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Nano-bio hybrid system for enhanced degradation of cefdinir using *Candida* sp. SMN04 coated with zero-valent iron nanoparticles

Adikesavan Selvi, Nilanjana Das*

School of Bio Sciences and Technology, VIT University, Vellore- 632014, Tamilnadu, India.

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ABSTRACT

The present study evaluates the effect of integrated nano-bio hybrid system involving nanoscale zero-valent iron (nFe^0) and yeast *Candida sp.* SMN04 on degradation of cefdinir in aqueous medium. The nanoparticle was chemically synthesised and characterized by atomic force microscopy (AFM), scanning electron microscopy (SEM), EDAX analysis and particle size analyser. Nano-bio hybrid system was prepared using optimal concentration (50 mg/mL) of chemically synthesized nFe^0, which were coated on the surface of yeast cells without causing any lethal effects to the cell. The survival and viability of the yeast cells were monitored by AFM and SEM images. Cefdinir (250 mg/L) degradation was studied, in both, the individual and hybrid system. The nano-bio hybrid system showed more effective cefdinir degradation compared to native yeast cell and nano zero-valent iron solely. The adherence of nanoparticles on the surface of the yeast cells increased the permeability of the cell membrane, thereby enhancing the entry of cefdinir into the cell. The kinetic data showed the half-life of cefdinir as 1.34 days for nano-bio hybrid system, 3.99 days for nFe^0 and 2.96 days for native yeast, *Candida sp*. SMN04 confirming that nano-bio hybrid system reduced the half-life to less than half of the time taken by the yeast alone. This study signifies the potential efficacy of the nano-bio hybrid system to serve as an effective remedial tool for the treatment of pharmaceutical wastewater.

INTRODUCTION

The presence of pharmaceutical compounds, namely antibiotics in the ecosystem has been known for many years and considered as emerging pollutants. The accumulation and persistence of antibiotics in the environment produce harmful effects even at low concentration in which they are detected (Homem and Santos, 2011). Pharmaceutical industries involved in the production of antibiotics discharge their wastes openly, which contains some quantity of these active compounds that are toxic in nature. Cefdinir is an advanced third generation semi-synthetic cephalosporin antibiotic, characterized by a vinyl group at C-3 and a (Z)-2- (2-amino -4 thiazolyl) -2- (hydroxyimino) acetyl moiety at C-7 and used for the treatment of acute respiratory related disorders and mild skin infections. The effluents released from cephalosporin production units are reported to release harmful compounds which are resistant to biodegradation, photo-transformation and natural degradation (Wang and Lin, 2012). The presence of high concentration of cephalosporin in the environment leads to very high chemical oxygen demand thus by increasing the toxic strength of the effluent (Duan, 2009). In the last few decades, considerable attention has been given to the treatment of pharmaceutical wastewater. A wide range of physico-chemical methods are being used for the treatment of pharmaceutical wastewaters which are of limited applicability because of the limitations such as inefficiency of remediating high strength wastewater, high operating cost, huge labour requirement, high equipment cost, intervention of toxic by-products etc. (Homem and Santos, 2011). Bioremediation using yeasts has attracted special interest in the present study since yeast species are found to be adaptable to changing environmental conditions, persist in natural habitats and polluted sites, degrade various toxic and stable organic substances like dyes, pharmaceutical compounds etc. (Recek and Raspor, 1999; Yu and Wen, 2005; Zheng *et al.*, 2010; Selvi and Das, 2014; Yang and Zheng, 2014).

* *Corresponding Author*
Email:nilanjana00 @ lycos.com

In recent years, zero-valent iron nanoparticles (nFe^0) have increasingly been utilized for the remediation of groundwater and hazardous waste treatment. It could be used as a potential degradation tool and reported to have been applied successfully for the degradation of environmental contaminants including β-lactam antibiotics, a major representative of pharmaceutically active compounds in wastewater (Li *et al.,* 2006; Ghauch *et al.,* 2009; Deng *et al.,* 2013).

Moreover, Fe^0 was found to be more effective than Fe^{2+} as activating agent and potentially more suitable for environmental applications (Ghauch *et al.,* 2009). Because of its super-magnetic property, iron nanoparticles also facilitate easy recovery and reuse and can serve as nanocatalyst making the degradation process more advantageous and economically feasible (Ansari *et al.,* 2009; Li *et al.,* 2013; Xu *et al.,* 2011). In addition, nFe^0 is less expensive and can reduce total dissolved solids (TDS) and electrical conductivity (EC) unlike ferrous and ferric salts that generally increase TDS and EC after treatment (Deng *et al.,* 2013).

Previously, we have isolated a yeast, *Candida* sp. SMN04, which showed an excellent capacity to degrade cefdinir at a concentration of 250 mg/L (Selvi and Das, 2014; Selvi *et al.,* 2015). The present research is an attempt for enhanced degradation of cefdinir using nFe^0 and cefdinir degrading yeast *Candida* sp. SMN04 together in a nano-bio hybrid system.

