road, 110007 Delhi, India*

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Abstract. Discharge of adsorbable organic halides (AOX) into the water bodies has resulted into many health and environmental problems such as endocrine disruption, aquatic toxicity, bioaccumulation and carcinogenicity. The already known physical, chemical and electrochemical methods are not economically viable for the control of water pollution. So this paper focuses on the biological reduction of AOX from pulp and paper mill effluent using isolated bacteria. The isolated bacteria were screened and finally *Pseudomonas aeruginosa* strain 1 was used further. The effect of various parameters such as, bacterial cell concentration, surface washing of bacterial cell and agitation were investigated and it was found that to some extend every parameter has resulted in the reduction of AOX from the effluent. It was inferred that the three time washed pellet inoculated in the ratio of 1:1 (sample: pellet) and incubated at 150 rpm at 37 °C for 24h has resulted in 78% of AOX removal.

Keywords: adsorbable organic halides, AOX, biological reduction, water pollution.

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Introduction

In terms of fresh water usage pulp and paper industry ranks third in the world (Santosh 2007). Bleaching of pulp by chlorine based agents is still practiced in India and in other developing countries (Deshmukh *et al.* 2009). Waste water from bleaching unit is toxic because of the presence of chlorinated organic compounds (Savant *et al.* 2006). These organochlorine compounds are collectively termed (Saima, Izharul 2012) as adsorbable organic halides (AOX). Formation of these chlorinated organics also contributes to the color of waste water (Clifford, Noemi 2010). It has been reported that production of one ton of paper contributes 100 kg of colorimparting substances and 2–4 kg of organochlorines (Kansal *et al.* 2008). About 500 different chlorinated organic compounds have been identified in paper mill effluent (Savant *et al.* 2006); among these chlorinated hydrocarbons, benzenes, phenols, catechols, guaiacols, syringols, vanillins, chloroform, dioxins and furans etc. are of major concern (Kansal *et al.* 2008; Freire *et al.* 2003).The chemical diversity of these pollutants causes a variety of clastogenic, mutagenic, carcinogenic, endocrine effects on fishes and other aquatic communities in recipient water bodies (Karrasch *et al.* 2006). Increased awareness for the harmful effects of these pollutants has resulted in stringent regulations on black liquor discharge into the environment (CPCB 2007). The Central Pollution Control Board of India has imposed AOX discharge limit amounting to 1.5 kg AOX/ton of paper produced (MoEF 1993). This discharge limit will be further lowered to 1kg AOX/ton of paper produced in next five years for large pulp and paper industries.

A number of approaches have been explored to reduce the AOX level in paper mill effluent, which can be categorized as physico-chemical and biological. Physico-chemical techniques include adsorption, ultrafiltration, nanofiltration, reverse osmosis, neutralization of bleach effluent and supercritical water oxidation. Major limitations of above mentioned physico-chemical approach are cost and generation of secondary sludge (Patel, Suresh 2008). Whereas advanced oxidation processes (ozonation, peroxidation, photocatalysis) were developed and used as potentially powerful methods capable of transforming the pollutants into harmless substances (Biljana *et al.* 2012). Combining some oxidizing agents such as O_2/ZnO/UV, O_2/TiO_2/UV, O_3/UV, UV/H_2O_2/TiO_2, O_3/H_2O_2 has resulted in excellent color reduction and hold the

Corresponding author: Anil Kumar
E-mail: anilk@nii.ac.in

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greatest promise for detoxification and mineralization of pollutants (Catalkaya, Kargi 2007; Hassan, Hawkyard 2002; Helmy *et al.* 2003; Daneshvar *et al.* 2004; Catalkaya, Kargi 2008; Ugurlu, Karaogly 2009). But none of them is industrially applicable due to high cost which is a limitation and infeasibility of the processes. However, biological treatment (Ruggaber, Talley 2006) methods for treating pulp and paper effluent are of great concern over physico-chemical methods due to their economical as well as ecofriendly impact. Biological approach involves the use of diverse kind of microorganisms like bacteria, fungi, algae and microbes of extreme habitat for reducing AOX and chromophores in pulp mill effluent. Such biological process can be performed under aerobic or anaerobic conditions or in combination of the both. The biological degradation of chlorinated organic compounds under aerobic conditions (Kim *et al.* 2002; Vasconcelos *et al.* 2006) and anaerobic conditions (Li *et al.* 2009; Maphosa *et al.* 2010; Futagami *et al.* 2008) has been reported in the literature. Researchers also employed the combination of both aerobic and anaerobic conditions like Pratibha (2007) studied the degradation of chlorophenols. The AOX removal was 84% on third day when 7 days anaerobically treated effluent was further treated by aerobic means.

