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ORIGINAL PAPER

The accelerating effect and mechanism of a newly functional bio-carrier modified by redox mediators for the azo dyes decolorization

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Abstract In this study, a functional bio-carrier modified by redox meditors was developed as a redox mediator for application in azo dye decolorization processes. Its accelerating effect and mechanism for azo dyes decolorization were also examined. The decolorization rates of 10 azo dyes were enhanced about 1.5–3 fold by the functional bio-carrier modified with disperse turquoise blue S-GL, and the ORP value during the acid red GR decolorization process was changed to a more negative value of 20–25 mV. Non-dissolved redox mediator on the functional bio-carrier played a similar role as NADH during the azo dyes decolorization process. At the same time, the functional bio-carrier exhibited good reusability and the combinational technology of the redox mediator and bio-carrier was a great improvement of the redox mediator application and represents a new bio-treatment concept.

Keywords Mechanism · Functional bio-carrier · Redox mediators · Decolorization

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Introduction

Azo dye, aromatic moieties linked together by azo bond (–N=N–), is the largest class of dyes used in the textile, cosmetic, printing, drug and food processing industries. All dyes are not bound to the fabric depending on the class of the dye. Its loss in wastewater vary from 2% for basic dyes to as high as 50% for reactive dyes, resulting in the release of dye-containing wastewater (Delée et al. 1998; Pandey et al. 2007). These dye wastewaters are characterized by extreme fluctuation in many parameters such as chemical oxygen demand (COD), biochemical oxygen demand (BOD), pH, color and high salinity (Robinson et al. 2001; Dos Santos et al. 2007). So the effective removal of dyes has been a major concern in wastewater treatment because of their environmental question. Many technologies, such as physical, chemical and biological technologies or the combination of these technologies, are used for the treatment of dye wastewater. Biological technologies are frequently applied to treat textile and dyestuff wastewater due to cost effectiveness. However, conventional biological technologies cannot achieve

a satisfactory efficiency in color removal, because anaerobic azo dye reduction is a time-consuming process and is reflected by the requirement of long reaction time.

In recent years, more attention has been focused on the accelerating biological decolorization of azo dye with the addition of dissolved and non-dissolved redox mediators (Van der Zee and Cervantes 2009; Guo et al. 2007; Field and Cervantes 2005; Cervantes 2002; Rau et al. 2002). But continuous dosing of the dissolved redox mediators implies continuous expenses as well as continuous discharge of this biologically recalcitrant compound. To overcome the limits of dissolved redox mediators on the azo dyes bio-transformation, immobilization technologies for redox mediators have been little studied by researchers. Van der Zee et al. (2003) have reported that activated carbon (AC) could enhance dye decolorization rate. However, the accelerating effects of AC gradually decrease for its continuing wash-out from the reactor. Another non-dissolved redox mediators technology is the immobilize anthraquinone technology with calcium alginate (Guo et al. 2007). The disadvantage of this technology is that the accelerating effect of redox mediator lost gradually with the disruption of the polymeric material owing to weak mechanical strength. Recently, the accelerating effect of functionalized polypyrrole (PPy) composites consisting of active carbon felt (ACF)/PPy/anthraquinonedisulphonate (AQDS) is studied during the biological decolorization processes of azo dyes (Li et al. 2009; Wang et al. 2009), which could not be used well in the practical engineering for its strength and effectively retained in the bioreactor. Therefore, the more effectively and desirable immobilized technology for redox mediator is needed to study in details for the practical application.

Here we examine new functional bio-carriers modified by addition of redox mediators and explore their accelerating effects during azo dye decolorization processes. At the same time, the accelerating mechanism of the redox mediators was conducted from the two aspects of electrochemistry and biochemistry, which were considered little by other researchers in this field. This newly functional biocarrier modified by redox mediators for the azo dyes decolorization would be a great improvement of the redox mediator application and the bio-treatment concept.

Materials and methods

Dyes and chemicals

The used dyes in this study were from School of Textile and Fashion, Hebei University of Science and Technology. The chemical structures of the dyes were shown in Fig. 1. All other reagents were analytical grade and were purchased from Xiandai Ltd. (Shijiazhuang, China).

Organism and medium

A *Halomonas* sp. GYW (EF188281) was obtained from "The key laboratory of Environmental Biotechnology of Hebei", whose relative characteristic was studied in details in other paper (Guo et al. 2009). And the strain GYW was acclimatized in a salt-tolerant medium (STM) and incubated at 30°C, pH 7.0 on a rotary shaker at 150 r min^{-1}.