MATERIALS AND METHODS

Chemicals

Cefdinir (99% purity) was kindly donated by Orchid Pharmaceuticals, Chennai, India. Dimethyl sulphoxide (DMSO) procured from SRL Chemicals, India Ltd., was used to prepare a stock solution of cefdinir (10 g/L). Sodiumborohydride were of analytical grade and are obtained from Hi-Media India Ltd., SRL Chemicals India Ltd. and Sigma-Aldrich Chemical Co. (USA).

Microorganism and culture

The cefdinir degrading yeast *Candida* sp. SMN04 (KF963314.1) isolated from pharmaceutical wastewater was used in this work (Selvi and Das, 2014). The yeast was maintained on yeast extract peptone dextrose (YEPD) slants supplemented with 100 mg/Lof cefdinir. The degradation studies of cefdinir were carried out in mineral broth (MB) containing the following per litre: ammonium sulphate 5.0 g, potassium dihydrogenphosphate 1.0 g, dipotassium hydrogen phosphate 2.0 g, magnesium sulphate 0.5 g, sodium chloride 0.1 g, manganese chloride 0.01 g, ferrous sulphate 0.01 g, sodium molybdate 0.01 g, pH 7.0±0.2.

Nano scale zero-valent iron synthesis (nFe^0)

nFe^0 particles were synthesised following the method of Sun *et al.* (2007) with minor modifications. Equal volumes of 0.94M $NaBH_4$ and 0.18M $FeCl_3$were mixed thoroughly and was slowly added drop-wise into IM sodium borohydride solution with vigorous stirring (~400 rpm).The solution turned black immediately due to formation of nanoparticulate iron. The formed iron nanoparticles were harvested using vacuum filtration through 0.2μm filter paper. The synthesized zero-valent iron particles were then washed several times with deionized (DI) water and ethanol before use or stored in water containing 5 % ethanol.

$$4Fe^{3+}_{(aq)} + 3BH_4 + 9H_2O \rightarrow 4Fe^{0}_{(s)} + 3H_2B0_3 + 12H^{+}_{(aq)} + 6H_{2(g)} \quad (1)$$

Characterization of nFe^0

XRD

The XRD patterns were recorded to study the structural properties of the synthesised nFe^0 particles by θ-2θ method of x-ray diffraction (XRD) with a Cu $K\alpha_1$ (λ=0.15406 nm) source at 40 kV and 30 mA using multipurpose x-ray diffractometer (Bruker D8 Advance, Germany). The crystalline size of the particles was calculated from the Debye- Scherrer equation as follows:

$$d = \frac{0.89\lambda}{\beta cos\theta} \quad (2)$$

where, d is the mean size of the ordered (crystalline) domains, λ is the x-ray wavelength, 0.154; β is the line broadening at half the maximum intensity (full-width at half-maximum (FWHM)) and θ is the Bragg angle.

AFM, SEM and EDAX

The morphological characterization of the synthesised nFe^0 was carried out using atomic force microscope (AFM; Nanosurf, Switzerland; Model: easyScan2) and scanning electron microscope (EVO series-MA15 SEM). EDAX analysis was carried out by SEM coupled with EDAX-EVO-MA15-SEM-Oxford instruments.

Particle size and size distribution

The particle size and its distribution were determined using particle size analyser (Horiba scientific SZ-100). A calibration program provided by the instrument manufacturer was used to calculate the particle size distribution (PSD) from the attenuation spectra.

Effect of nFe^0 on the growth of strain SMN04

To study the effect of nFe^0 on growth of the yeast, *Candida* sp. SMN04, agar well diffusion method was followed with various concentration of nFe^0 ranging from 5-100 mg/mL.nFe^0 was added onto the lawn culture of the yeast streaked on YEPD agar plate. The test plates were incubated for 2 days at 30 °C and 120 rpm. Zone of inhibition around the wells were indicative of growth inhibition. The least concentration which, showed inhibition was taken as minimal inhibitory concentration (MIC). The test was supported with MIC assay in broth cultures and the cell dry weight was monitored regularly.

Development of nano-bio hybrid system

Nano-bio hybrid system was developed following the method of Li *et al.* (2013). Yeast cells were grown in YEPD broth until the mid-exponential growth phase and harvested by centrifugation at 8400×g for 10 min.

The cell pellets were washed twice with phosphate buffer and re-suspended back in YEPD at a concentration of 3 g/L of cell dry weight. 10 mL of the suspension containing 50 mg of nFe^0 per millilitre of water was mixed with 100 mL of the cell suspension in YEPD broth. The ratio of mass of nanoparticles to yeast biomass was 3.0 w/w. At this ratio, nanoparticles were sufficient enough to coat the yeast cells. The nFe^0 coated yeast cells termed as nano-bio hybrid system were visualized by AFM and SEM analysis.

