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Distillery effluent - An analysis

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ABSTRACT

In India, the wastewater at large from distilleries is known as spent wash, which is highly acidic in nature. In India 2004, distillery industry number has gone up to 319, producing 3.25x109 l of alcohol and generating 40.4x1010 l of wastewater annually (Uppal, 2004). Because of using large quantities of water in distillery industries it is essential to treat and reuse their waste water. In the most of time the discharge standards applied for distilleries are often too tough and below the level that can be achieved with appropriate biological treatment technologies (Pant and Adholeya, 2007a,b). In distillery industry, the production and characteristics of spent wash is highly variable and dependent on feed stocks and various aspects of the ethanol production process. The molasses spent wash (MSW) is a potential water pollutant in two ways. First, the highly coloured nature of MSW can block out sun light from rivers and streams thus reducing oxygenation of the water by photosynthesis and hence becomes injurious to aquatic life. Secondly, it has a high pollution load which would result in eutrophication of contaminated water sources (FitzGibbon et al., 1998). The first reason is due to the presence of water soluble recalcitrant colouring compound called melanoidin (Evershed et al., 1997). Melanoidin are dark brown to black coloured natural condensation product of sugar and amino acids produced by nonenzymatic browning reactions called maillard reactions (Plavsic et al., 2006). Ohmomo et al. (1988a) concluded that microbial decolourization of melanoidin is due to two decomposition mechanisms; in the first the smaller molecular weight melanoidin are attacked and in the second the larger molecular weight melanoidin are attacked. Satyawali and Balakrishnan (2008 a b) have investigated that the degradation of low molecular weight compound occurred in the membrane bioreactor (MBR) while the higher molecular weight compounds comprising the colour imparting melanoidin remained unaffected. As melanoidins are recalcitrant to biodegradation, the elimination of colored effluents in molasses-based distillery wastewater treatment system is mainly based on physical or chemical procedures such as adsorption, coagulation, precipitation, and oxidation. Although these methods are effective, they suffer from such short coming as requiring high reagent dosage, high cost, and formation of hazardous byproducts and intensive energy consumption.

Introduction

Wastewater at large from distilleries and fermentation industries are the major source of soil and aquatic contamination due to presence of water soluble recalcitrant coloring compounds called melanoidin (Evershed et al., 1997). Melanoidin are high molecular mass amino-carbonyl compounds. Due to their structural density, dark colour and unpleasant order, these pose serious risk to soil and aquatic eco system that release of melanoidin because increased load of recalcitrant organic material to water bodies. This then causes the problem, like reduction of sun light penetration, decreased photosynthetic activity and dissolved oxygen concentration whereas on land, it causes reduction in soil alkalinity and inhibition of seed germination. Further due to the possibility of complication reaction of introduced melanoidin with metal ions, they could influence the biogeochemical succession of many constituents in natural water (Chandra et al., 2008), which are highly resistant to microbial show aggression. Hence the wastewater requires pre treatment before its safe disposal in to the environment (Mohana et al., 2007; Kumar and Chandra, 2006).

Conventional biological processes such as activated sludge management process are insufficient to treat these melanoidin containing wastewater released from distilleries. Degradation and decolourization of these wastewater by chemical methods (Chandra and Singh, 1999), flocculation treatment and physicochemical treatment such as ozonation (Kim et al., 1998) and activated carbon adsorption have been accomplished, but these methods are not economically feasible on large scale due to cost limitation where as biological decolourization by using fungi such as Coriolus, Aspergillus, Phanerochaete and certain bacterial species such as Bacillus, Alkaligenes and Lactobacillus (Kumar and Chandra, 2006; Kumar et al., 1997; Ohmomo et al., 1987, 1985) have been successfully achieved and thus can be applied as a bioremediation technique.

Biological treatment methods-

Biological treatment of molasses wastewater is either aerobic or anaerobic but in most cases a combination of both is used. Aerobic treatment of anaerobically treated effluent using

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different microbial populations has also been explored. Majority of biological treatment technologies remove color by either absorbed the color into sludge or by partial or complete breakdown of the color molecules.