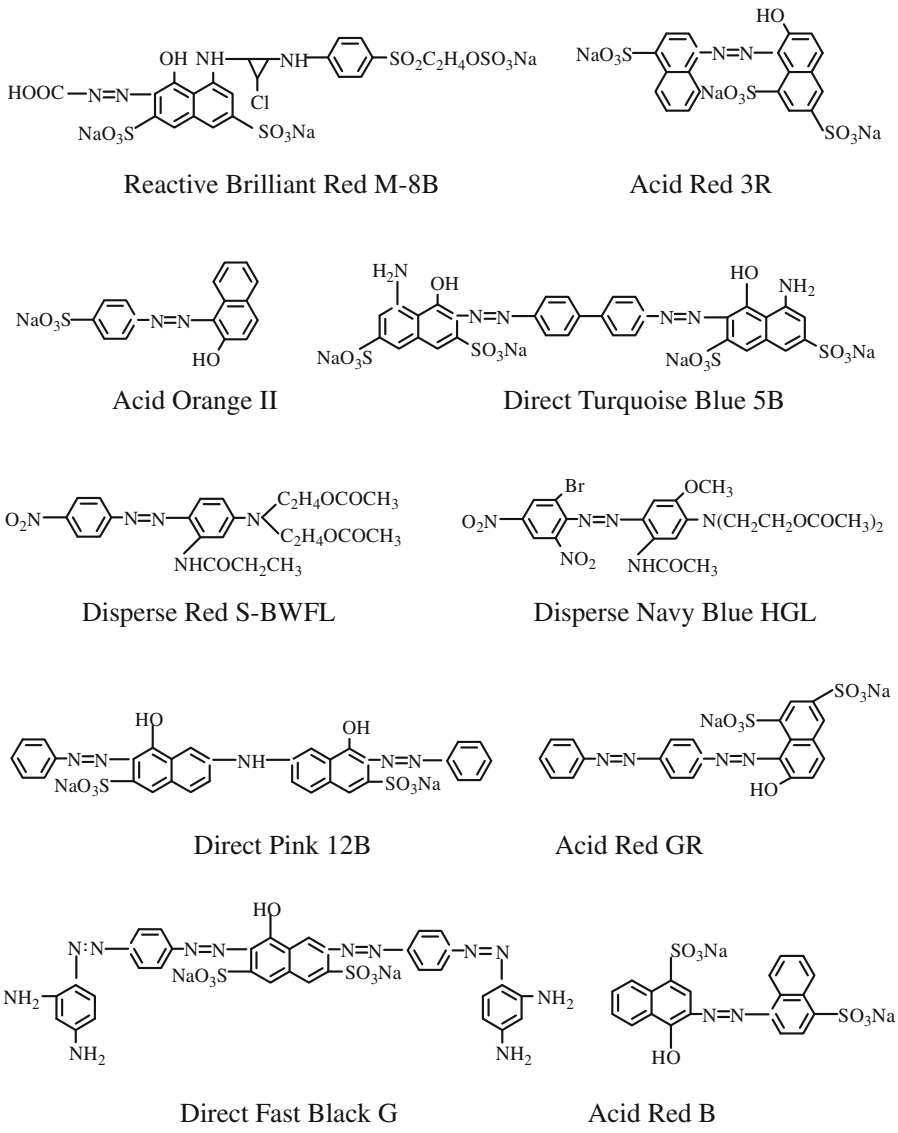
The salt-tolerant medium contained yeast extract 5 g l^{-1}, peptone 10 g l^{-1} and NaCl 100 g l^{-1} (pH 7.0).

The modification method of functional bio-carriers

Four anthraquinone dyes, disperse turquoise blue S-GL, disperse red 3B, disperse violet HFRL and disperse blue 2BLN (Fig. 2), were selected as redox mediators. One kind of terylene fiber and one kind of cotton textile were selected as the modified bio-carriers.