Use of fungi for the treatment of pulp mill effluent was also reported by some researcher such as Basak *et al.* (2004) who reported 76% AOX reduction with 0.2 g/l feed acetate concentration and 70% AOX could be removed from chlorinated pulping waste in 7.3 h of contact with 0.2 g/l of carbon supplement by using *Penicillium camemberti*.

The major drawback associated with the fungal treatment is that fungi can effectively degrade lignin but they are unable to survive under extreme environmental conditions (high temperature, pH and presence of toxic chemicals) which usually exist in the treatment plants. In addition, fungal filaments cause structural hindrance so their utilization is not feasible for biological treatment of pulp and paper industry effluent (Amr *et al.* 2008).

Considering the above drawbacks this study explores the possibility of naturally occurring resident bacteria on the reduction of adsorbable organic halides present in the effluent emanating from pulp and paper mill located in Northern India. Various parameters were optimized in order to increase the rate of degradation and decrease the time of treatment like bacterial cell concentration, surface washing of bacterial cell and agitation. Later the isolate, exhibiting the best AOX reducing capability, was identified by 16s rRNA studies at DSMZ, Germany.

1. Material and methods

1.1. Sample collection

Sludge and Wastewater samples were collected from the inlet and outlet ETP streams of pulp and paper mill in clean amber glass containers. Samples were transported within 12 hrs. Under refrigerated conditions and stored at 2–4 °C till use.

1.2. Isolation of bacteria

Sludge sample was collected from effluent treatment plant of pulp and paper mill for the isolation of autochthonous population of bacteria. Sludge extract was prepared by dissolving 300 g of dried (50 °C) sludge in 960 ml of distilled water. Then autoclaved at 15 psi for 1h followed by centrifugation at 7000 rpm for 10 min. The supernatant (extract) was collected and used for the preparation of enrichment medium. The enrichment medium used for bacterial isolation was composed of sludge extract and nutrient broth in the ratio of 1:2. Candid B (purchased from Glenmark) was added to the medium at a concentration of 0.25 µl ml^{-1} in order to prevent fungal growth during isolation. The enrichment medium was inoculated with 5 g sludge from the said site and incubated on orbital shaker for 48 h at 30 °C, 120 rpm. The enriched sludge extract sample was serially diluted in 0.85% saline solution and spread onto plates containing enrichment medium with 2% agar. The plates were incubated at 37 ± 2 °C for 24–96 h. Single isolated colonies were picked and streaked on fresh plates containing the NB to obtain pure cultures. The isolates obtained were then screened for their capacity to reduce AOX and color.

1.3. Screening of bacterial isolate for AOX reduction

a) Experimental setup

The AOX reduction experiment was set up to screen out effective bacterial isolate. The mother culture was prepared by inoculating one loopful of all the individual bacterial isolates separately in 25 ml of sterilized nutrient broth having 0.01% tween 80 (surfactant). The inoculated broths were incubated in an orbital shaker at 37 °C for 16–24 h so as to obtain actively growing mother cultures. The actively growing individual bacterial cultures were inoculated separately in 2000 ml of flask containing 1000 ml of sterilized nutrient broth and incubated at 37 °C, 200 rpm for 16–24 h. After the growth was achieved the cultures were centrifuged at 8000 rpm for 15 min 4 °C. Pellets were washed with phosphate buffer (pH 6.8) and stored at 4 °C till further use. The flasks containing 1 liter of wastewater sample were inoculated with the pellets and incubated in shaker at 200 rpm at 37 °C for 24 h. The ratio of wastewater: bacterial isolate was 1:0.5. After 24 h of incubation, the samples were analyzed for AOX reduction.

b) AOX (Adsorbable organic halides)

