Studies On Immobilized Yeast For Decolorization Of Azo Dyes

Dastagir, Sindhu Sameera, P. Naga Padma

Abstract: Azo dyes that are extensively used in textile industries are recalcitrant xenobiotic compounds causing a serious damage to the environment. Degradation of azo dyes being a necessity diverse dye dumped soils were screened for dye degrading yeasts. One efficient yeast isolate capable of decolorizing both mono-azo dyes and di-azo dyes was selected, immobilized by entrapment method and studied for decolorization efficiency. The isolate was morphologically, culturally and biochemically identified as Saccharomyces sp. Sodium alginate entrapped yeast cells were studied for decolorization of commercially available textile dyes having azo groups. The extent of colour removal in the culture medium was assessed through the decrease in dye absorbance of the supernatants. The immobilized yeast's decolorization abilities were different and varied with inoculum size, incubation time and nutrients like carbon and nitrogen sources. The fermentation conditions like pH, temperature and aeration had no profound effect. The decolorization ranged from 70-100%. Complete decolorization was seen within 12 and 18 hrs depending on inoculum size. The immobilized beads with yeasts could be reused efficiently for three cycles. The dye degrading immobilized Saccharomyces isolate could be commercially significant as it is efficiently degrading both mono-azo and di-azo dyes, different dye waste waters in a semi-continuous manner and so could also be used for detoxification of industrial effluents.

Key words: Azo dyes, Mono-azo dye, yeasts, immobilization, dye decolorization.

1 INTRODUCTION:

Azo dyes represent the largest group of dyes used in the textile industry. They are characterized by the presence of one or more azo groups -N=N-, which are responsible for their coloration, recalcitrant nature and hence are less biodegradable [1]. Over 10% of the dye used in textile processing does not bind to the fibers and is therefore released to the environment. The percentage of unchanged dye after such treatments lost to waste waters more than 50%[2][2]. Some of these compounds pose a serious threat because of their carcinogenic potential or cytotoxicity [3]. Currently the major methods of textile waste water treatment involve physical and chemical process [4]. These methods usually involve complicated procedures which are economically unfeasible. Microbial decolorization and degradation is an alternative approach which is both environment friendly and cost effective [3], [5]. Most studies on azo dye microbial biodegradation have focused on bacteria and fungi [6], [7]. Especially anaerobic bacterial species isolated from the intestinal micro flora [8]. Due to the inherent drawbacks of physical, chemical and photochemical approaches to dye removal, the use of biological methods for the treatment of textile waste waters has received attention as a more cost effective alternative [4], [9], [10]. Anaerobic microbial wastewater treatment can be very effective in removing color, primarily by the activity of azo reductases that cleave the azo bond yielding the corresponding amines, which are frequently toxic, mutagenic and carcinogenic and resist further degradation under anaerobic conditions [4], [11]. Bacteria, yeasts and molds are capable of aerobic decolorization and mineralization of dyes [12], [13], & [14].

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The present study concentrated on degradation of Azo dyes by an efficient isolated yeast culture. Degrading yeast was immobilized by entrapment method and used for dye degradation studies, It showed nearly more than 90% decolorization ability for mono-azo dyes and nearly 50-60% for di-azo dyes. The co-metabolic role of carbon source was also studied along with effect of nitrogen source on decolorization. The isolate also responded well to dye waste water decolorization. Thus the present potential yeast isolate could be used efficiently for dye waste water treatment in semi-continuous manner.

2 MATERIALS AND METHODS:

2.1 MICROORGANISM:

Yeasts isolated from dye dumped soils were maintained on YEPD slants in refrigerator.

2.2 INOCULUM PREPARATION:

Yeast cells were scrapped from YEPD slants to water suspension and then used as inoculum at a concentration of 10^{6-8} cells/ml.