Anaerobic method-

The high organic content of molasses wastewater makes anaerobic treatment attractive in comparison to direct aerobic treatment. Anaerobic digestion is viewed as a complex ecosystem in which physiologically diverse groups of microorganisms operate and interact with each other in a symbiotic, synergistic, competitive or antagonistic association. Molasses wastewater treatment using anaerobic process is a very promising re-emerging technology which presents interesting advantages as compared to classical aerobic treatment. It produces very little sludge, requires less energy and can be successfully operated at high organic loading rates. Further, low nutrient requirements and stabilized sludge production are other associated benefits (Jimenez et al., 2003). However, the performance and treatment efficiency of anaerobic process can be influenced both by inoculum source and feed pretreatment. These processes have been sensitive to organic shock loadings, low pH and showed slow growth rate of anaerobic microbes resulting in longer hydraulic retention times (HRT).

Aerobic method-

Anaerobically treated distillery wastewater still contains high concentrations of organic pollutants and then cannot be discharged directly. The partially treated spent wash has high BOD, COD and suspended solids. It can reduce the availability of essential mineral nutrients by trapping them into immobilized organic forms, and may produce phytotoxic substances during decomposition. Stringent regulations on discharge of colored effluent impede direct discharge of anaerobically treated effluent (Nandy et al., 2002). Therefore, aerobic treatment of sugarcane molasses wastewater has been mainly attempted for the decolorization of the major colorant, melanoidins, and for reduction of the COD and BOD. A large number of microorganisms such as bacteria (pure and mixed culture), cyanobacteria, yeast and fungi have been isolated and are capable of degrading melanoidins and thus decolorizing the molasses wastewater.

Bacterial treatment: Pure bacterial culture for microbial treatment has been reported frequently in past and recent years. Under aerobic condition Bacillus sp. has been decolourize molasses wastewater upto 35.5% within 20 days at 55oC (Nakajima et al., 1999). A detailed list of bacteria tried by different researchers for decolourization of distillery effluent is given in Table 2. Kumar and Viswanathan (1991) isolated bacterial strains from sewage and acclimatized on increasing concentrations of distillery waste. These strains were able to reduce COD by 80% in 4-5 days without any aeration. The major products left after treatment were biomass, carbon dioxide and volatile acids. Dahiya et al. (2001a) isolated Pseudomonas fluorescens from reactor liquid and found that these bacterial strains are capable of decolourizing melanoidin wastewater upto 76% under nonsterile condition and upto 90% in sterile condition. The difference in decolourization might be due to the fact that melanoidin stability varies with pH and temperature. At higher temperature during sterilization melanoidin-pigments decompose to low molecular weight compounds (Ohmomo et al., 1988b). The pH of distillery spent wash increases from 4.5 to 8.5 during the anaerobic treatment process. Sirianuntapiboon et al., 2004; Benito et al., 1997). Ohmomo et al. (1988a) used calcium alginate immobilized cells of Lactobacillus hilgardi to decolourize melanoidin solution which resulted in 40% decolourization. It requires a small amount of oxygen continuously with limited aeration for the decolourization. Some researchers carried out melanoidin decolourization by using immobilized whole cells. Decolourization of molasses wastewater by immobilized cells of Pseudomonas fluorescence on porous cellular carrier was attempted achieving 76% decolourization in 24 hr at 30oC. Jain et al. (2002) isolated three bacterial strains from the activated sludge of a distillery effluent identified as a B. megaterium, B. cereus and B. fragariae which were found to remove colour and COD from the distillery effluent in the range of 38-58 and 55-68%, respectively. An Acetogenic strain was isolated by Sirianuntapiboon et al. (2004). from vegetables and juice samples which decolorizes molasses pigment medium and anaerobically treated distillery effluent to 73-76% within 5 days when supplemented with glucose and nitrogen sources. Patel et al. (2001) have reported 96, 81 and 26% decolourization of distillery effluent through bioflocculation by Oscillateria sp., Lyngbya sp. and Synechocystis sp. respectively. Pseudomonas, Enterobacter, Stenotrophomonas, Klebsilla and Acinetobacter, all of which carried out degradation of PMDE and maximum 44% COD reduction either singly or collectively A COD removal of 77% was achieved under non-optimal conditions. Marine cyanobacteria such as Oscillatoria boryna have also been reported to degrade melanoidin due to production of H2O2, hydroxyl, perhydroxyl and active oxygen radicals, resulting in the decolourization of the effluent (Kalavathi et al., 2001). Sirianuntapiboon et al. (2004) used an acetogenic bacterium to obtain a decolourization yield of 76.4% under optimal nutrient conditions. However, this value was only 7.3%, by using anaerobic pond. Also, it required sugar, especially glucose and fructose for decolourization of MSW. The decolourization activity might be due to a sugar oxidase.