AOX was measured according to the standard method given in (APHA 98). The samples were

collected (before and after treatment) and centrifuged, the supernatant was analyzed. The three main steps involved were: adsorption of organic matter onto activated carbon, organically-bound chlorine mineralization through combustion and determination of released chloride by micro-coulometric titration. AOX was estimated by using the 686 – Titroprocessor analyzer (Metrohm, Switzerland).

c) Adsorption and desorption assay

Desorption studies were carried out to the extent of adsorbed color to the bacterial biomass during the process of color removal which ultimately leads to the reduction in AOX. Different desorbing agents like 1N NaOH, 1N HCl, triple distilled water and 85% HCOOH were used for the desorption studies. Known volumes of thoroughly shaken samples were taken and centrifuged to obtain pellets. The pellets were then treated with the above chemicals and extracted twice to remove the adsorbed color, till no more color could be extracted from the biomass. The extent of color adsorbed was estimated by comparing with the actual color of control and the color removed by the bacterial biomass. Controls of pure inocula in buffer, uninoculated effluent and non-decolorizers were also run parallely and subjected to same desorption protocol, to eliminate any color being produced due to the intracellular contents of the bacteria and the chances of a false reading.

1.4. Analysis of the color adsorption data

The decolorization data was analyzed using the general equation for multilayer adsorption by Freundlich, expressed as

$$x/m = K.C^{1/n}, \quad (1)$$

where: x/m = amount of solute adsorbed per unit weight of the adsorbent;

C = is the equilibrium concentration of color remaining;

K and n = empirical constants.

The constant K is positively related to the extent of degree of adsorption, while the constant n provides a rough estimate of the intensity of adsorption. When n = 1, adsorption is considered to be linear.

A linear form of the expression will yield the constants K and n, hence:

$$\text{Log } x/m = \log K + 1/n \ \log C, \quad (2)$$

where: 'x' was calculated by subtracting the color value after decolorization, from the initial value; 'm' was calculated by estimating the dry weight of the biomass used for decolorization and C was the final color value after which there was no significant decrease.

1.5. Optimization of AOX reduction parameters

a) Bacterial cell concentration

The innoculum was prepared by inoculating one loopful of selected isolate in a 50 ml of sterilized nutrient broths having 0.01% tween 80. The inoculated broths were incubated in an orbital shaker at 37 °C for 16–24 h so as to obtain actively growing mother cultures. This actively growing culture was used to inoculate 500 ml, 1000 ml and 2000 ml of sterilized nutrient broths separately and incubated at 37 °C, 200 rpm for 16–24 h. After the growth was achieved the cultures were centrifuged on 8000 rpm at 4 °C for 15 min. The pellet of the cultures was washed with phosphate buffer (pH 6.8) and used to inoculate three 1liter of wastewater samples individually in ratios (wastewater:pellet); (1:0.5), (1:1), (1:2). These inoculated wastewater samples were incubated in shaker at 200 rpm at 37 °C for 24 h. After 24 h of incubation, the samples were analyzed for AOX reduction.

b) Surface washing of bacteria

The innoculum was prepared by inoculating one loopful of selected isolate in a 50 ml of sterilized nutrient broth having 0.01% tween 80. The inoculated broths were incubated in an orbital shaker at 37 °C for 16–24 h so as to obtain actively growing mother cultures. This actively growing culture was used to inoculate three 1000 ml sterilized nutrient broth separately and incubated at 37 °C, 200 rpm for 16–24 h. After the growth was achieved the cultures were centrifuged at 8000 rpm for 15 min at 4 °C. The pellet of first 1000 ml culture was washed only once with phosphate buffer (pH 6.8) and used to inoculate 1liter of wastewater sample. The pellet of second flask was washed twice with phosphate buffer (pH 6.8) and used to inoculate 1liter of wastewater sample. The pellet of third flask was washed three times with the same phosphate buffer (pH 6.8) and used to inoculate another 1liter of wastewater sample. These inoculated wastewater samples were incubated in shaker at 200 rpm at 37 °C for 24 h. The ratio of wastewater: bacterial isolate was 1:1. After 24 h of incubation, the samples were analyzed for AOX reduction.

c) Agitation

The actively growing culture was used to inoculate three 1000ml sterilized nutrient broth separately and incubated at 37 °C, 200 rpm for 16–24 h. After the growth was achieved the cultures were centrifuged at 8000 rpm for 15min 4 °C. Pellets were washed thrice with phosphate buffer (pH 6.8). The 3 flasks containing 1 liter of wastewater sample were inoculated with the pellets and each was incubated in shaker at

different rpm i.e. (150 rpm, 200 rpm, and 250 rpm) at 37 °C for 24 h. The ratio of wastewater:pellet was 1:1. After 24 h of incubation, the sample was analyzed for AOX reduction.