Cefdinir degradation assay

Batch degradation studies were carried out in 100 mL of MB containing cefdinir at a final concentration of 250 mg/L in 250 mL Erlenmeyer flask incubated on a rotary shaker at 120 rpm and 30°C. Degradation experiments employed the following treatments in MB containing cefdinir, which include (1) nano-coated yeast cells (nano-bio hybrid degradation), (2) native yeast cells (biodegradation), (3) nFe^0 (nanodegradation) and (4) uninoculated control.

These treatments were used to compare the efficiency of nano-bio hybrid system and native yeast cells. The experiments were run for 6 days under aerobic conditions. The supernatant was collected at regular intervals and residual cefdinir was analysed by UV-spectrophotometric analysis (Shimadzu UV-2450), following the method of Cabri *et al.* (2006) with minor modifications. The residual cefdinir concentration was calculated using the formula given below,

$$\text{Residual cefdinir concentration (\%)} = \frac{C_i - C_f}{C_0} \times 100 \qquad (3)$$

The obtained degradation data were fitted with pseudo-first order reaction kinetics (Capellos and Bielski, 1972) for the calculation of half-life and degradation rate constant using the following equations:

$$C_t = C_0 . e^{-k't} \qquad (4)$$

$$t_{1/2} = \ln 0.5 / - k' \qquad (5)$$

where, C_0 is the initial concentration of cefdinir in the medium, C_t is the concentration of cefdinir at time 't', k' is the degradation rate constant, $t_{1/2}$ is the biodegradation half-life period of cefdinir.

Enzyme analysis

To study the enzymatic response of native and nano-coated yeast cells, activities of various enzymes viz. β-lactamase (Wayne, 2002), NADPH reductase (Kappeli *et al.*, 1982), amylase (Yalchin and Corbaci, 2013), manganese peroxidase (Hussaini *et al.*, 2011) were assayed following the standard protocols collecting the samples at regular time intervals. The crude extracts from yeast cells grown in MB without cefdinir were used as controls. One unit is equivalent to that amount of enzyme required to catalyze the 1.0 mg of substrate per minute under standard assay conditions.

RESULTS AND DISCUSSION

Characterization of nFe^0

X-ray diffraction (XRD) pattern of chemically synthesized zerovalent iron nanoparticlesis shown in Fig. 1. The diffraction peaks of nFe^0 exhibited at $2\theta = 44.6^o$, 64.9^o and 82.2^o which are corresponding to the (111), (200) and (211) diffraction plane of cubic structured Fe phase (JCPDS 87-0721). The diffraction peaks are in good agreement with an earlier report by Dickinson and Scott (2010).

The estimated crystalline sizes using Scherrer's formula for the nFe^0 was found to be 31.7 nm. The full width half maximum (FWHM) of 0.270 and θ value of 0.389 (in radians) corresponding to the high intensity peak of the nanomaterial was observed.

The surface morphology of the synthesized nanoparticles was examined by atomic force microscope and scanning electron microscope. The AFM and SEM micrographs of nFe^0 powder was shown in Fig. 2(a,b). The microscopic studies revealed the synthesized zero-valent iron nanoparticles were of spherical in shape. Similar findings were reported by Sun *et al.*(2007). The corresponding particle size distribution of nFe^0 revealed that the average particle diameter of the synthesized nFe^0 was in the nanometer range (10-30 nm). Toxicity concerns are often based on its large size and mobility, whereas nFe^0 used in the present study is of small size (crystalline size-31.7 nm), which prevents their dispersion and persistence in the environment thus making the degradation process eco-friendly (Wiesner *et al.*, 2006).

The intensity weighed particle size distribution histogram is presented in Fig. 2(c). Another study conducted by Ponder *et al.* (2000) gave an estimation of the average nano scale iron particle diameter as 10-30 nm. Sun *et al.*(2007) stated that the particle size of iron nanoparticles ranged from tens to hundreds of nanometers. The differences in the particle sizes obtained using the microscopic technique were due to differences in sample preparation and due to the polydispersity of the sample which will depend upon the particle numbers, volume, mass or optical property (Vimala *et al.*, 2014).

The EDAX spectrum of nFe^0 showed a single strong peak at 0.7 KeV corresponding to Fe element, therefore confirming the synthesized nanoparticle was free from other impurities (Fig. 2d). These characterization results confirmed that the nFe^0 formed was in nanosize and hence these nanoparticles were used as nanocatalyst for cefdinir degradation experiments in mineral medium.

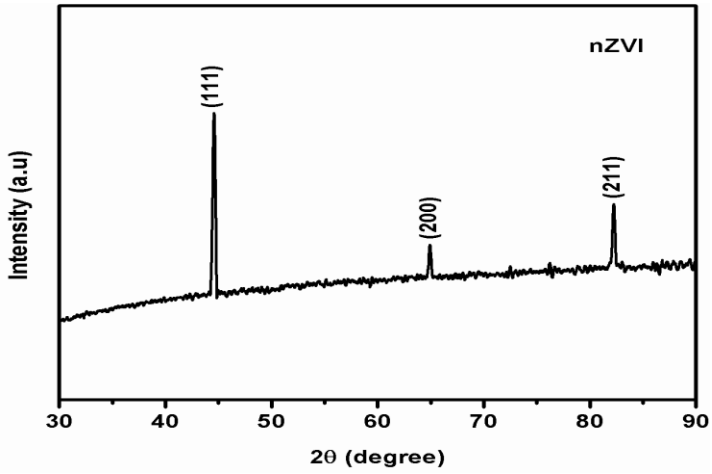

Fig. 1: X-Ray diffraction pattern of nFe^0.