Biocomposting method

Biocomposting is a process of activated bioconversion through the aerobic trail, whereby heterotrophic microorganisms act on carbonaceous materials depending on the availability of the organic source and the presence of inorganic materials fundamental for their augmentation. Composting is particularly effective in converting the wet resources to a usable form thereby stabilizing the organic materials and destroying the pathogenic organisms in addition to significant drying of the wet substrates. In the composting process, under aerobic conditions, thermophilic biodegradation of organic wastes at 40-60% moisture content occurs to form relatively stable, humus-like materials (Kannan and Upreti, 2008).

Phytoremediation approach

Phytoremediation of effluents is an promising low cost method for the exclusion of toxicants including metals from industrial effluent and is still in an tentative stage (Mohana et al., 2009). Kumar and Chandra (2004) successfully treated distillery effluent in a two stage process involving transformation of recalcitrant colouring components of the effluent by a bacterium Bacillus thuringiensis followed by subsequent reduction of remaining load of pollutants by a macrophyte Spirodela polyrrhiza. A similar biphasic treatment of the effluent was carried out in a constructed wetland with B. thuringiensis and Typha angustata by Chandra et al. (2008) which resulted in 98-99% BOD, COD and colour reduction after 7 days. Recently, macrophyte Potamogeton pectinatus was used for bioaccumulating heavy metals from distillery effluent (Singh et al., 2005). Trivedy and Nakate (2000) employed Typha

latipholia for distillery effluent treatment in a constructed wetland and found that the system resulted in 78 and 47% reduction in COD and BOD respectively in a period of 10 days. Increasing concentration of the effluent greatly reduced the biomass of the plant with maximum accumulation of Fe being recorded in plants growing in 100% effluent. Valderrama et al. (2002) reported 52% colour removal from distillery effluent when using a combined treatment with Lemna minuscula and Chlorella vulgaris. The micro algal treatment removed nutrients and organic matter from wastewater and produced oxygen for other organisms.

Cyanobacterial treatment-

Cyanobacteria are considered ideal for the treatment of distillery effluent as they, apart from degrading the polymers also oxygenated water bodies, thus reduce the BOD and COD levels (Mohana et al., 2009). Kalavathi et al. (2001) explored the possibility of using a marine cynobacterium for decolourization of distillery spent wash and its ability to use melanoidin as a carbon and nitrogen source. A marine filamentous, non heterocystous form Oscillatoria boryana used the recalcitrant biopolymer melanoidin as nitrogen and carbon source leading to decolourization. First the microalgal treatment led to removal of organic matter and further treatment with macrophytes removed other organic matter, colour and precipitated the microalgae.