1.6. Repeatability and reproducibility experiment

The innoculum was prepared by inoculating one loopful of selected bacterial isolate separately in 50 ml of sterilized nutrient broth having 0.01% tween 80. The inoculated broth was incubated in an orbital shaker at 37 °C for 16–24 h so as to obtain actively growing mother culture. The actively growing individual bacterial culture was inoculated separately in 2000 ml of flask containing 1000 ml of sterilized nutrient broth and incubated at 37 °C, 200 rpm for 16–24 h. After the growth was achieved the cultures were centrifuged at 8000 rpm for 15 min 4 °C. Pellet was washed thrice with phosphate buffer (pH 6.8) and stored at 4 °C till further use. The flask containing 1 liter of wastewater sample was inoculated with the pellet and incubated in shaker at 200 rpm at 37 °C for 24 h. The ratio of wastewater: bacterial isolate was 1:1. After 24 h of incubation, the sample was analyzed for AOX reduction.

1.7. Bacterial identification

The selected bacterium was identified by 16S rRNA studies at DSMZ, Germany. The selected bacterial isolate was identified by 16S RNA gene analysis. Genomic DNA was extracted by using kit (Real Genomics kit). 16S rRNA gene of DNA sample was amplified using universal primer 16S F = 5'- AGCAGCCGCGGTAATAC-3' and 16S R = 5'-TACGGCTACCTTGTTACG-3' synthesized by the centre for genomic application (TCGA). The PCR product purified by gel elution (Gel extraction Kit, Qiagen) was sequenced. 16S RNA gene was compared with available sequences using NCBI-BLAST tool (Johnson *et al.* 2008).

2. Results

2.1. Isolation and characteristics of bacterial population

Nine bacterial isolates were purified from all 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 wrinkled periphery; shining to dry and so on. Table 1 show the morphological characteristics of those isolated bacterial population obtained during the process of isolation.

2.2. Screening of bacterial isolate for AOX reduction

In the present study, selected (on the basis of growth rate) isolates were screened for their capability to survive and actually degrade the pollutional components present in the wastewaters to which they are exposed. The bacteria responded in varying degrees with a few bacteria not being able to survive in the given conditions. A few bacteria were able to grow in the wastewaters but were not actually able to bring down the pollutional parameters, i.e. AOX. Adsorbable organic halides (AOX) were particularly a challenging parameter. Bacterial isolates were screened individually to observe their capacity to reduce AOX of the paper mill effluent. Studies were conducted with the selected isolates obtained from pulp and paper mill. The results are depicted in Figure 1, however as can be seen from the figure, there was a marked increase in AOX values of these sample in case of some isolates some will give good response. The isolates were able to give the results after 24 h of incubation. Isolates 4 exhibited the best results giving a reduction of 2.8 mg/l (standard deviation – 0.11 with standard error – 0.05) of AOX while comparing with control showing AOX value of 10.4 mg/l after 24 h of incubation followed by isolate 7 giving reduction of 5.7 mg/l.