The immobilization mechanism of the tested redox mediators was a chemical immobilization. The functional bio-carrier modified by redox mediator were carried out at 40:1 liquor to goods ratio in sealed stainless steel dye pots of 100 ml capacity in a Roaches-Rotee dyeing machine. An appropriate volume of the dye solution and dispersing agent NNO (Sodium salt of polynaphthalene sulphonic acid) was placed in each pot to give the required quantity of dye. The dyebath pH was adjusted to 5.0 with sodium acetate/acetic acid buffer solution. A sample of preweighed fabric was placed in each pot, and the modifying process was carried out by raising the dyebath temperature from 40 to 95°C and holding at 95°C for 60 min before cooling to 40°C at 3°C/min. After clearing, the modified fabrics were rinsed

Fig. 1 The chemical structure of the tested dyes



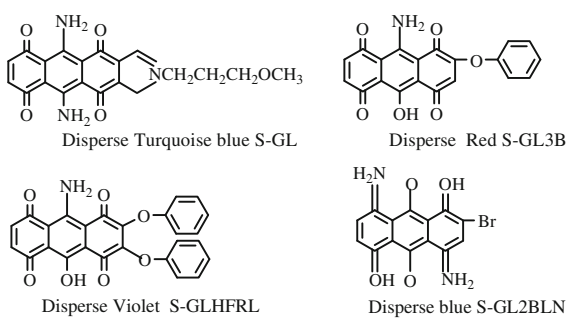


Fig. 2 The chemical structure of the tested anthraquinone dyes (as redox mediators)

successively with cold water and finally air-dried (Yang 1992).

Decolorization with functional bio-carriers

To eliminate the effect of adsorption, adsorption isotherms were conducted to estimate the adsorption extent of dye by non-modified bio-carriers. The same amount of non-modified bio-carriers was added into 100 ml of sterile deionized water containing the same concentration of dye.

The modified bio-carrier were placed in 100 ml STM medium containing designated concentrations of dye for decolorization, and the same amount of non-modified bio-carrier was also performed as the blank control. To evaluate the efficiency of decolorization, the batch decolorization experiments were carried out under the optimal conditions of temperatures (30°C), pH values (7.5) and the static-incubation conditions.

The effect of functional bio-carriers on the change of oxidation–reduction potential (ORP) during the decolorization processes

To explore the mechanism of bio-accelerating from the electrochemistry aspect, the characteristics of ORP during the decolorization processes were compared for the modified bio-carrier with redox mediators and non-modified bio-carrier without redox mediators system.

The accelerating effect of functional bio-carrier on different dyes decolorization

To demonstrate the general applicability of functional bio-carrier, 10 azo dyes (Fig. 1) were tested to examine the accelerating effect of functional bio-carrier on the decolorization. The bacteria without bio-carrier and with non-modified bio-carrier were as the controls.

The mechanism or biochemistry role of the selected redox mediator during the bio-accelerating decolorization processes

The different combinations of crude enzyme (including azo reductase), functional bio-carrier, coenzyme NADH and acid red GR were conducted to explore the mechanism of selected redox mediator during the azo dye decolorization processes.

To extraction crude azoreductase, the strain was incubated over night. Then the culture was centrifuged twice at 8,000*g* for 20 min and the cell pellets were washed by sodium phosphate buffer (pH 7.0) at each time. The cells from 2,000 ml culture were preserved over night at −20°C. The cells were completely lysed by ultrasonic processor (Model cp ·750, USA) with 20 mM sodium phosphate buffer (pH 7.0, 30 min, 225 W). Finally the lysed cells were centrifuged (12,000*g*, 20 min, −4°C) and the supernatant was used as crude azoreductase for the decolorization experiment. Protein concentration was determined by the modified Lowry protein assay (Markwell et al. 1978), using bovine serum albumin as the standard.

The total volume of the reaction mixture was 2.0 ml. The different mixture was the different combination of 20 mM sodium phosphate buffer (pH 7.0), 10 mg l^{-1} acid red GR, 0.4 μg crude azoreductase, 50 μl 10 mM NADH and functional bio-carrier for 35 min reaction. The reaction mixture was preincubated at 35°C in a cuvette that was placed inside an UV/VIS Spectrophotometer (China, UV-2600). The temperature of the cuvette holder was held constant by means of circulating thermostatic oven. The decolorization experiments were performed under static condition. Each experiment was repeated three times. The anaerobic reaction was performed in gastight cuvettes by gassing N_2.

Repeated-batch operations

To examine the reusability of the functional bio-carrier modified by redox mediator for azo dye decolorization, the functional bio-carrier was placed into a medium containing 100 mg l^{-1} dye (pH 7.5) and was then statically incubated at 30°C for decolorization. After complete decolorization, the functional bio-carrier were collected, rinsed twice with sterile deionized water and transferred into a fresh medium containing 100 mg l^{-1} dye for the second decolorization experiment. The same procedures were repeated four times.

