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Removal of Orange-II dye from water by adsorption on enzyme immobilized and sodium alginate supported layered double hydroxides

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ABSTRACT

Wastewater from various industries contains noticeable amounts of azo dyes which are unfortunately not efficiently removed using conventional wastewater treatment. The chemical Aniline, the basis for the Azo dyes (specifically group III A1 and A2) are considered to be poisonous giving off carcinogenic amines. Biosorption can be considered as an emerging green, cost-effective, and efficient alternative technology for removal of such dyes from wastewater. In the present study, peroxidase and alpha amylase enzymes were immobilized on sodium alginate incorporated Zinc-Aluminum LDH and tested for the removal of orange-II dye from water. The adsorption capacity of the prepared biosorbents was measured. The equilibrium adsorption capacity(q_t) for Alpha-amylase immobilized Sodium alginate incorporated Zinc-Aluminum LDH (ZA-NA-Amy LDH) was found to be 1.78 mmol/g and that for peroxidase immobilized Sodium alginate incorporated Zinc-Aluminum LDH (ZA-NA-HRP LDH) was found to be 1.00 mmol/g. The composites were found to be more effective than just enzymes immobilized LDHs.

Keywords: Biosorption, wastewater, Amylase, peroxidase.

INTRODUCTION

Wastewater from various industries contains noticeable amounts of dyes which are then discharged in various water bodies causing serious environment threat. The organic dyes are soluble and are known to decrease the levels of dissolved oxygen in water affecting the entire aquatic flora and fauna (Lellis *et al*, 2019). The chemicals used as dyes are often highly toxic and carcinogenic (Khatri *et al*., 2018). Mainly the azo dyes are persistent in the environment and causes biomagnification through the food chain. Orange II is a sulfonated dye that is used in pharmaceuticals, cosmetic and textile industries as colourants (Marmion, 1991). Most of the methods employed for removal of the contaminants from waste water are inefficient and are costly (Fernando *et al*., 2018).

So, it is of paramount importance to develop environment friendly cheaper technology for removal of azo dyes from water. Biomaterials can provide sustainable solution for this problem. Biosorption can be considered as an eco-friendly cost-effective approach for removal of contaminants like dyes from water (Fernando *et al*., 2018). Many researchers have studied the application of layered double hydroxides (LDH) as sorbent materials for removal of contaminants from water (Johnston, *et al.* 2021). They are synthetic inorganic clay with positively charged layers and charge balancing anions in the interlayer region. They can be prepared in aqueous medium from low cost precursors and can be easily regenerated. Studies have also shown that immobilized enzymes could prove to be a better alternative at industrial scale under harsh conditions increasing the productivity of the entire process (Zdarta *et al*, 2021). In the present study, peroxidase and alpha amylase enzymes were immobilized on sodium alginate incorporated Zinc-Aluminum LDH and tested for the removal of orange-II dye from water.

MATERIAL AND METHODS

Synthesis of LDH: Aluminum chloride and zinc chloride is co-precipitation in 2 M NaOH solution at 60 ^{0}C at pH of 9.5± 0.2. Precipitate formed was aged for 12 h at the same temperature with continuous stirring and repeated washing with distilled water and centrifuged at 9000 rpm for 15min. It is dried at 60° C for about 24 h (Mandal et.al., 2008).

Synthesis of Sodium alginate incorporated LDHs: Sodium alginate was added initially to 100 ml distilled water at 60 °C. Sodium alginate - water suspension is formed. Zn/AL LDH were prepared by following the same co-precipitation procedure as described earlier.

Enzyme immobilization: 5g of each LDH was taken in separately clean and dry beakers. Each sample was dipped in 10 ml of enzyme solution separately (Alpha-amylase and Peroxidase) and incubated at room temperature for 3 days. Samples were filtered using Whatman qualitative filter paper no1 and dried at room temperature. LDHs were crushed and weight of the samples were taken before and after immobilization. Alpha-amylase immobilized Sodium alginate incorporated Zinc-Aluminum LDH (ZA-NA-Amy LDH) and Peroxidase Immobilized Sodium alginate incorporated Zinc-Aluminum LDH (ZA-NA-HRP LDH) were synthesized.