Table 1. Morphological characteristics of isolated bacteria

Lab Name	Configuration	Margin	Elevation	Color	Size in mm
Isolate 1	Round	Smooth	convex	bluish Cream	3 mm
Isolate 2	Irregular	Lobate	umbonate	yellowish	2–3 mm
Isolate 3	Round	Smooth	drop like	yellowish	1 mm
Isolate 4	Round	Smooth	flat	creamy white	2 mm
Isolate 5	Punchi form	Smooth	convex	yellow	Pinpoint
Isolate 6	Round	Smooth	raised	orangish	1–2 mm
Isolate 7	Round	Smooth	convex	white	1 mm
Isolate 8	Round	Smooth	drop like	yellow glossy	1 mm
Isolate 9	Spindle	Smooth	flat	thick white	2 mm

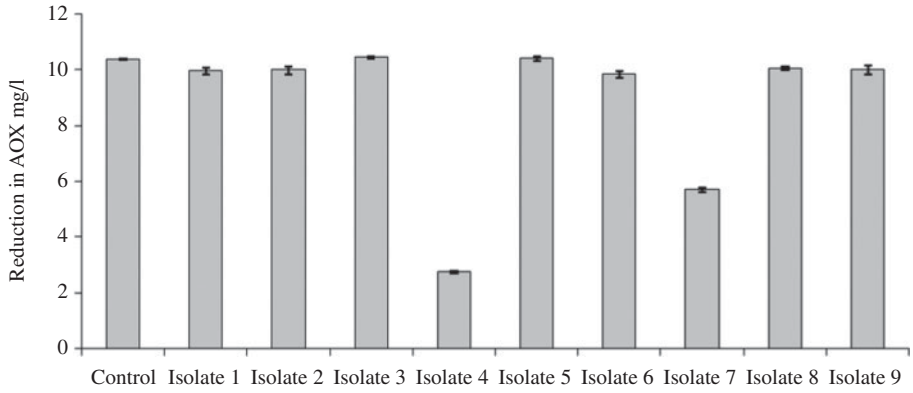

Fig. 1. Reduction in AOX level of Pulp and paper samples treated with different individual bacterial strains

2.3. Adsorption and desorption assay

Different process units of the paper manufacturing plant, contribute to the overall color of the effluent, of which Black liquor, which comes out from the pulping stage, is the major contributor. The bleaching section release organochlorine compounds, which form chromophoric compounds with lignin breakdown compounds and impart color to the effluent. This also increases the absorbable organic halide (AOX) content of the effluent. So, the bacterial isolates were screened for their capacity to reduce the color of wastewater, individually which ultimately affects the AOX level present in the effluent. It was evident from the obtained data (Fig. 2) that about 65% (standard deviation = 0.7 with standard error of = 0.31) of the color was removed by isolate 4 in comparison to other isolates which shows the reduction in the range of 30.6 to 57.2%. Isolate 4 was selected for further studies.

The colored components present in the effluent generally arise from lignin and its derivatives, which are responsible for AOX present in the effluent. They are negatively charged, in general, with mostly hydroxyl and carboxyl functional groups. In view of the results obtained during the study of various parameters, it is this charge repulsion that caused lesser decolorization in certain bacteria. Therefore, production of some exopolysaccharides or capsular elements by the best decolorizers could be actual reason for adsorption of color from the wastewater.

2.4. Optimization of parameters for AOX reduction

Effect of inoculum size: the effect of biomass on the AOX reduction ability of isolate 4 was studied. Different effluent:biomass ratios of 1:0.5, 1:1 and 1:2, were tried. Results suggested that isolate 4 produced the best results when used in the 1:1 effluent: biomass