2.3 CULTURE IMMOBILIZATION:

Yeast cells at a concentration of 10^6-10^8 CFU/ml were taken, added to sodium alginate which was then used to prepare beads. The size of beads varied in two ranges 0.002 and 0.02 cu.sq.mm (Bead size was standardized using syringes).

2.4 FERMENTATION CONDITIONS:

The number of immobilized beads used in a fixed volume of medium (25 beads for 25 ml) were used to study the effect of pH of medium, incubation temperature and inoculum size and incubation time. The experiments were carried out in 250 ml flasks.

2.5 DECOLORIZATION ASSAY:

Different dyes and dye waste waters were tested for decolorization which was read spectrophotometrically at 540nm. Formula used was:

% Decolorization = {(O.D i – O.D r) / O.D i}*100

Where O.D i – initial concentration & O.D r – resultant concentration of dye

2.6 EFFECT OF CARBON & NITROGEN SOURCE ON DECOLORIZATION:

Decolorization response of immobilized yeast to glucose and yeast extract in media was tested. The culture was also tested for alcohol production and also amine production during dye decolorization.

4 RESULTS:

The yeast isolate, isolated from dye dumped soil by enrichment culture technique was immobilized by entrapment method in sodium alginate. This was identified by microscopic and biochemical studies as belonging to genus *Sachharomyces sp*, though it showed similarity to both *cerevisiae* species and *italicus* species it was not identified till species level (Table 1). The selected isolate showed good decolorization ability for both mono azo dyes like methyl red and di-azo dye like tryphan blue at different concentrations. The percentage decolorization ranged from 70-80% for methyl red and 65-75% for tryphan blue (Figure 1) of dyes. The isolate showed good response to immobilization as decolorization increased from 80% to 100% (Figure 2). A bead size of 0.02 cu.sq,mm and an inoculum size of 10^6 cells/ml showed 100% decolorization (Figure 2). The decolorization increased with incubation time and maximum decolorization was till 24 hours only for methyl red, red dye and red dye waste water (Figure 3). It produced alcohol which could enhance decolorization. The dye decolorized medium and dye waste waters did not show any presence of amine (Table 2). The beneficiary aspect of the immobilization is that the beads could be recycled. The beads were recycled four times comparatively the 1^{st} cycle showed maximum decolorization within 24 hrs, the percentage decolourization of the dyes gradually decreased with the increase in the number of cycles (Figure 4). The effect of simple carbon source (co-metabolite) like glucose and nitrogen source like yeast extract on decolorization of methyl red (Figure 5) and red dye waste water (Figure 6) was studied to ascertain the decolorization efficiency of the isolate under study. The response of the isolate was good with 100% decolorization even both in presence and absence of the cometabolites (Figures 5 & 6).

5 DISCUSSION:

There are reports of azo dye decolorization by bacteria, molds and yeasts by both aerobic and anaerobic process [11], [13], & [14]. This prompted screening of an efficient strain from dye dumped soils and so an efficient isolate was obtained from dye dumped soil. The isolate could show decolorization with increased concentration of both mono and di-azo dyes (Figure 1) as it could have been acclimatized to the dyes in the dye dumped soil. This could also be the reason for 100% decolorization ability by 24 hours for the yeast isolate under study as indicated in (Figure3.). Decolorization efficiency due to acclimatization is in concurrence with earlier reports [15]. The present isolate responded positively to immobilization and decolorization increased from initial 80% to 100% (Figure 2). Bacteria are known to be metabolize efficiently as whole cells by adsorption of azo dyes [16] and also degrade azo dyes by immobilization as a consortium rather than single cultures [17], [18]. The present yeast isolate is efficient in decolorization of azo dyes in immobilized state, did not produce amines on dye degradation and produced alcohol that could enhance decolorization. The decolorization was efficient till three recycles of decolorization by the immobilized cells. These characters could make the isolate a potential azo dye degrader that could be exploited for decolorization azo dye waste waters. The dye decolorized with same efficiency both in presence and absence of cometabolites like glucose or yeast extract which is a unique feature that can enhance dye degradation and hence used for bioremediation of dye contaminated water.