Batch adsorption experiments: 50 ml of the dye solution was contacted with 0.2 g of the adsorbents taken in a stoppered conical flask in a thermostatic water-bath shaker at a constant shaking speed of 130 rpm for 24 h. 2ml of the samples are taken out after the time intervals of 5 min, 10 min, 20 min, 30min, 60 min, 120 min, 240 min, 360 min and 24 h. Samples were centrifuged in microcentrifuge at 10000 rpm for 10 mins. Dye concentrations were measured using UV-Visible spectrophotometer at a wavelength of 483 nm. The adsorption capacity was measured by the formula:

Adsorption capacity (Q_t) = (C_o – C_t) *V/(W*1000)
Were,

Q_t= Adsorption capacity at time‘t’ (mg/g)
C_o = Initial concentration (mg/l)
C_t = Concentration at time `t’ (mg/l)
V = Volume of the solution (ml)
W = Weight of the adsorbent (g)

Surface Area Determination by Gas Adsorption: 15 mg sample was taken in cell. Sample was degassed. Weight of sample was taken and sample tube is immersed in a coolant bath of liquid nitrogen. Total surface area was determined by Brunauer, Emmett and Teller (BET) analyzer. Surface area is expressed in meters^2 per gram. It is a measure of surface roughness as well as quantity and size distribution of open pores.

Stability Analysis: The Alpha-amylase and Peroxidase activities were determined periodically over a total duration of 63 days using standard methods (Fernandez *et al*, 2001, Alemzadeh *et al.* 2009). The initial enzyme activities were set as 100%, and the relative activities were defined as the ratio of the initial activities.

RESULTS

Weight of the LDHs before and after immobilization: The Zn/AL LDH were prepared by co-precipitation and then sodium alginate was incorporated Alpha-amylase

and Peroxidase was immobilized with Zn/AL LDH alone as well as after encapsulation with sodium alginate. The weights before and after immobilization was determined which is represented in table no. 1. The results showed increase in the weight after immobilization. Encapsulation of LDH in sodium alginate is a cost-effective approach for wastewater treatment as it stabilizes the LDH and protect it from acidic conditions. Moreover, as a result of the protective effect of the matrix, immobilized enzymes become more resistant to changes of environmental parameters such as temperature, pH or inhibitory effect of different compounds (Guzik *et al.* 2014).

Table No. 1: The comparative weights of LDHs before and after immobilization

No.	LDHs	Weight of LDHs(g) Alpha-amylase immobilization		Weight of LDHs(g) Peroxidase immobilization	
		Before immobilization	After immobilization	Before immobilization	After immobilization
1.	ZA LDH	5	6.01	5	5.98
2.	ZANA LDH	5	5.83	5	6.487

Table No 2: Equilibrium adsorption capacity of ZA LDH for adsorption of Orange-II dye

Surface area (m^2/g)	Equilibrium Time(min)	Equilibrium adsorption capacity(q_t)(mmol/g)
44.47	360	0.93

Table 3: Equilibrium adsorption capacity of only sodium alginate beads for adsorption of Orange-II dye

Surface area (m^2/g)	Equilibrium Time(min)	Equilibrium adsorption capacity (q_t)(mmol/g)
0.0	1440	0.1

Table 4: Equilibrium adsorption capacity of ZANA-Amylase LDH for adsorption of Orange-II dye

Surface area (m^2/g)	Equilibrium Time (min)	Equilibrium adsorption capacity(q_t)(mmol/g)
37.8	1440	1.78

Table 5: Equilibrium adsorption capacity of ZANA-HRP LDH for adsorption of Orange-II dye

Surface area (m^2/g)	Equilibrium Time (min)	Equilibrium adsorption capacity(q_t) (mmol/g)
45.2	1440	1.00