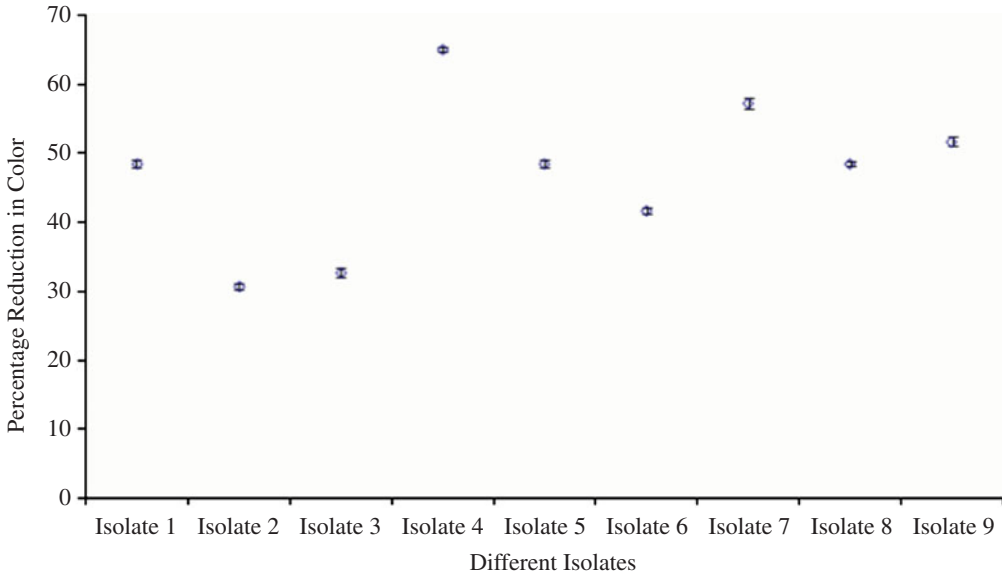

Fig. 2. Percentage reductions in color of pulp and paper mill effluent with different individual bacterial isolates

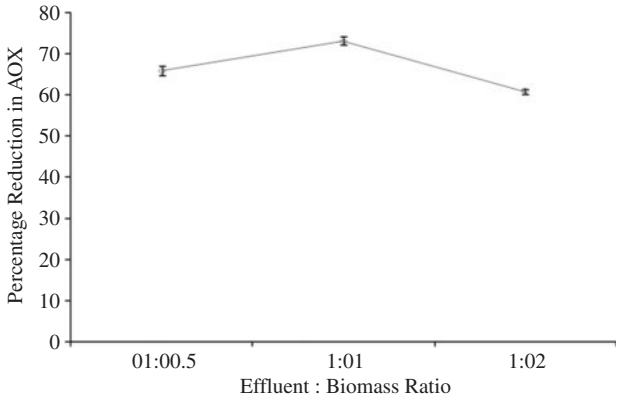

Fig. 3. Effect of rate of inoculum size on the AOX reduction of pulp and paper mill effluent

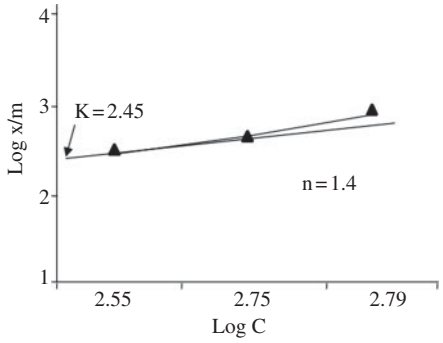

Fig. 4. Color adsorption data at different effluent: biomass ratio. 'x/m' is the amount of color adsorbed per unit biomass and C' is the amount of color remaining in the effluent after decolorization

ratio. The reduction in AOX was observed up to 73% after 24 h of incubation period (Fig. 3).

2.5. Analysis of the color adsorption data

It is evident from the graph that the process of decolorization is following Freundlich's adsorption isotherm well with an n value equal to 1.4 and K value 2.54 (Fig. 4). The logarithmic version of Freundlich's equation is straight line in the form of $y = mx + b$, where, b is the y-intercept for $x = 0$. In our case, K equals x/m intercept, when the log of the concentration C (color remaining) equals one unit, the log of which is zero. The value of m, the slope of the line, is equal to 1/n. our data shows a considerable linearity till an effluent:biomass ratio 1:1, however, upon increasing the biomass proportion after this, a linear adsorption pattern is not observed. This indicates the presence of some other mechanism for color removal apart from true adsorption, which was also indicated by the adsorption desorption assay. Based on the results obtained it can be concluded that although adsorption plays an important role in the decolorization process, assimilation of some chromophoric material is also taking place.

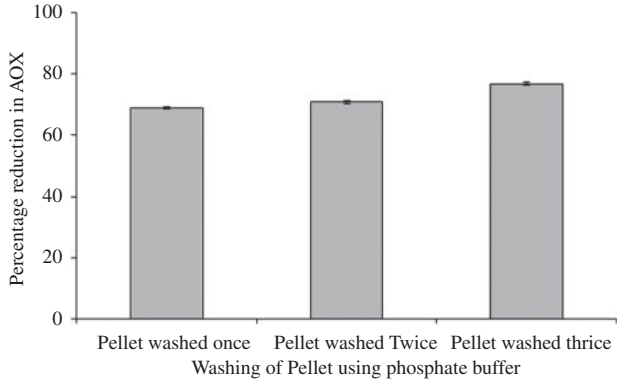

Fig. 5. Effect of surface washing of bacterial cell on the AOX reduction of pulp and paper mill effluent (n = 5)