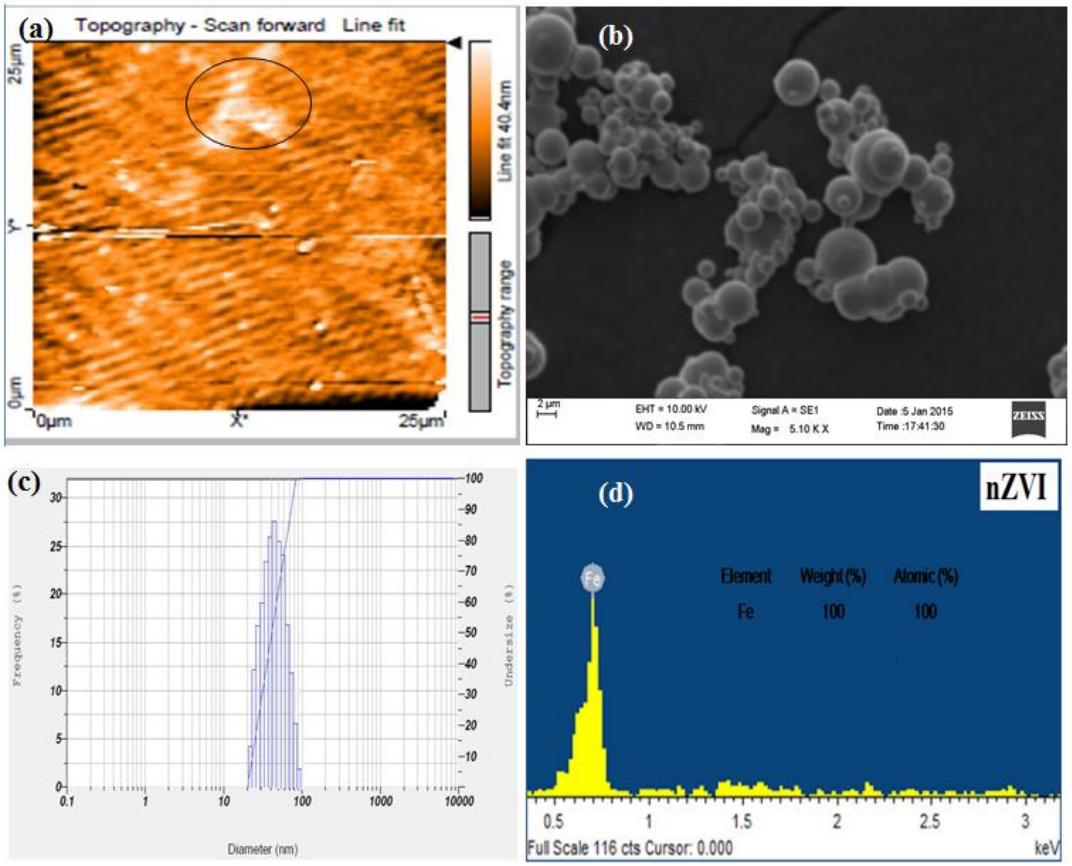

Fig. 2: Characterization studies of nFe^0. (a)AFM image; (b) SEM image; (c) histogram showing particle size distribution and (d) EDAX spectrum.

Effect of nFe^0 on growth of *Candida* sp. SMN04

In general, nanoparticles exert toxic effects on microbes by disrupting the cell membranes, increasing the membrane permeability, interrupting the energy transduction, producing reactive oxygen species etc. (Diao and Yao, 2009; Li *et al.*, 2010). The obtained results showed that, the yeast cells showed the positive effect of growth until 50 mg/mL concentration of nFe^0 (Figure not shown). Above this concentration, the yeast cells showed poor or no growth, indicating the cell damage and microcidal activity of nFe^0 and complete inhibition of cell growth was noticed at 75 mg/mL of nFe^0, therefore this concentration was noted as MIC for *Candida* sp. SMN04. Hence, concentration of 50 mg/mL was used for yeast coating to form a nano-bio hybrid system for cefdinir degradation experiments.