6 ACKNOWLEDGEMENT:

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7 TABLES:

Table 1: Microscopic and Biochemical identification of yeast isolate.

Character	*Saccharo -myces italicus*	*Saccharo-myces cerevisiae*	*Isolate*
Colony Morphology	Flat, cream to tannish cream in color	Pale cream & smooth with regular edge	Flat cream colony with regular edge
Microscopic-Presence of Pseudomyceliu m	Absent	Absent	Absent
Shape of the cell	Oval	Spherical	Oval
Fermentation of Glucose	+	+	+
Galactose	+	+	+
Sucrose	+	+	+
Maltose	+	+	+
Lactose	-	-	-
Nitrate utilization	-	-	-
Urease production	+	+	+
Alcohol production	+	+	+

Table 2: Yeast isolates fermentation response in terms of alcohol and amine production

Character	12 Hours	18 Hours	24 Hours
Growth	+	++	++
Alcohol production	+	++	++
Amine production	-	-	-
Decolorization	+	++	++
pH	5	4	4

8 FIGURES:

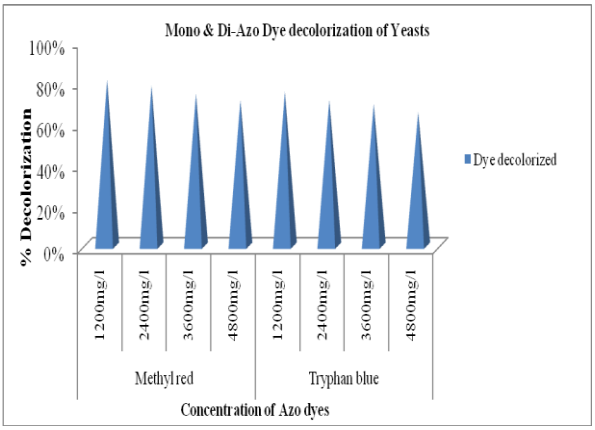

Figure 1: Decolorization of different concentrations of mono azo and di azo dyes by yeast isolate.

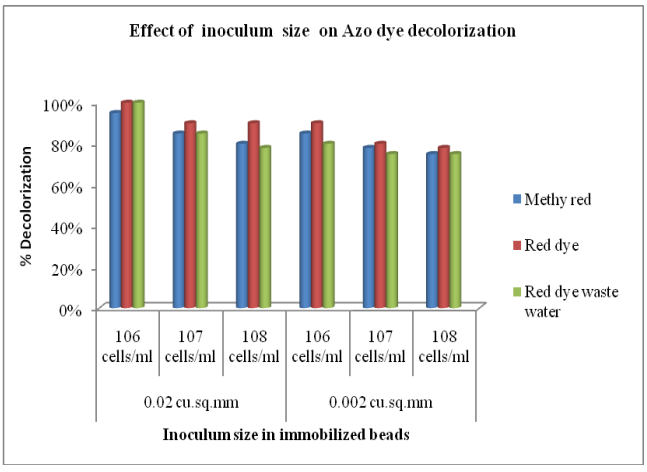

Figure 2: Dye decolorization by immobilized yeast isolate at different inoculum levels.

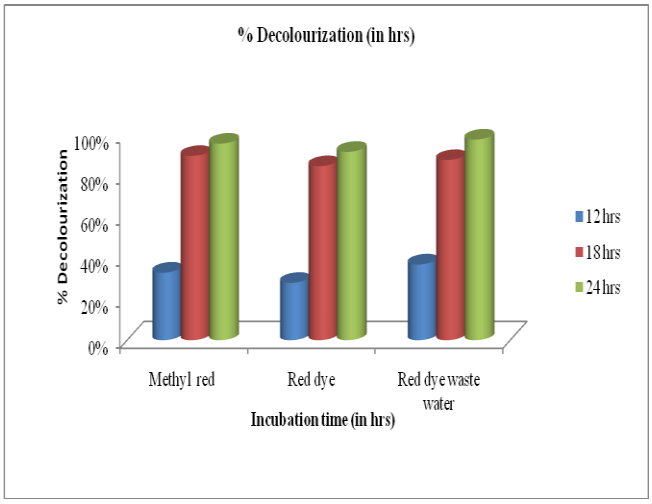

Figure 3: Effect of incubation time on Dye decolorization by immobilized yeast .