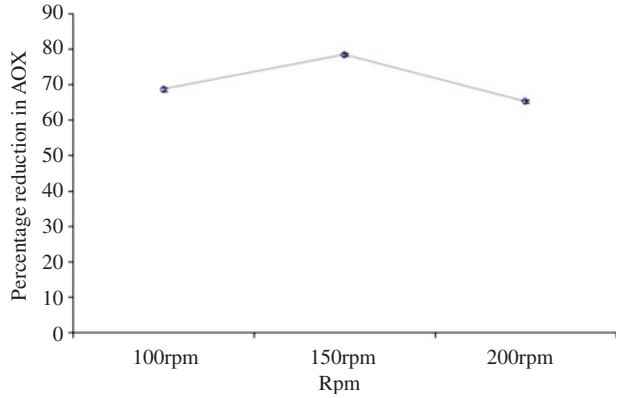

Fig. 6. Effect of rate of agitation on the AOX reduction of pulp and paper mill effluent (n = 5)

Effect of surface washing of bacteria: effect of surface washing on AOX reduction was studied and observed that the pellet washed thrice with phosphate buffer show effective reduction in AOX, i.e. up to 76% (standard deviation of = 1.3 with standard error of = 0.5) in comparison to the pellet washed once and twice showed reduction up to 68% and 70% respectively (Fig. 5).

Effect of Agitation: the effect of rate of agitation on the AOX reduction was also investigated. A range of 100–200 rpm was selected and AOX studies conducted after a period of 24 h. It was observed that percent AOX reduction was highest, i.e. 78.4% (standard deviation = 1.1 with standard error of = 0.5) at about 150 rpm (Fig. 6).

2.6. Repeatability and reproducibility

After optimizing the various parameters the experiment was performed 5 times in order to check the reproducibility and repeatability among the results. Results showed three time washed pellet inoculated in the ratio of 1:1, incubated at 150 rpm for 24 h. remove 77–78% (standard deviation = 1.1 with standard error of = 0.5) of the AOX affectively from the pulp mill effluent.

2.7. Identification of bacterial isolates

Identification of selected bacterial strain: the isolate number 4 were identified by 16S RNA studies at DSMZ, Germany, as *Pseudomonas aeruginosa strain 1*.

3. Discussion

Color of pulp mill effluent is dark-brown and obnoxious. It is not only unaesthetic but also increases the amount of AOX in the effluent. Color of pulp mill effluent is primarily due to lignin, which is an important part of the woody raw materials utilized for paper manufacture. Physico-chemical treatments of pulp and paper wastewater were mentioned in literature but none of them is industrially applicable due to cost limitation and infeasibility of the processes. Biological treatment includes aerobic and anaerobic treatment or the combinations of both. Under aerobic conditions, reduction of AOX has been studied by Fulthorpe and Allen (1995) who compared three bacterial strains, *Pseudomonas*, *Ancylobacter* and *Methylobacter*, for their dehalogenating capabilities and reduction of AOX of bleached Kraft mill effluent from three different sources. AOX reduction was poor to negligible in the case of *Ancylobacter* and *Pseudomonas*, whereas *Methylobacterium sp.* exhibited some reduction in overall AOX levels of the effluents. Jaspers *et al.* (1994) reported reduction in AOX levels of E1 stage of the Kraft process by adsorption on pellets of *Phanerochaetechrysosporium,* under controlled incubation conditions. In anaerobic treatment, color (70%), lignin (25%), COD (42%), AOX (15%) and phenol (39%) were reduced in 15 days retention time. After this, such anaerobically treated effluent was treated in a bioreactor in presence of a fungal and bacterial strain separately. The reduction efficiency with fungal strain was color (95%), AOX (67%), lignin (86%), COD (88%), Phenol (63%), while with bacterial strain the reduction efficiency was: color (76%), lignin (69%), COD (75%), AOX (82%), phenol (93%). Singh and Thakur (2004) studied the sequential aerobic and anaerobic treatment of pulp and paper mill effluent and found the 15% AOX 70% color, 255 lignin and 42% COD reduction. In a study by Singh and Shekhar (2006) sequential anaerobic and aerobic treatment of pulp and paper mill effluent was done in a two-step bioreactor. Chen *et al.* (2003) designed a process by combining the co-agulation, anaerobic acidification and aeration package reactor for the treatment of bleaching effluents and observed the COD, BOD, AOX reduction efficiencies of 88.1%, 81%, 98.4% respectively. By using activated granular carbon as a medium for biofilm growth and 24 h retention time in a sequencing batch biofilm reactor, AOX removal in the range of 10–100% can be achieved with selected retention time (Mohamad *et al.* 2008). For further study fungi and algae was used for the treatment of the effluent. The effluent toxicity also reduced considerably. MyCoR process, where fungus has been used in the form of fixed film reactor that converts 70% of organic chlorides into inorganic chlorides in 48 h with 50% reduction in BOD and COD (Savant *et al.* 2006). The 67% of AOX reduction and 97% of color reduction has been achieved by using fungus *Penicilliumcamemberti* in a hemp based pulp and paper mill bleachery effluent (Taseli 2008). Mixed algal culture was used by Esra *et al.* (2002) and Tarlan *et al*. (2002) for AOX and color removal. The efficiency of algal culture for AOX and color removal was up to 70%, 80% respectively. Saravanan and Sreekrishnan (2005) studied a combination of chemical and biological process for treatment of bleached kraft pulp mill effluent. The efficiency of this combined process for AOX removal was 81%.