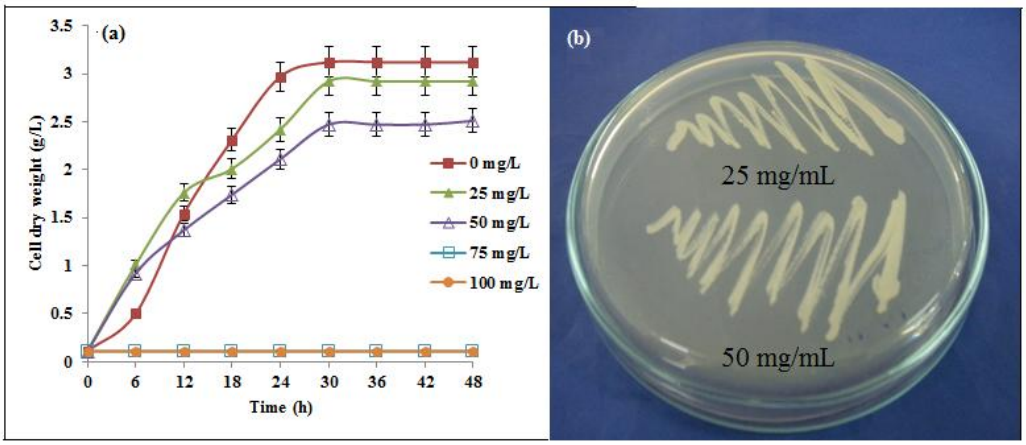

Fig. 3: Survival assay of *Candida* sp. SMN04 grown with nFe^0 (a) Growth curves of *Candida* sp. SMN04 grown at various concentrations of nFe^0, (b)Survival assay of strain SMN04 grown in presence of nFe^0 at 25 and 50 mg/mL

Thus, MIC & survival assay was performed to determine the lowest concentration of nFe^0 that inhibits the visible growth of the microbe in an overnight culture. After the preliminary experiments of MIC determination, survival assay of the yeast cells were demonstrated in YEPD broth (Fig. 3a) and YEPD plate (Fig. 3b) containing nFe^0 concentration below its MIC value. The results were analysed in terms of cell dry weight and cell growth (g/L) respectively. Fig. 3(a) explains the growth curve of the yeast strain with various concentrations of nFe^0 ranging from 0-100 mg/mL, where the maximum cell dry weight was observed in the medium containing no nFe^0, indicating an inhibitory effect of nFe^0 on yeast growth. A significant amount of biomass was produced in the medium containing 25 and 50 mg/mL of nFe^0. The growth of the yeast colonies on YEPD plates showed that the yeast cells could tolerate up to 50 mg/mL of nFe^0. When the concentration of nFe^0 was increased up to 75 mg/mL and above, yeast growth was found to be completely inhibited (Fig. 3a). The results of this experiment were supported with yeast survival assay on YEPD plates (Fig. 3b). From the results, it was implied that nFe^0 at a concentration of 50 mg/mL was tolerated by the yeast with no lethal effects or cell damage. Therefore, 50 mg/mL concentration of nFe^0 was fixed as the optimum concentration for yeast coating.

Nano-bio hybrid system

The images of atomic force micrographs (AFM) of the native cells and yeast cells with nFe^0 (nano-bio hybrid system) are presented in Fig. 4, which shows both topography (1a-d) and deflection (2a-d) images. As observed, the native cells showed normal morphology with oval-shaped cells with smooth and intact cell wall structure (Fig. 4a).

The AFM images of the nano-bio hybrid system showed increased surface area due to the adsorption of nFe^0 nanoparticles onto the surface of the yeast cells. At lowest concentrations of nFe^0, i.e., 25 mg/mL, even though the nanoparticles were attached to the cell surface, the yeast was in a growing phase with intact cell wall structure (Fig. 4b). On further increasing the concentration of nFe^0 to 50 mg/mL, the yeast cells were still found in growing condition, which indicated that the adsorption of zero-valent nanoparticles did not show any negative impact on the growth of yeast cells (Fig. 4c). In both cases, the yeast cells were viable in nature as evident from the presence of intact cell structures. This implied that the concentration of 25 and 50 mg/mL were not lethal to the yeast strain SMN04. According to Lee *et al.* (2008), formation of oxide layer by iron nanoparticles can decrease the toxicity of iron nanoparticles towards microbial cells supporting their survival. Kim *et al.* (2010) also reported that inactivation of microbial cells by iron nanoparticles was less under aerated conditions than de-aereated environments. The yeast cells grown in 75 mg/mL of nFe^0 showed the absence of proper cell wall structure which might be due to the rupture of the cell because of the increased concentration of nFe^0 (Fig. 4d). These observations suggested that accumulation of the nanoparticles by the yeast cell was directly proportional to the concentration of the nanoparticle. Since, at nFe^0 concentration of 50 mg/mL in the growth medium of the yeast strain SMN04 could stimulate the coating of nFe^0 nanoparticles without causing any lethal effect to the cell, this concentration was considered optimum for the development of a nano-bio hybrid system, which will induce membrane permeabilization to facilitate the entry of cefdinir into the cell. These findings were also reported by other workers in case of pollutant removal (Ansari *et al.*, 2009; Diao and Yao, 2009; Li *et al.*, 2010). Figure 5 shows the scanning electron micrographs of the surfaces of the native yeast cells and nFe^0 –yeast hybrid system. In Fig. 5(a), the native cells had normal ovoid cell morphology, whereas, the yeast cells with nFe^0 (Fig. 5b) clearly showed that the nFe^0 particles were efficiently assembled on the surface of the yeast cell. In this condition, the yeast cells were alive and in actively dividing phase with budding cells. The SEM images revealed that there were no morphological distortions in case of control and nano-hybrid yeast cells. Fig.5(c) shows that the yeast cells recovered from the stationary phase of cefdinir degradation. Recently, Li *et al.*(2013) reported the same findings while demonstrating carbazole biodegradation using *Sphingomonas* cells/Fe_3O_4 nanoparticles.