Analytical methods

The cell concentration was measured by optical density at 660 nm.

The ORP was measured by with a digital pH Meter (Delta-320, China) and the ORP composited electrode (Leici-501, China).

The pH was determined with a digital pH Meter (Delta-320, China).

Absorbance of the dye-containing solution was measured at its λ_{max} values using an UV–Visible spectrophotometer (China, UV-2600, UV/VS spectrophotometer), and absorbance was proportional to concentration in the tested range. The relationship

between absorbance and concentration was unaffected by pH in the range of 5–9.

To prevent oxygen contamination during sampling, bottles were opened only once, and many bottles were incubated as measurements were planed. The assays were performed in triplication.

Results and discussion

The accelerating effect of the selected redox mediators

Different selected redox mediators had different effects on the acid red GR decolorization rate (Fig. 3). The results indicated that: disperse red 3B did not enhance the decolorization rate of acid red GR and inhibited this decolorization process; disperse violet HFRL and disperse blue 2BLN had little accelerating effect compared to the control; and disperse turquoise blue S-GL accelerated more 2-fold rate than the control. So disperse turquoise blue S-GL was selected as the ideal redox mediator to modify bio-carrier.

The accelerating effect of the functional bio-carrier

Figure 4 suggested that the terylene fiber modified by disperse turquoise blue S-GL enhanced the decolorization rate higher than the cotton textile modified by

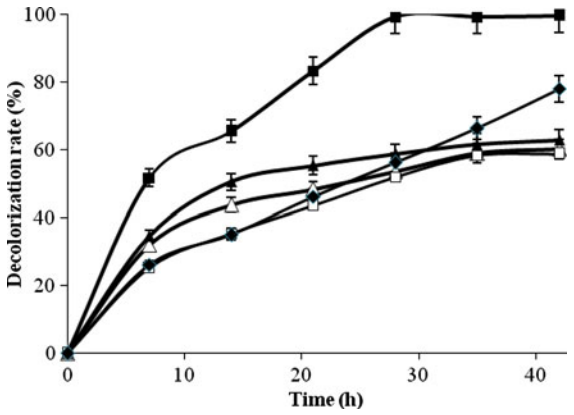


Fig. 3 The effects of different redox mediators on the acid red GR decolorization rate. *Filled square*: disperse turquoise blue S-GL; *filled diamond*: disperse violet HFRL; *triangle*: disperse blue 2BLN; *open square*: disperse red 3B; *open diamond*: control (bacteria without redox mediator)

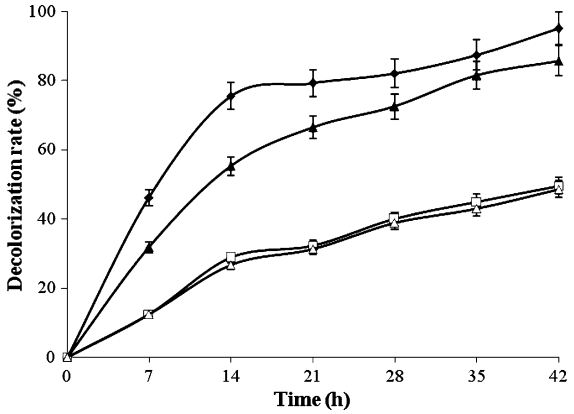


Fig. 4 The effect of functional bio-carrier on the acid red GR decolorization by bacteria strain GYW. *Filled diamond*: terylene fiber modified by disperse turquoise blue S-GL; *triangle*: cotton textile modified by disperse turquoise blue S-GL; *square*: terylene fiber without redox mediator; *open diamond*: cotton textile without redox mediator

disperse turquoise blue S-GL at 50 mg l^{-1} acid red GR, 30°C and the bacteria concentration of OD_{660} (0.6). This difference of two functional bio-carriers was that terylene fibers exhibited the best shade depth, while cotton textile was hard to be colored and showed the poorest shade depth. At the same time, by comparing the combined degree of the redox mediators and the bio-carrier, the stabilization of bio-carrier, the growth ability of biofilm, the accelerating effect of the selected redox mediators and the mechanical intensity, the terylene fiber bio-carrier modified by disperse turquoise blue S-GL was the perfect bio-carrier (the data did not showed). So the used functional bio-carrier in next experiment was terylene fiber modified by disperse turquoise blue S-GL.