The present work involved the study in reduction of adsorbable organic halides of pulp and paper mill sector India. Isolation of naturally occurring bacterial stains and their utilization for the treatment of wastewaters emanating from such industrial units was the main focus of this work. Bacteria were isolated from different sources in the vicinity of a paper mill. The sources included activated sludge and old soil samples, which had been accumulating over a number of years. Over 9 pure bacterial isolates were isolated during this exercise, using selective enrichments and subculturing. After extensive screening of bacterial isolates, one of the bacteria, *Pseudomonas aeruginosa str.1* was able to reduce the approximately 65% within a period of 24 h. Various parameters were optimized like effluent: biomass ratio which results in increase in reduction of AOX up to 73% when pellet was inoculated in the ratio of 1:1 (effluent:biomass). On the other hand the surface cell washing was studied which showed that washing the pellet thoroughly will be helpful in removing the extra media attached on the surface of bacteria. Results showed pellet washed thrice will helpful in reducing the AOX further to 76%. At last different speed for shaking the flasks were studied and the flask incubated at 150 rpm shows the maximum reduction of 78.4%. Laterally the repeatability experiment was performed in which the three time washed pellet was inoculated in the ratio of 1:1 incubated at 150 rpm for 24 hrs was results in 79% of AOX removal from pulp mill effluent.

Conclusion

The results show that autochthonous bacteria isolated from the site of pulp and paper industry have the ability to remove the AOX efficiently. The AOX reduction was significantly affected by various parameters including the amount of inoculum size, rate of agitation and surface cell washing. The 78% (standard deviation = 1.1 with standard error of = 0.5) of AOX

was removed when the pellet was washed thrice and inoculated in 1:1 ratio and incubated at 150 rpm for 24 h.

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Anil KUMAR is a Staff Scientist in National Institute of Immunology, New Delhi, India. He received his PhD in Botany from University of Delhi, Delhi, India, in 2008. His research interests include intellectual property rights, white biotechnology, biosensor, bioremediation and bioinformatics.

Purnima DHALL is a PhD student in Institute of Genomics and Integrative Biology, Delhi, India. She is working on development of BOD biosensor. Her research interests are BOD biosensor and bioremediation.

Rita KUMAR is a head and senior scientist in the Division of Environmental Biotechnology, Institute of Genomics and Integrative Biology. She received her PhD in medical microbiology from University of Delhi, in 1983. Her research interest includes Intellectual property rights, White biotechnology, biosensor, bioremediation and waste water treatment.

Note "Experiments mentioned in this paper were conducted at Institute of Genomics and Integrative Biology, New Delhi".