Assorted consortium treatment:

All through preceding two decades, numerous attempts have been made to investigate the possibility of using cell immobilization in the technology of aerobic wastewater treatment (Fedrici, 1993; Sumino et al., 1985). Early experiments were restricted to the use of selected pure cultures immobilized on solid supports for the degradation of specific toxic compounds. Later, immobilized consortia of two or more selected strains were employed Kowalska et al., 1998; Zache and Rehm, 1989) but of late activated sludge has been immobilized on different carriers and used for wastewater treatment (Shah et al., 1998). Adikane et al. (2006) studied decolourization of molasses spent wash in absence of any additional carbon or nitrogen source using soil as inoculum. A decolourization of 69% was obtained using 10% (w/v) soil and 12.5% (v/v) MSW after 7 day incubation.

Fungal treatment:

Numerous basidiomycetes and ascomycetes type fungi have been used in the decolourization of natural and synthetic melanoidin in connection with colour reduction of wastewaters from distilleries. The fungus have capability to purify the effluent by consumption of organic substances, thus, reducing its COD and BOD, and at the same time to obtain some valuable product, such as fungal biomass for protein-rich animal feed or some specific fungal metabolite. In comparison to bacteria filamentous fungi have lower sensitivity to variations in temperature, pH, nutrients and aeration and have lower nucleic acid content in the biomass (Knapp et al., 2001). One of the most studied fungus having ability to degrade and decolourize distillery effluent is Aspergillus sps. such as Aspergillus fumigatus G-2-6, A. niger, A. niveus, A. fumigatus Ub²60 brought about an average of 69-75% decolourization along with 70-90% COD reduction (Ohmomo et al., 1987; Miranda et al., 1996; Jimnez et al., 2003). Jimnez et al. (2003) reported the treatment of distillery spent wash with ascomycetes group of fungi such as Penicillium sps. for example P. decumbens, P. lignorum resulted in about 50% reduction in colour and COD and 70% phenol removal. Sirianuntapiboon et al. (1995) studied that Rhizoctonia sp. D-90 decolourized molasses melanoidin medium and a synthetic melanoidin medium by 87.5 and 84.5% respectively, under experimental growth conditions. Electron microscopy revealed that the mycelia absorbed melanoidin pigment, which was in the form of electron dense material in the cytoplasm. However, melanoidin could be eluted from the mycelia by washing in a solution of NaOH and the relative amount of melanoidin eluted from the mycelia increased with increase in the concentration of NaOH. Kida et al. (1995) has investigated that Aspergillus awamori var. kawachi used for production of single cell protein from Japanese distillery (Shochu) wastewater after aerobic cultivation. The supernatant after cultivation could be anaerobically treated, at a high TOC loading rate, by the addition of Ni2+ and Co2+. Also, NH+4, accumulated in the anaerobically treated wastewater, was efficiently removed by utilization of residual volatile fatty acids (VFA) as electron donors during biological denitrification and nitrification and the residual organic matter could be removed simultaneously. Miranda et al. 1996) studied that Colour elimination from MSW by using Aspergillus niger and they found that under optimal nutrient concentration 83% of the total colour removed was eliminated biologically and 17% by adsorption on the mycelium. Benito et al. (1997) have investigated that anaerobically treated distillery effluent when supplemented with sucrose and inorganic N source has capability to decolourize by the Trametes versicolar. It was found that reduction in COD was 75% and decolourization was 80%. Under nutrient limiting conditions, fungal cells generally cannot remain active during a long-term cultivation.

Therefore, the continuous-culture method is not practical and the semi-batch or repeated-batch method can be an alternative for long-term cultivation. The immobilization of the fungus on a solid support is an appropriate means for controlling the thickness of the biofilm. The immobilization of the fungus offers advantages such as short retention time, easy recovery of the cells and increased activity. Furthermore, in the presence of the foam matrix, pellet size is restricted by the size and the physical properties of the foam (Kim and Shoda, 1999). Recently, Pant and Adholeya (2007a,b) isolated three fungal strains and identified them by molecular methods as Penicillium pinophilum TERI DB1, Alternaria gaisen TERI DB6 and Pleurotus florida EM 1303. These cultures were found to produce liglotic enzymes and decolourize the effluent upto 50, 47 and 86% respectively. Miyata et al. (2000) suggested an inhibitory effect of organic nitrogen on melanoidin decolourization by fungus Coriolus hirsutus. At the same time glucose was also required for enhancing decolourization as the peroxidases require H2O2, which is generated by glucose oxidation, to decolourize melanoidin. In another study it was reported that presence of additional nitrogen could not inhibit activity of fungus C. versicolour sp. no. 20 considerably, as significant decolourization and COD reduction occurred even in the absence of it (Chopra et al., 2004).

