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## Color removal of distillery effluent by immobilized cells on artificial soil

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### ABSTRACT

The Melanoidin Pigment (MP) is hardly decolorized due to recalcitrant as well as typical inhibitory effect of microbes available in treatment process. Therefore any discharge of treated effluent (after secondary treatment) can cause nuisance not only to natural surface water but also to ground water quality. Because, the available technologies are highly energy intensive, costly, land oriented and can not give any guarantee to full proof system, the development of the low cost technology with some Return on Investment (ROI) is realized, so that distilleries could be attracted and accept the technology. Qualitative studies of color removal of distillery effluent were carried out continuously in packed bed reactor to screen out the suitable combination of artificial soil. The continuous reactor (voidage:0.39-0.4, packed density:1.01-1.02kg/L) studies enable to remove color and Chemical Oxygen Demand (COD) degradation of the effluent (after secondary treatment with COD 22000-23000mg/L) to the tune of 97-99% and 96-97% respectively for the period of more than 10 days. Formulations of artificial soil, kinetic data, void age, space velocity were important parameters useful in designing 10 KLPD pilot plant. © 2009 Trade Science Inc. - INDIA

### KEYWORDS

COD;  
Capillary sepage system;  
Immobilized Cells;  
Melanoidin;  
Packed density;  
Voidage.

### INTRODUCTION

The treatment of distillery waste is a gigantic problem in terms of quality and quantity. In India, there are more than three hundred distilleries having installed capacity of  $32 \times 10^7$  L. They discharge huge volume of wastewater at the rate of 8-15L/L of rectified spirit. The wastewater consisted of not only high COD and Biochemical Oxygen Demand (BOD) values but also high color substances as melanoidin. With the available tech-

nology of wastewater treatment, the treated waste can not meet the prescribed norms of 30 or 100mg/L set by Central Pollution Control Board (CPCB) and Ministry of Environment and Forest (MOEF), India. Moreover, the Melanoidin Pigment (MP) is hardly decolorized due to recalcitrant as well as typical inhibitory effect of microbes available in treatment process<sup>[7]</sup>.

In India, researcher have demonstrated the ability of aquatic macrophytes (either single or two-three aquatic plant system) in reducing the level of toxic met-

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als of polluted water<sup>[2-4]</sup>. In the global scenario, scientists have tackled the problem of decolorisation in laboratory scale by chemical precipitation, chemical adsorption or combined biological and chemical processes<sup>[6,8]</sup>. In the industrial sector, virtually no information is available except the pilot plant (10m<sup>3</sup>/day) operated for two years at M/s Ashoka Alcho-Chem Ltd, Maharashtra, India with natural soil capillary seepage trench system, with technical guidance of M/s Somtech Co.Ltd, Japan<sup>[9]</sup>. The COD of seepage water from the system was around 350-400mg/L with decolorisation efficiency around 99%. This water was recycled back to the bed for irrigation of various crops (sunflower, mustard, cane etc) and do not need to draw fresh water input. However in India, it is observed that a wide variety of top soils, other than black cotton is unsuitable for this technology. So formulation of artificial soil having adequate properties (high water retention and base changing capacity) of Maharashtra, India soil was realized. In addition to that, color removal efficiency by combined chemical and biological process still has disadvantages due to the high operating cost, high consumption of chemical agents and large volume of generated solid waste etc. Under this context the development of a new technology was realized on the principle of whole cell immobilization coupled with capillary seepage trench system on artificial soil which would perform identically in color removal comparable to natural soil and also can give some guarantee for a low cost technology with some Return on Investment (ROI) from the cultivated crops. Objectives of this paper (first in the series) are on formulation of artificial soil, cell immobilization on it, reactor studies on suitability of color removal and COD degradation and operational stability of the system.