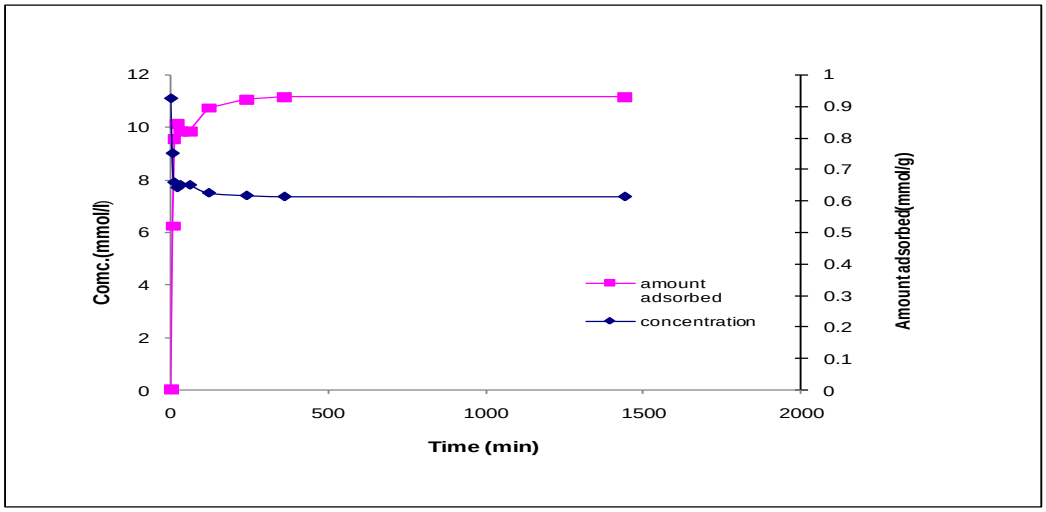

Fig 1: Concentration vs Time and Adsorption capacity vs Time Graphs for ZA LDH

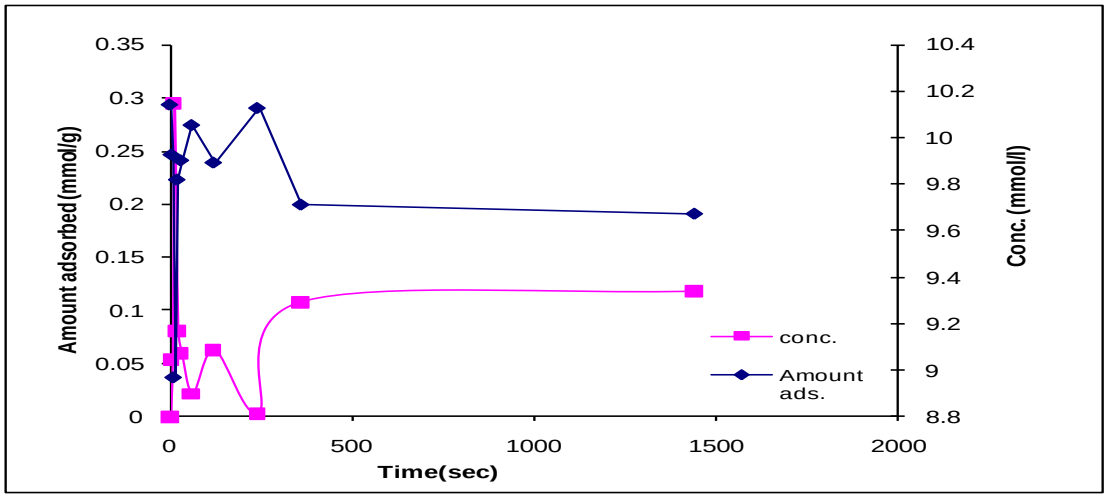

Fig 2: Concentration vs Time and Adsorption capacity vs Time Graphs for only sodium alginate beads