The effect of functional bio-carrier on the change of ORP during the decolorization processes

Redox mediators are compounds that accelerate the electron transfer from a primary electron donor to a terminal electron acceptor, which may increase the reaction rates by its lower the reaction's activation energy (Van der Zee and Cervantes 2009). Therefore, its E_0' should ideally be between the reduction of an azo dye and the oxidation of a primary electron donor. In this study, the ORP values decreased gradually and stabilized about a constant value during the decolorization processes (Fig. 5). The ORP value

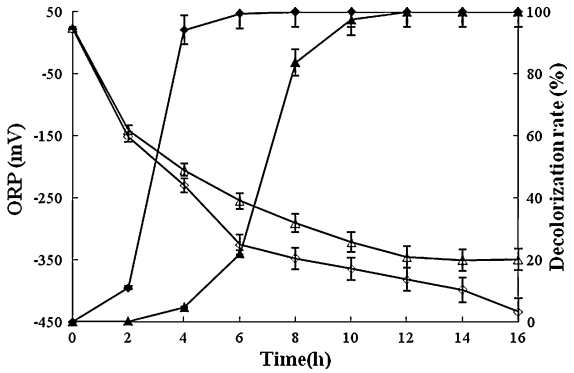


Fig. 5 Decolorization of acid red GR and ORP profiles by bacteria strain GYW with non-modified bio-carrier and functional bio-carrier; *filled triangle*: Decolorization of acid red GR (bacteria with non-modified bio-carrier); *open diamond*: ORP (bacteria with functional bio-carrier); *filled diamond*: decolorization of acid red GR (bacteria with functional bio-carrier); *open square*: ORP (bacteria with non-modified bio-carrier)

was about −150 mV after 2 h decolorization and stabilized around −280 to 290 mV after 6 h anoxic conditions. Compared with the control, the decolorization time was shorted about 4 h and the ORP value was stimulated more negative values of 20–25 mV for the functional bio-carrier. The similar results were also reported in other paper (Guo et al. 2008). The reason for the above-mentioned results might be explained in item 3.6.

The accelerating effect of functional bio-carriers on different dyes decolorization

The results suggested that the decolorization rates of all tested azo dyes (Fig. 6) was enhanced about 1.5–3 fold by functional bio-carrier. The different decolorization rates were usually affected by their molecular weights, substitution group of the dye molecules, and the intramolecular hydrogen bond between the azo and hydroxyl groups. The low decolorization rates of direct turquoise blue 5B, reactive brilliant red M-8B and direct fast black G might be attributed to their lager molecular weight, which affected their solubility and its degradation (Supaka et al. 2004).

Repeated-batch operations

Repeated-batch operations were performed to examine the reusability of the functional bio-carrier for azo dye decolorization. After four cycles, the specific

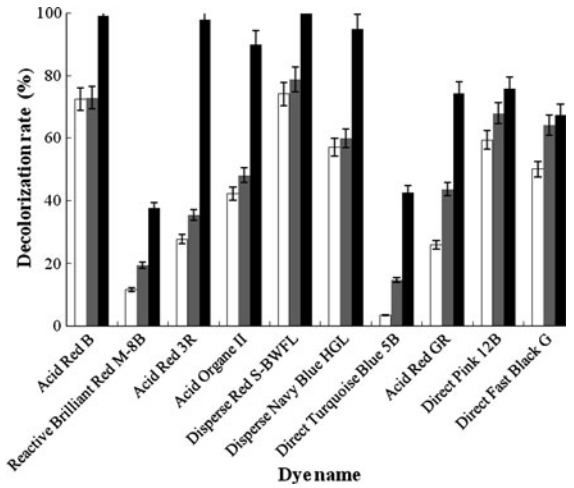


Fig. 6 The accelerating effect of functional bio-carrier on the strain GYW decolorization by bacteria strain GYW. *White bar*: bacteria without bio-carrier; *gray bar*: bacteria with non-modified bio-carrier; *black bar*: bacteria with functional bio-carrier

decolorization rates decreased less than 2% and the functional bio-carrier exhibited good reusability (Fig. 7). This also suggested that redox mediator on the functional bio-carrier was be recycled during the transfer of electron from an electron donor to the terminal electron acceptor.

