Supplementary Material (ESI) for Chemical Communications

SUPPLEMENTARY INFORMATION

Coimmobilization of a redox enzyme and a cofactor regeneration system

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EXPERIMENTAL METHODS

Expression and purification of the nitrobenzene nitroreductase (NBNR) from *Pseudomonas pseudoalcaligenes* JS45[6].

PCR was used to amplify the *nbzA* gene[4a] from *P. pseudoalcaligenes* JS45 genome (G. Zylstra, unpublished data). The amplified fragment was cloned into pBAD-HisA, (Invitrogen, Carlsbad, CA). The resulting plasmid was transformed into *E. coli* Top10 (Invitrogen, Carlsbad, CA), to give the strain *E. coli* Top10 pBAD-*nbzA*. An overnight pre-culture from a single colony (1/100, v/v) of *E. coli* Top10 pBAD-*nbzA* was inoculated into Luria broth containing ampicillin (100 μg/mL). Cultures were grown at 37°C to an optical density of 0.6 – 0.8 at 600 nm. *L*-arabinose (0.002%) was added and the cells were incubated at 22°C for an additional 16 hours to induce the expression of the recombinant NBNR.

Cells were washed twice in saline potassium phosphate buffer (50 mM, pH 7.0, 500 mM NaCl) via centrifugation (7000 x g for 10 minutes at 4°C) and lysed by 3 passes through a French pressure cell (16,000 psi). Cell debris and unbroken cells were removed by centrifugation (10,000 x g for 20 minutes at 4°C). The supernatant containing NBNR

was purified by affinity chromatography (Co^{2+} NTA column, HiTrap Chelating HP™, GE Healthcare, Picataway, NJ). Fractions with nitroreductase activity were dialyzed overnight against potassium phosphate buffer at 4°C, using a Slide-A-Lyzer 10,000 MWCO (Pierce Biotechnology, Rockford, IL).

Enzyme entrapment in PEI induced silica.

Samples (0.25 mL) of enzyme solutions (0.025 and 0.012 mg/mL for NBNR and G6PDH respectively) in potassium phosphate buffer (25 mM, pH 8.0) were mixed with 0.125 mL of 10% PEI (pH 8.0) and 0.125 mL of a hydrolyzed tetramethyl orthosilicate (TMOS) solution. The TMOS was hydrolyzed by dilution in hydrochloric acid (1 mM) to a final concentration of 1 M. The mixture was agitated for 2 minutes at 22˚C and the particles were collected by centrifugation for 10 seconds (14,000 x g) and washed twice in phosphate buffer before use in subsequent experiments.

Enzyme assays:

Nitrobenzene nitroreductase activity was determined spectrophotometrically by monitoring the decrease in absorbance at 340 nm due to the oxidation of NADPH. The reaction mixture contained 100 μM nitrobenzene and 240 μM NADPH in potassium phosphate buffer. One enzyme unit (U) was defined as the amount of enzyme that catalyzes the oxidation of 1 μmol of NADPH per minute at 25˚C.

The activity of G6PDH was monitored using glucose-6-phosphate (3 mM) and NADP (0.67 mM) and measuring the increase in absorbance at 340 nm due to the formation of NADPH. One enzyme unit (U) was defined as the amount of enzyme that catalyzes the reduction of 1 μmol of NADP per minute at 25˚C.

Kinetics parameters for NBNR and G6PDH were determined in the coimmobilized suspension. The Michaelis constants for NBNR were determined by the initial rate of NADPH consumption in a reaction buffer containing 100 μM nitrobenzene and a range from 1 to 420 μM NADPH. The Michaelis constants for G6PDH were determined by the initial rate of NADP consumption in a reaction buffer containing 3 mM glucose-6-phosphate and a range from 1 to 600 μM NADP. Kinetics curve fitting were performed with the Prism 4 program (GraphPad software Inc, San Diego, CA).

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Nitrobenzene conversion:

Nitrobenzene conversion was performed in sodium phosphate buffer 25 mM pH 7 containing 100 µM nitrobenzene, 240 µM NADPH and 3 mM glucose-6-phosphate unless otherwise stated in the Results section, using 0.22 and 1.1 IU of NBNR and G6PDH respectively. For continuous conversion of nitrobenzene, 1.5 mM nitrobenzene and 6 mM glucose-6-phosphate were added to the system by increments of 250 µM and 3 mM respectively.

Conversions were monitored by HPLC using a Spherisorb C8 column (5U, 250 mm, Alltech, Deerfield, IL) with a mobile phase of acetonitrile and water (containing 0.05% and 0.1% trifluoroacetic acid respectively). The concentration of acetonitrile was increased from 20 to 60% over 13 min, with a flow rate of 1.5 mL/min. Nitrobenzene and HAB were monitored by UV detection at a single wavelength of 240 nm.

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SUPPORTING DATA

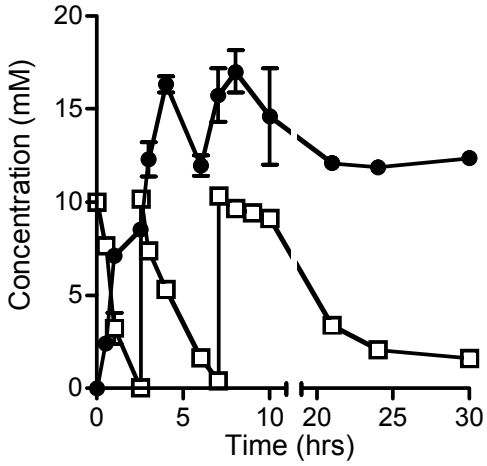

Fig. 3: Continuous conversion of nitrobenzene to HAB by 1 U NBNR:5 U G6PDH. Nitrobenzene was added in 10 mM increments and 240 μM NADPH was added at 0 hrs. Nitrobenzene (□), HAB (●)

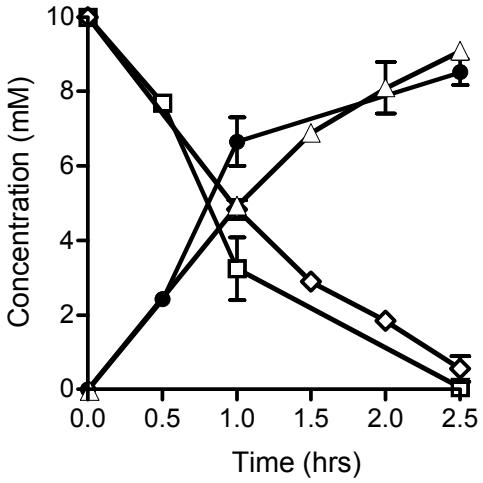

Fig. 4: Reuse of the immobilized preparation 1 U NBNR:5 U G6PDH.

Nitrobenzene (□), HAB (●), 240 μM NADPH added at 0 hrs. After 10 hrs, the sample was centrifuged and resuspended in fresh reaction buffer with 240 μM NADPH. The conversion was monitored for 2.5 hrs. Nitrobenzene (◇), HAB (▲)