### MATERIALS AND METHODS

#### Organism and medium

A mixed culture population was isolated (not identified) from the soil of Maharashtra and Bundelkhand region of Uttar Pradesh, India, having high water retention capacity as well as base exchange capacity by acclimatization with effluent.

For medium, 3.5% molasses solution was used for the cultivation and maintenance.

#### Artificial soil preparation

Artificial soil consists of five components and traces of inorganic salts. The composition of artificial soil for the study is natural Balrampur soil (35%), river sand (25%), compost of mixed culture on lignocellulosics (15%), lignocellulosic derivative-1 (15%), lignocellulosic derivative-2 (10%) and traces of inorganic salts.

#### Cell immobilization

Although mixed culture compost contains highly dense population of microbes in the artificial soil but prior to laboratory studies in columns, active culture (in stationary phase) isolates was passed continuously for 2 days to activate the columns to attain cell numbers in the range of  $2.5-3 \times 10^{10}$  per ml.

#### Continuous experiments

A mild steel column (1.1m height, 5.25cm internal diameter, having five different ports at 30,50,70,90 and 110cm high) was employed for the studies. Effluent of different COD values (9500-22000mg/L) after secondary treatment (removing suspended solids after passing through 30cm deep sand bed) was pumped through the top of the column to maintain constant level in the column. Samples were drawn in every four hour interval from the sampling ports to assess the color removal and COD degradation. The residence time in the column was maintained at 12hours. A steady state was assumed when the color removal and COD degradation leveled off as evidence by four successive samples assayed.

#### Batch experiments

Qualitative studies of suspended cells were carried out in 500 ml conical flask containing 200 ml effluent (COD ranging 9500-22000mg/L) inoculated with 10% inoculum and incubated at 30°C for six days. After incubation, degradation of COD and decolorisation of effluent were computed.

#### Analytical procedure

COD was measured as per the standard methods for the examination of water and wastewater (APHA,

1995). Estimation of the color intensity of effluent was carried out by diluting the sample with 0.1 M acetate buffer solution, pH 6 after being centrifuged at 6000xg for fifteen minutes. The extent of color removal of the diluted solution was measured at 475nm. The percentage of color removal was calculated as the color intensity of decolorized sample against that of original effluent. The removal yield was expressed as the degree of decrease in the absorbency at 475nm (pastel yellow color) against the initial absorbency (dark brown color) at the same wave length. This is further quantified from the standard curve drawn against COD and optical density of decolorized samples.

Cell counts were computed by colony counter after developing the colonies of the properly diluted samples spread on nutrient agar plates.

**RESULTS AND DISCUSSION**

**Batch studies**

The extent of COD degradation and color removal of suspended cells are represented in TABLE 1. From the table it is clear that for lower strength of effluent (COD 5000-5500mg/L) although COD degradation is marginal (15-18%) but color removal is in the tune of 45-50%. But in case of high strength effluent (20000-22000mg/L) the COD reduction and color removal were as low as 8-10% and 20% respectively.