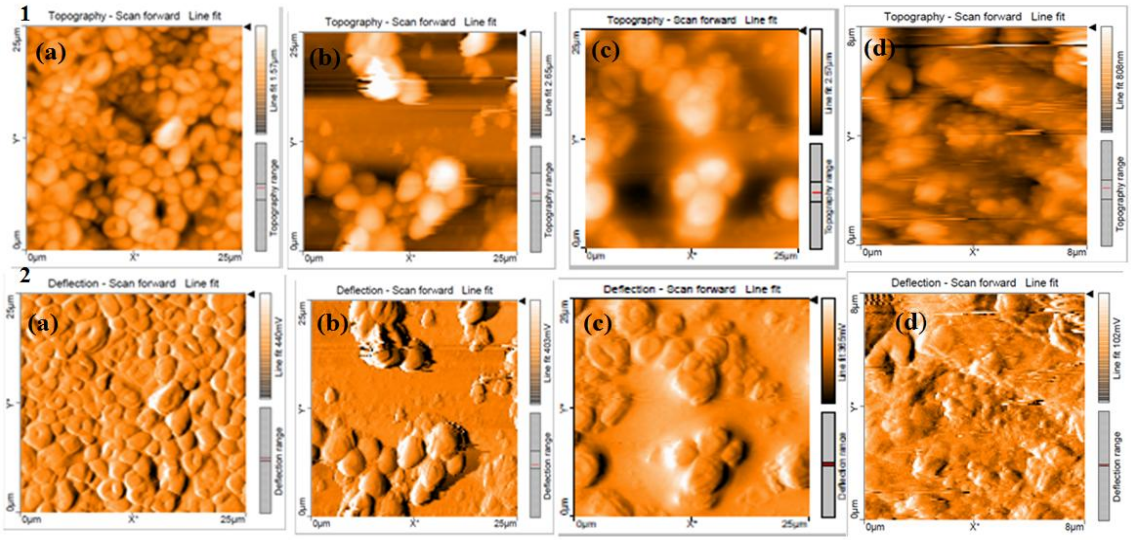

Fig. 4: AFM images of *Candida* sp. SMN04. (a) Native yeast cells; (b) yeast cells grown with 25mg/mL of nFe^0; (c) yeast cells grown with 50 mg/mL of nFe^0; (d) yeast cells grown with 75 mg/mL of nFe^0 showing topography (1) and deflection (2) of each image.

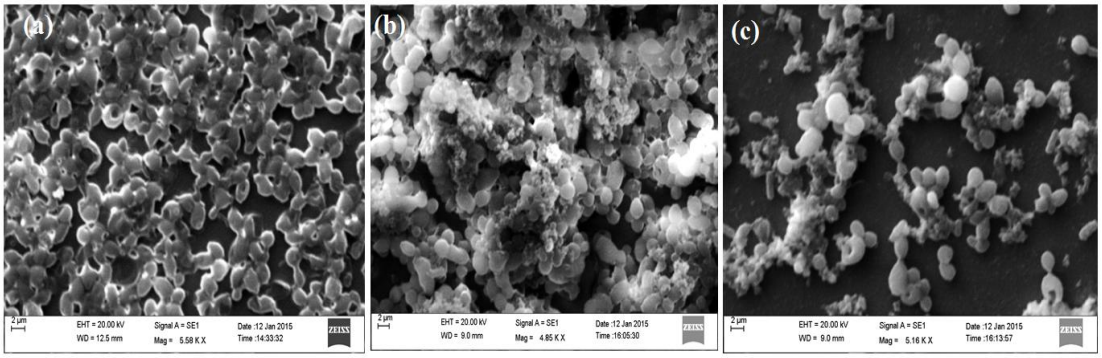

Fig. 5: SEM Images showing (a) Native yeast cell; (b) nano-bio hybrid nFe^0 coated yeast cells at optimal concentration of 50 mg/mL and (c) cefdinir-interacted nano-bio hybrid cells recovered from stationary phase.