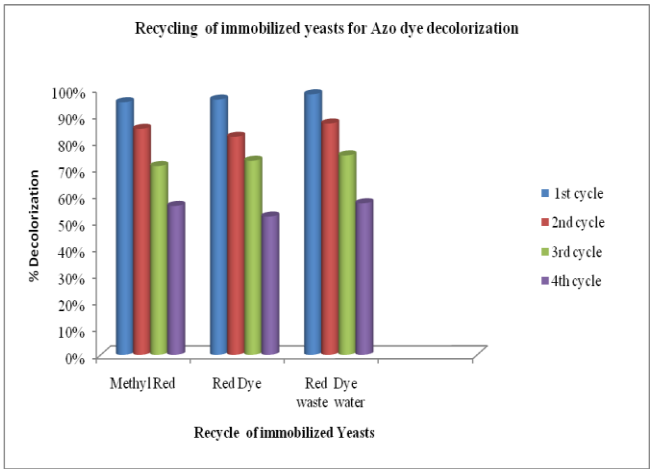

Figure 4: Azo dye decolorization by recycled immobilized yeasts.

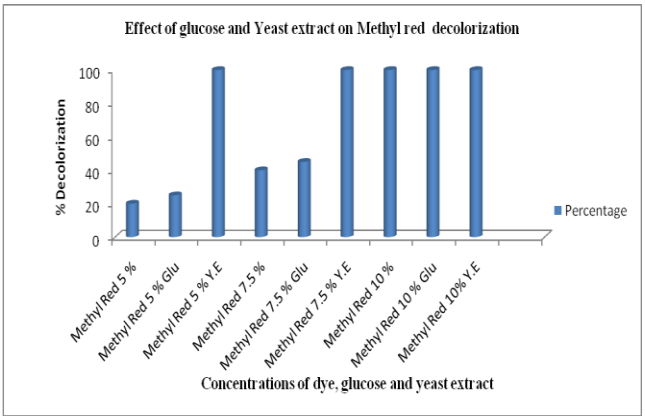

Figure 5: Effect of glucose and Yeast extract on Methyl red decolorization by immobilized yeast.

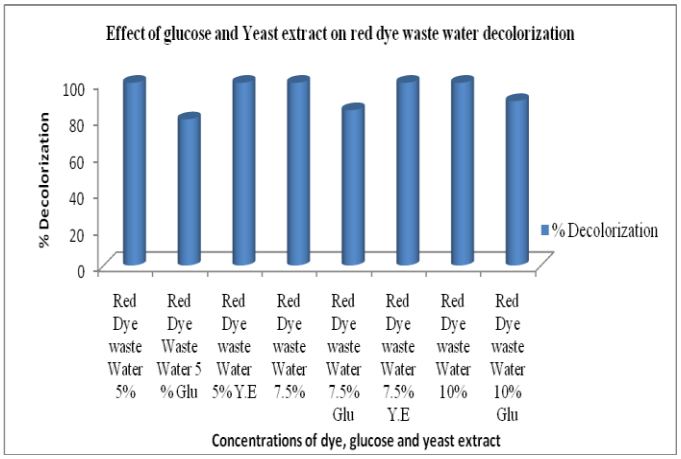

Figure 6: Effect of glucose and Yeast extract on decolorization of red dye waste waters by immobilized yeast

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