Physicochemical treatment-

After a multistage biological treatment of distillery spent wash, most of the organic load is removed. However, the brown colour does not disappear and may even increase due to repolymerization of the coloured components, melanoidin (Pena et al., 2003). Conventional anaerobic and aerobic treatment can accomplish degradation of the melanoidin up to only about 6-7%. Therefore, it is necessary to study about additional treatments required to decolourize distillery effluent (Pena et al., 2003).

Table 1. Bacteria employed for the decolourization of distillery effluent

	Name	Colour	Color removal %	Reference
	Xanthomonas fragariae	All the three strains needed glucose as carbon source and NH4Cl as nitrogen source. The decolourization efficiency of free cells was better than immobilized cells.	76	Jain et al., 2002
	Bacillus smithii	Decolourization occurred at 55oC in 20 days under anaerobic conditions in presence of peptone of yeast extract as supplemental nutrient. Strain ould not use MWW as sole carbon source	35.5	Kambe et al., 1999
	Lactobacillus hilgardii	Immobilized cells of the hetero fermentative lactic acid bacterium decolourized 40% of the melanoidin solution within 4 days aerobically	40	Ohmomo et at., 1988a
	Acetobacter acetii	The organism required sugar especially, glucose and fructose for decolourization of MSWs	76.4	Sirianuntapiboon et al.,2004
	Pseudomonas Fluorescens	The decolourization was obtained with cellulose carrier coated with collagen. Reuse of decolourized cells reduced the decolourization efficiency	94	Dahiya et al., 2001a
	Bacillus thuringiensis	Addition of 1% glucose as a supplementary carbon source was necessary	22,	Kumar and Chandra., 2006
	Pseudomonas aeruginosa	The three strains were part of a consortium which decolourized the anaerobically digested soebt wash in presence of basal salts and glucose	67	Mohana et al., 2007

Table 2. Fungi employed for the decolourization of Distillery effluent

	Name	Comments	Color removal %	References
	Phanerochaete chrysosporium	Free cells as well as calcium alginate immobilized cells decolourized the distillery effluent	85 (free) 59 (immobilized)	Fahy et al., 1997
	Phanerochaete chrysosporium	Both the fungi required a readily available carbon source for melanoidin decolourization while nitrogen source has no effect. Maximum decolourization was observed in 6.2% spent was.	53.5	Kumar et al., 1998
	Trametes versicolour	Anaerobically treated distillery effluent supplemented with sucrose and inorganic nitrogen sources was decolourized by the culture in shake flask studies	80	Benito et al., 1997
	Penicillium sp.	All fungi produced decolourization from first day of incubation, with maximum being show by P.decumbens at forth day with a reduction of 70% of the phenolic content of the wastewater	30	Jimnez et al., 2003
	Aspergillus niger UM2	Decolourization was more by immobilized fungus and it was able to decolourize up to 50% of initial effluents concentrations.	80	Patil et al., 2003
	Corilus hirsutus	Synthetic as well as wastewater melanoidin was and decolourized by the fungus in a median containing glucose and peptone.	80	Miyata et al., 1998 Miyata et al., 2000
	Flavodon flavus	Distillery effluents are decolourized using these marine basidiomycetes in presence of 5% glucose.	80	Raghukumar and Rivonkar 2001; Raghukumar et al., 2004
	Coriolus versicolour	The cultures were incubated along with cotton stalks in vinasses media in static condition. No synthetic carbon and nitrogen sources were used	63	Kahraman and Yesilada, 2003
	Aspergillus - UB2	This was with diluted wastewater with optimum values of supplemented materials.	75	Shayegan et al., 2005
	Pleurotus florida Eger EM1303	Hydroponically treated distillery effluent was subjected for treatment by fungus	86.3	Pant and Adholeya, 2009

Majority of these methods remove colour by either concentrating the colour into sludge or by partial or complete breakdown of the colour molecules.