Degradation of cefdinirusing nFe^0 and nano-bio hybrid system

Fig. 6(a) compares the degradation of cefdinir in MB with the native yeast cells, nFe^0 as well as nano coated yeast as nano-bio hybrid system. The results showed that the amount of residual cefdinir was significantly less in the case of nano-bio hybrid system. The percent removal of cefdinir was found to be 84% in case of native yeast cells and 73% in case of nFe^0 particles within 6 days of treatment, whereas, in the culture of the nano-bio hybrid system, 91% removal was observed within a period of 2.68 days. The faster cefdinir removal might be due to synergistic effect of the nano-bio hybrid system. However, after the measurement of residual concentration of cefdinir in various treatments after 10 days, it was found that complete removal of cefdinir was achieved by nano-bio hybrid system (data not shown). The enhanced entry of cefdinir into the yeast cells occurred due to the increased permeability as a results of nFe^0 coating on yeast cells, which could positively influence the rate of cefdinir utilization by the yeast. Ghauch *et al.* (2009) reported the complete removal of amoxicillin and ampicillin using nFe^0 particles with final antibiotic concentration of 20 mg/L. Our study reports the complete removal of cefdinir using nano-bio hybrid system with final concentration of 250 mg/L. The increased permeability of the yeast cells due to nFe^0 adsorption on their surface might be another factor, which could have positively influenced faster cefdinir degradation. This adsorption of nanoparticles with yeast cells facilitates easy transport of cefdinir into the intracellular matrix through a transmembrane system, leading to the enhanced entry of the pollutant (Grigoriev, 2002; Ansari *et al.*, 2009). Additionally, nFe^0 can be easily recovered and reused from the medium using "magnetic separation" technique, which is a low-cost, simple and highly reliable. Xu *et al.* (2011) studied the recovery of Fe_3O_4 magnetic nanoparticles from the harvested microalgal biomass and maintained the same microalgal recovery efficiency as the newly synthesized ones after five times of reuse. To understand the reaction kinetics of cefdinir removal, the degradation data were fitted to pseudo-first order kinetic model (Fig. 6b). The degradation rate constant and half-life were calculated from the kinetic plot and presented in Table 1. The half-life of cefdinir

degradation was 2.97 days (almost equal to 71.2 h) for the yeast *Candida* sp. SMN04, which was further reduced in the case of the nano-bio hybrid system being 1.34 days (almost equals to 32 h). The results suggested that, the degradation of cefdinir by the nano-bio hybrid system proved to be more efficient than the individual systems which could bring down the half-life to more than half of the value as shown by the individual yeast.

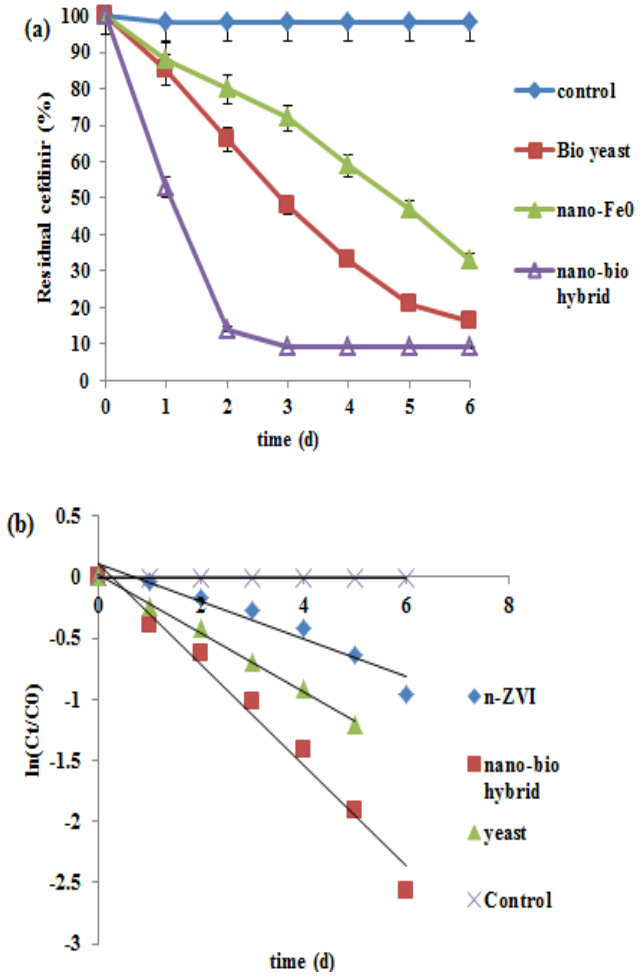

Fig. 6: Cefdinir degradation in mineral medium. (a) Residual cefdinir percentage in the culture medium treated with various degradation agents; and (b) pseudo first-order kinetic plot of cefdinir degradation by various treatments.

Table 1: Comparision of kinetic parameters of cefdinir degradation in various systems.