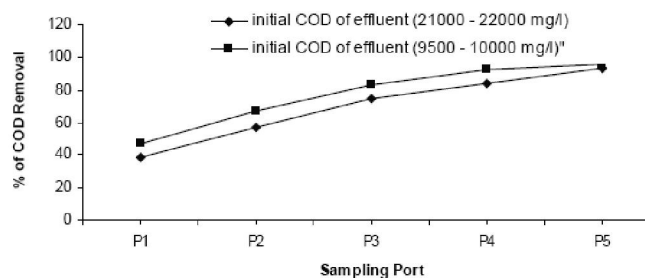
**Continuous reactor studies**

**Point to point analysis of color removal and COD degradation along the height of column reactor**

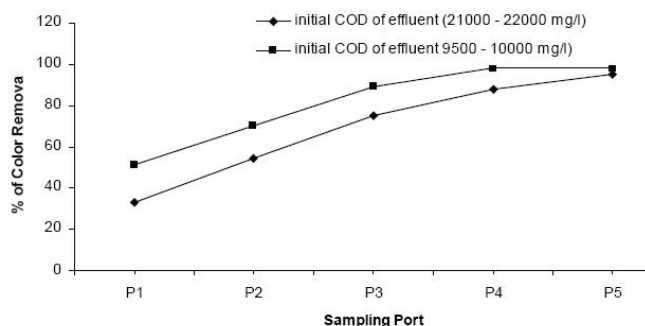
Point to point analysis of COD degradation and color removal were monitored in the reactor employing immobilized artificial soil with different strength of effluent (COD 10000-22000mg/L). The objective of this work was for better understanding of the system at different sections of the reactor and its thorough analysis to establish the kinetic pattern and to asses minimum depth of the bed required for scaling up the data in pilot plant or commercial scale. Figure 1 and 2 show that under the condition of operation (mentioned in the figure) around 77-79% of reactor volume was sufficient to attain COD degradation and color removal to the extent of 82-92% and 92-96% respectively. Balance

**TABLE 1 : COD reduction and color removal of distillery effluent by suspended cells**

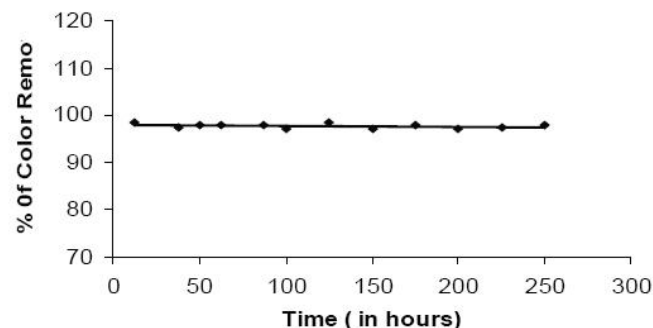
COD of effluent (mg/L)	Color removal (%)	COD reduction (%)
5000-5,500	50	15-18
10,000-12,000	40	15-18
20,000-22,000	20	8-10



**Figure 1 : COD removal of effluent at various sections of the reactor. Voidage : 0.39-0.4, Packed density (kg/L): 1.03, Linear velocity(cm/h): 1.98-2.01, Temperature (°C):30**



**Figure 2 : Color removal of effluent at various sections of the reactor. Voidage : 0.39-0.4, Packed density (kg/L): 1.03, Linear velocity(cm/h): 1.98-2.1, Temperature (°C):30**



**Figure 3 : Operational stability of immobilized cell reactor. Residence time (h) : 12, Temperature (°C): 30, Initial COD of effluent (mg/L): 21000- 22000**

20-30% of reactor volume is essential as polishing reactor to attain final COD of seepage water in the range of 300-500 mg/L and color removal of 97-99% depending on the strength of the effluent.

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### Operational stability

The reactor performance was found to be steady for more than 10 days and required no cell input. Figure 3 shows the pattern of color removal to the extent of 97-98% constantly for the input effluent (COD 21000-22000mg/L) fed to the reactor at 30°C. Continuous removal of color might be due to combined effect of melanoidinase activity of cells (can be confirmed from suspended cell data) and adsorbing effect of some color substances on artificial soil. Microbial decolorisation of melanoidins is due to successive decomposition mechanisms, that is the smaller molecular weight melanoidins are firstly attacked and the larger molecular weight melanoidin are finally attacked is supported from the work of Ohmomo<sup>[5]</sup>.

### CONCLUSIONS

In recent years considerable interest in removal of melanoidin pigments present in various types of effluents has stipulated several kind of research on improving clean environment and eco-system. Continuous decolorisation of distillery effluent using immobilized whole cells is one of them. In the present study it was possible to remove color and COD of effluent to the extent of 97-99% and 95-97% respectively for a period of more than ten days through whole cell immobilization on artificial soil by capillary seepage system.

### ACKNOWLEDGEMENT

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