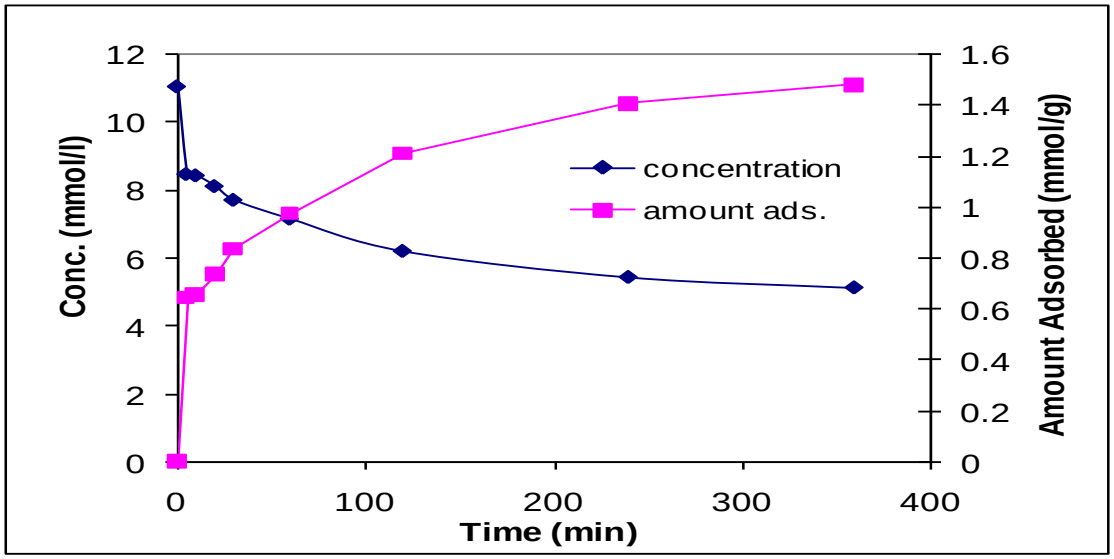

Fig 3: Concentration vs Time and Adsorption capacity vs Time Graphs for ZANA-Amylase LDH

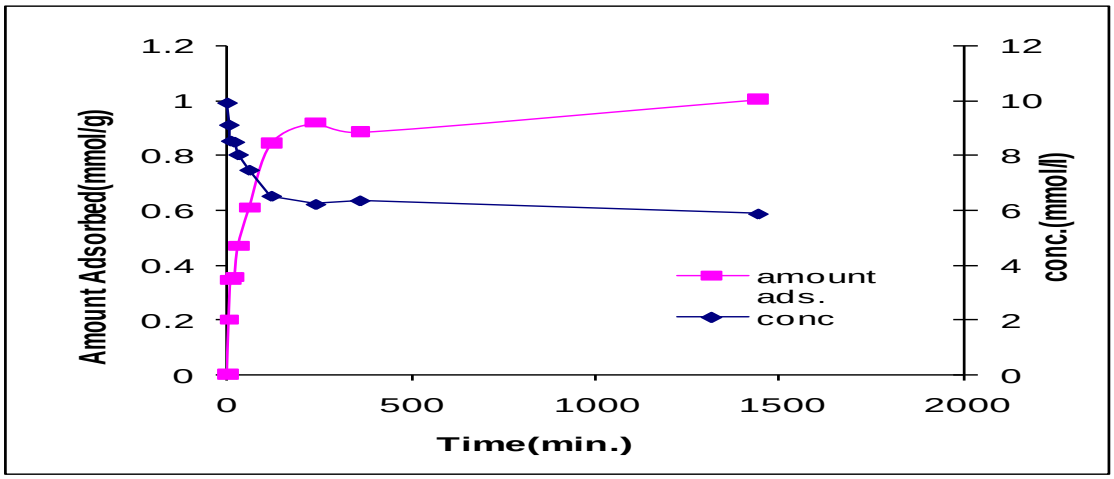

Fig 4: Concentration vs Time and Adsorption capacity vs Time Graphs for ZANA-HRP LDH