Treatments	Rate constants; K per day	Half life; $T_{1/2}$ (days)
Control	0.0013	533.19
nFe^0	0.1736	3.99
Candida sp. SMN04	0.2335	2.97
Nano-bio hybrid	0.5171	1.34

Enzyme analysis

The role of degradative enzymes in cefdinir degradation using native yeast cells and nano-bio hybrid system was evaluated through standard enzyme analysis. In the present study, activity of β-lactamase (Fig. 7a), NADPH reductase, amylase, manganese peroxidase was tested (Fig. 7b). The antibiotic activity of cefdinir was tested growing *E.coli* in the test plates where, a zone of no growth around the well was noted. Cefdinir degraded products collected at the end of day 1 was tested on the same organism where no inhibitory zone of growth was found which confirmed the loss of antibiotic property demonstrating the role of the enzyme β-lactamase during cefdinir degradation. This is considered as one of the vital steps in the antibiotic degradation process (Okamoto *et al.*, 1996; Cabri *et al.*, 2006; Mashelkar and Renapurkar, 2010). A rapid rupture of β-lactam ring within 40 min of treatment with nano scale iron particles was reported by Ghauch *et al.* (2009) during removal of β-lactam antibiotics from water. The enhanced activities of other three enzymes viz., NADPH reductase, amylase, manganese peroxidase were also noted in nano-bio hybrid system compared to native yeast cells. Enhanced activity of enzymes immobilized on nanoparticles was reported in other studies (Dyal *et al.*, 2003; Li *et al.*, 2007; Song *et al.*, 2012).

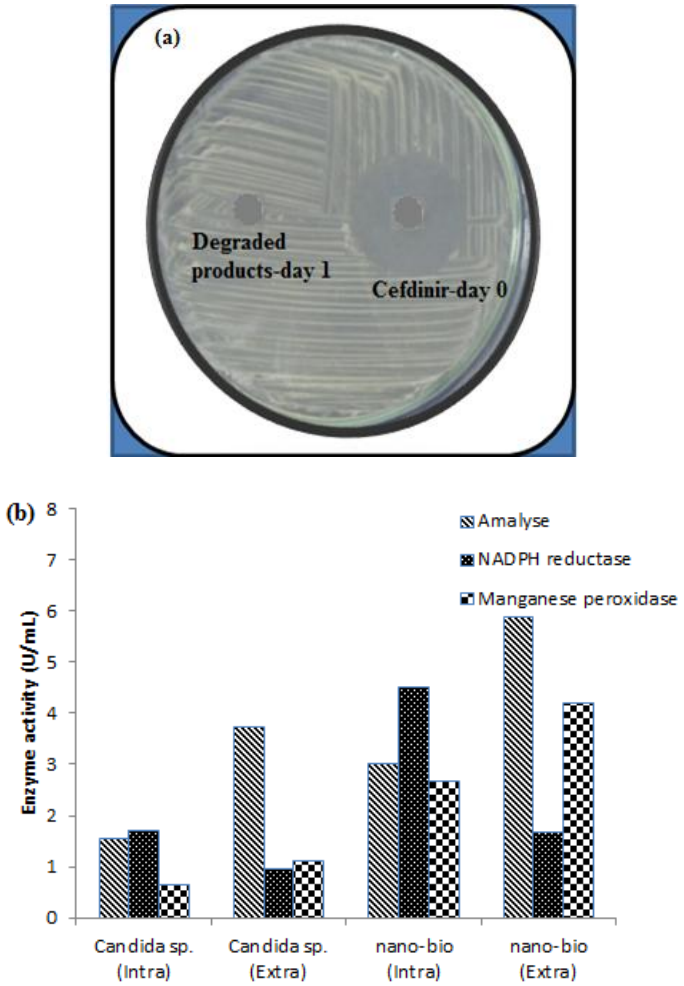

Fig. 7: Enzyme assays involved in cefdinir degradation. (a) Demonstatation of loss of antibiotic activity (β-lactamase) on day 0 (cefdinir) and day 1 degraded products after degradation; and (b) Other degradative enzymes in intra and extracellualr fractions of native *Candida* sp. SMN04 and nano-bio hybrid systems.

CONCLUSION

The present research showed the applicability of a novel nano-bio hybrid system using nFe^0 coated on *Candida* sp. SMN04 for enhanced degradation of cefdinir. Experiments conducted in batch mode revealed that the degradation of cefdinir by nano-bio hybrid system was more than the individual systems. The coated yeast showed more activity than the non-coated ones. The concentration of nFe^0 for coating on the yeast was optimized. The nano-bio hybrid system showed 91% degradation of cefdinir at concentration of 250 mg/Lwithin two and half days, which was a remarkable decrease in time compared to the results reported earlier.

Further, the involvement of major enzyme β-lactamase along with other enzymes was also noted during cefdinir degradation. The enhanced activity of the enzymes may be possibly due to the immobilization of both intracellular and extracellular degradative enzymes onto nFe^0 particles. Enhanced cefdinir degradation might have occurred through an integrated approach of nano-bio hybrid system involving nFe^0 and *Candida* sp. SMN04 in aqueous medium may serve as an effective remediation tool for the treatment of pharmaceutical wastewater containing cephalosporin antibiotics.

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