The accelerating mechanism of the functional bio-carrier during the decolorization processes

The accelerating role of functional bio-carrier during the azo decolorization processes was explored by the

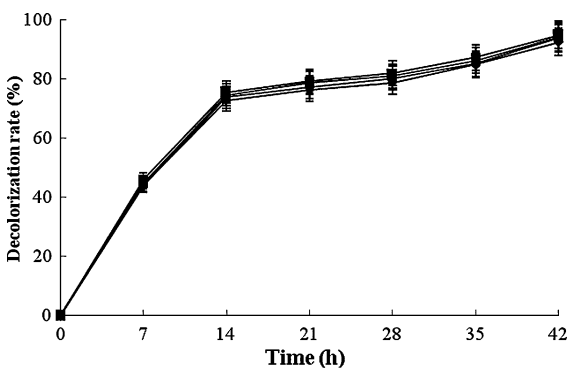


Fig. 7 The repeated batch decolorization of acid red GR with functional bio-carrier and bacteria strain GYW. *Square*: first cycle; *triangle*: second cycle; *circle*: third cycle; *diamond*: fourth cycle

different combinations of crude azoreductase, functional bio-carrier, coenzyme NADH and acid red GR. When different system reacted 35 min, different combined systems had the different decolorization rates, and the order of the decolorization rate was the combination of crude azoreductase, NADH and functional bio-carrier (96%), the combination of crude azoreductase and NADH (45%), and the combination of crude azoreductase and functional bio-carrier (25%). Compared the above results, non-dissolved redox mediator was speculated to be similar role of coenzyme NADH (Fig. 8), because redox mediator with quinine group had special chemical and electric chemical reduction–oxidation character. This first presumed mechanism of non-dissolved redox mediator was similar to dissolved redox mediator by other researcher (Cervantes 2002).

At the same time, the biological decolorization processes was an oxidation–reduction reaction in theory, in which transfer of electrons matched with proton needed the help of coenzymes, like NADPH/$NADP^+$ and NADH/NAD^+. The oxidation–reduction potentials of the couples of NADPH/$NADP^+$ and NADH/NAD^+ were −324 and −320 mV, respectively (Rau et al. 2002; Bourbonnais et al. 1998). The least $\Delta G_0^{'}$ of the conversion NADPH/$NADP^+$ and NADH/NAD^+ is 44 kJ (Zhu and Zheng 2003). Therefore, −93 mV obtained from Eq. 1 could be as a rough limited ORP value for primary electron donors of biological azo dye reduction. Hence, the observed failure of cyanocobalamin (Dos Santos et al. 2004) and ethyl viologen (Kudlich et al. 1997) to act as a mediator was most probably due to their too low $E_0^{'}$ values: −530 and −480 mV, respectively.

$$\Delta G_0' = 2F\Delta E_0'(F = 96.6\,KJ/(V\,mol)) \quad (1)$$

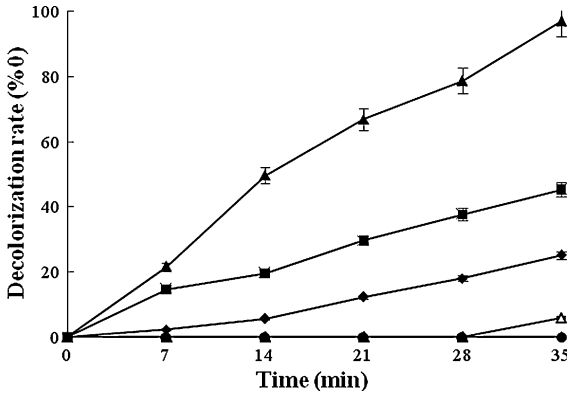


Fig. 8 Decolorization of acid red GR by the strain GYW under different combinations (crude enzyme, dye, functional bio-carrier and NADH). *Filled triangle*: crude enzyme, dye, functional bio-carrier and NADH; *square*: crude enzyme, dye and NADH; *diamond*: crude enzyme, dye and functional bio-carrier; *open triangle*: crude enzyme and dye; *circle*: dye

Conclusion

(1) The functional bio-carrier modified by disperse turquoise blue S-GL was demonstrated to increase about 1.5–3 fold decolorization rate of 10 azo dyes, and the ORP value during the acid red GR decolorization process was stimulated more negative values of 20–25 mV for the functional bio-carrier;

(2) Non-dissolved redox mediator on the functional bio-carrier was firstly put forward as the similar role of NADH in the azo dyes decolorization process, which was be recycled during the transfer of electron from an electron donor to the terminal electron acceptor.

(3) The functional bio-carrier exhibited good reusability and the combinational technology of the redox mediator and bio-carrier was a great improvement of the redox mediator application and the new bio-treatment concept.

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