Adsorption:

Among the physicochemical treatment methods, adsorption on activated carbon (AC) is widely employed for removal of colour and specific organic pollutants. Activated carbon is a well known adsorbent due to its extended surface area, microporous structure, high adsorption capacity and high degree of surface reactivity. Previous studies on decolourization of distillery spent wash include adsorption on commercial as well as indigenously prepared activated carbons (Satyawali and Balakrishanan 2008a,b). Bernardo et al. (1997) investigated decolourization of synthetic melanoidin using commercially available activated carbon as well as activated carbon produced by sugarcane bagasse. Chandra and Pandey (2000) observed that significant decolourization was achieved when used packed bed on anaerobically treated spent wash using commercial activated charcoal with a surface area of 1400 m^2 g^{-1}. Almost complete decolourization (> 99%) was obtained with 70% of the diluted sample.

Coagulation and flocculation:

Coagulation is the decay of colloids by neutralizing the forces that keep them apart. Cationic coagulants provide positive electric charges to reduce the negative charge (zeta potential) of the colloids. As a result, the particles collide to form larger

particles (flocs). Flocculation is the action of polymers to form bridges between the flocs, and bind the particles into large agglomerates or clumps. Bridging occurs when segments of the polymer chain adsorb on different particles and help particles aggregate. Generally coagulation seems to be an expensive step taking into account expenses of chemicals and sludge disposal. Thus, there is a need for development of low cost alternatives for post biomethanated effluent. Migo et al. (1993) used a commercial inorganic flocculant, a polymer of ferric hydroxysulphate for the treatment of molasses wastewater. The treatment resulted in around 87% decolourization for biodigested effluent. These findings have been in disaggrement with those of Inanc et al. (1999) who reported that coagulation with alum and iron salts was not effective for colour removal. They explored lime and ozone treatment with anaerobically digested effluent. The optimum dosages of lime was found to be 10 g l-1 resulting in 82.5% COD removal and 67.6% reduction in colour in a 30 min period. Later FeCl3 and AlCl3 were tested for decolourization of biodigested effluent and showed similar removal efficiencies, about 93% reduction in colour and 76% reduction in total organic carbon (Sowmeyan and Swaminathan, 2008).

Oxidation processes:

Ozone is a powerful oxidant for wastewater treatment, dissolved in water, ozone reacts with a great number of organic compounds in two different ways: by direct oxidation as molecular ozone or by indirect reaction through formation of secondary oxidants like free radical species, in particular the hydroxyl radicals. Equally ozone and hydroxyl radicals are strong oxidants and are capable of oxidizing a number of compounds (Bes-Pia et al., 2003). Ozone destroys hazardous organic contaminants and that have been applied for the treatment of dyes, phenolics, pesticides, etc. (Pena et al., 2003). The Fenton's oxidation technology is based on the production of hydroxyl radicals •OH, which have an extremely high oxidation potential. Fenton's reagent, which involves homogeneous reaction and is environmentally acceptable, is a mixture of hydrogen peroxide and iron salts (Fe2+ or Fe3+) which produces hydroxyl radicals which ultimately leads to decolourization of the effluent (Pala and Erden, 2005).

In broad-spectrum a biological management employing fungi and bacteria have been investigated essentially for decolourize the distillery spent wash. The microbial decolourization is an environment- friendly and cost viable alternative to chemical putrefaction process. Most favorable microbial activities and optimum results are found when effluent is supplemented with additional nutrients as well as diluting the effluent. So it is felt that the ideal cost effective and commercial treatment scheme should comprise of physico-chemical treatment followed by biological management.

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