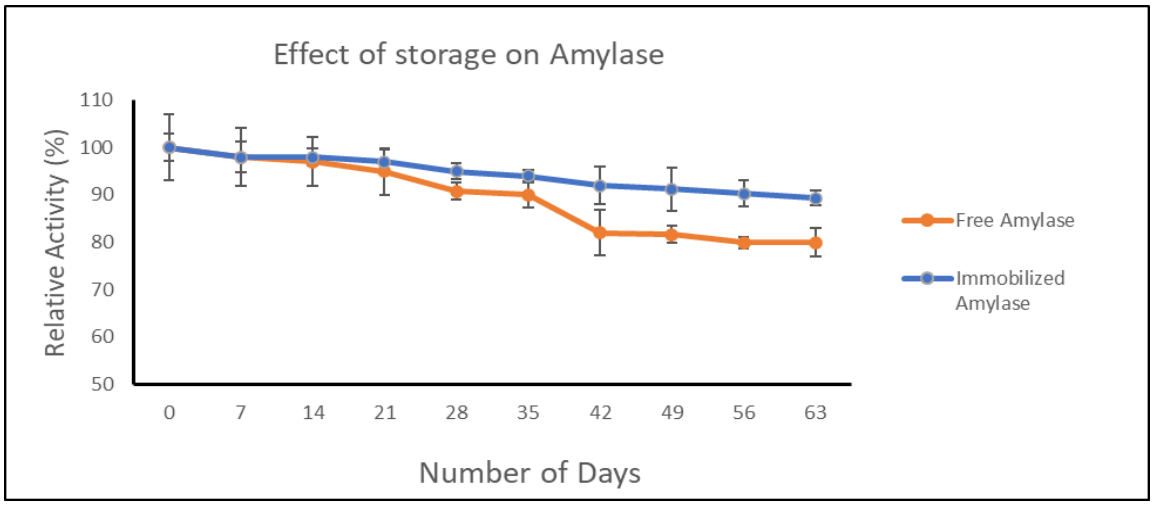

Fig 5: Effect of Storage on Free and Immobilized Amylase

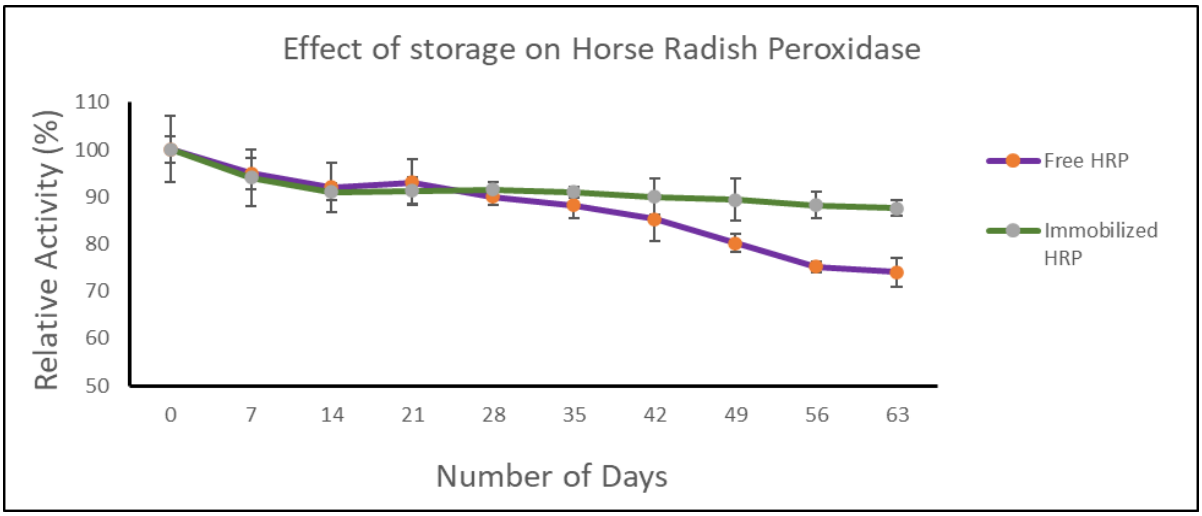

Fig 6: Effect of Storage on Free and Immobilized Horse Radish Peroxidase

Batch adsorption experiments: The kinetic data for the removal of orange II from water using ZA LDH, sodium alginate, ZANA-Amy LDH and ZANA-HRP LDH is presented in the figures 1, 2, 3 and 4. respectively. It revealed that the reaction reached equilibrium after 360 min of reaction time for LDH with is not encapsulated in sodium alginate whereas for all the materials encapsulated in sodium alginate with and without enzymes reached equilibrium after 1440 minutes. The results show that the adsorption capacity of the unsupported LDHs could be improved by supporting them on sodium alginate. The adsorption capacity of amylase immobilized Zn/Al LDH was 1.78 which was higher than peroxidase immobilized Zn/Al LDH which was found to be 1.

Stability Analysis: After storing the free enzymes and the immobilized enzymes, it was observed that the relative activity decreased from 100% which is represented in the Figures 5 and 6. The free enzymes, both amylase and HRP showed a higher decreased activity as compared to immobilized enzymes. These results are in accordance to studies by other investigators where encapsulated enzymes showed higher storage stability (Amid *et al.* 2014, Alemzadeh *et al.* 2009, Swarnalatha *et al.* 2013).

CONCLUSION

In this study, ZANA-Amy LDH and ZANA-HRP LDH were synthesized and used for orange II dye removal from

water. Results showed that blending of amylase and Horse radish peroxidase with the LDH-alginate beads significantly improved their stability as compared to the free enzymes. The results also indicated that the orage II dye removal removal reaction in ZANA-Amy LDH beads reached equilibrium at 1440 minutes post reaction with a adsorption capacity as compared to ZANA-HRP LDH. The adsorption study data suggest that the sodium alginate incorporated LDHs and enzyme immobilized LDHs can be an effective biocatalyst for the treatment of dye effluents. Optimizing the adsorption study to achieve most efficient adsorption by changing important variables such as different types of LDHs at the different temperatures can be further studied.

Conflicts of interest: The authors stated that no conflicts of interest.

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