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ORIGINAL PAPER

# Effect of metal ions and metal nanoparticles encapsulated in porous silica on biphenyl biodegradation by *Rhodococcus erythropolis* T902.1

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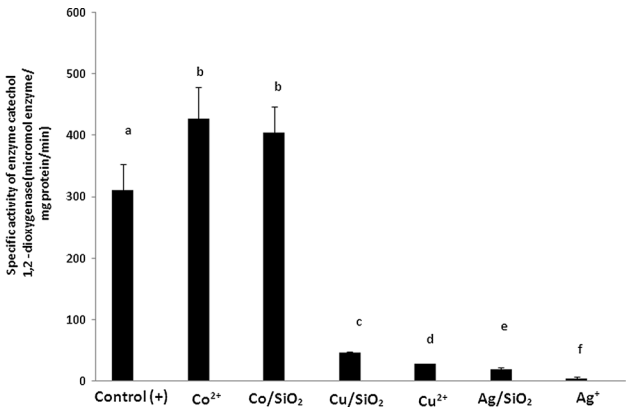
**Abstract** Biodegradation of biphenyl was carried out by *Rhodococcus erythropolis* T902.1 in presence of nanometer-sized metallic (Co, Pd, Ag and Cu) nanoparticles ($NP_S$) synthesized by the sol–gel process. In order to prevent their agglomeration, the metallic NPs (1–2 nm diameter) were anchored inside microporous silica crystallites and named $Co/SiO_2$, $Pd/SiO_2$, $Ag/SiO_2$ and $Cu/SiO_2$ samples, respectively. They were added at low concentrations of $10^{-6}$, $10^{-5}$ and $10^{-4}$ M of metal in the culture medium, and their impact was compared with that of the simple metal ions added as cobalt, palladium, silver or copper salts. The cultures containing $Pd/SiO_2$ or $Co/SiO_2$ samples at $10^{-4}$ M of metal achieved a 50 % higher biphenyl degradation yield after 18 days of incubation and improved *R. erythropolis* T902.1 growth compared with those without (positive control) or with silica particles only. The highest biodegradation performance, i.e., 107 ± 3 ppm/day, which was about 85 % higher than in control conditions without NPs, was recorded in 250-mL baffled flasks stirred at 150 rpm with $Co/SiO_2$ sample at $10^{-4}$ M Co. Furthermore, the stimulating effect of NPs on biphenyl biodegradation seems to also depend on the thermal treatment conditions applied to NPs since the experimental results indicated that, after calcination, the cobalt oxide NPs at a concentration of $10^{-4}$ M were more effective than the reduced cobalt NPs with a degradation yield of 81 ± 1 and 77 ± 2 %, respectively, after 18 days. On the other hand, the results showed that the addition of $10^{-4}$ M of $Cu^{2+}$ or $Ag^{+}$ ions or the addition of $Cu/SiO_2$ or $Ag/SiO_2$ samples at $10^{-4}$ M of metal have an inhibitory effect on biphenyl biodegradation. However, $Cu^{2+}$ and $Ag^{+}$ ions were more toxic to the *R. erythropolis* T902.1 bacteria than the respective Cu or Ag $NP_S$ anchored inside silica particles. Moreover, this work showed that in these conditions, the activity of catechol 1,2-dioxygenase (a critical enzyme in aromatic biodegradation pathway) was severely inhibited, whereas the presence of $10^{-4}$ M of $Co^{2+}$ ions or $Co/SiO_2$ sample stimulated the enzyme activity compared to the conditions without NPs.

*Graphical Abstract*

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**Keywords** Biodegradation · Biphenyl · *Rhodococcus erythropolis* · Sol–gel process · Encapsulated nanoparticles

## 1 Introduction

Polycyclic aromatic hydrocarbons (PAH) such as biphenyl, fluorene, phenanthrene, anthracene, and fluoranthene are worldwide manufactured and used in a variety of industrial processes and products such as fungicides and pesticides [1, 2]. Therefore, large amounts of these compounds or derivatives are diffused in ground waters and soils [3, 4]. Due to their carcinogenic, genotoxic and mutagenic properties, PAH were widely studied regarding their environmental effects [5–7].

For some decades, different physico-chemical techniques were used for the treatment of wastewater and soil contaminated with PAH, i.e., ozonation, adsorption, solvent extraction, Fenton effects and photodegradation in presence of iron [8–10]. Biological methods are also available for the reduction of aromatic pollutants though they need a longer process time. These methods are based on the potential of some microorganisms to use the pollutants for growth and source of energy [11, 12]. Compared with physico-chemical methods, the PAH biodegradation methods are preferred because of lower costs and their potential to degrade almost completely the pollutants without release of toxic end-products in the environment. Indeed, the physico-chemical techniques are relatively expensive and they frequently produce toxic and undesirable products that require further treatment steps [13, 14].

Numerous studies have demonstrated that many PAH can be biodegraded under aerobic or anaerobic conditions by different microorganisms such as *Pseudomonas* species [15–17], *Mycobacterium* [18–20], *Nocardioides* [21], *Citrobacter freundii* BS2211 [22] and *Rhodococcus* [8, 23–25]. However, most of the PAH are stable and highly hydrophobic, thus having a low availability for microorganisms since they need an aqueous environment for their growth. As a consequence, bioremediation processes are very limited. Long time periods of a few weeks to a few months are required for microbial degradation of highly hydrophobic substances [26].

Several strategies have been proposed to accelerate bioremediation processes using mixed cultures of suspended bacteria [27], cell immobilization [11, 28] or genetically engineered microorganisms [12]. Furthermore, the addition in the microbial environment of a conventional carbon source such as ethanol or glucose as co-substrate [11, 29] and of nitrogen sources [3, 29] was often reported to promote growth and biodegradation performances. Moreover, numerous studies have demonstrated that bioremediation can be accelerated with some metals at low concentrations [8, 30, 31]. Aldric et al. [30] showed that the presence of iron at concentrations up to $10^{-3}$ M increased the degradation of isopropylbenzene by *Rhodococcus erythropolis* T 902.1. Sridevi el al. [31] also reported that the presence of 0.01 g/L of $Co^{2+}$, 0.02 g/L of $Mn^{2+}$ or $Cu^{2+}$ ions (i.e., $1.7 \times 10^{-4}$, $3.6 \times 10^{-4}$ and $3.1 \times 10^{-4}$ M, respectively) enhanced the phenol biodegradation yield by *Pseudomonas putida* NCIM 2102. On the other hand, Bunescu et al. [8] studied the degradation of 2-aminobenzothiazole (ABT) under various conditions: (1) a photodegradation process with UV light in presence of Fe(III)-nitrilotriacetic acid (FeNTA) only, (2) a biodegradation process using *Rhodococcus rhodochrous* OBT18 with FeNTA at concentrations ranging from $10^{-4}$ to $10^{-3}$ M and (3) the combined processes (FeNTA plus *R. rhodochrous* in presence or absence of UV light). The degradation of ABT in the combined system, with or without UV light, was more efficient (99 % degradation after 25 h) than in the separated systems (respectively 37 % photodegradation and 26 % biodegradation after 125 h). It is also reported that the presence of FeNTA increased the biodegradation of ABT without any inhibition up to $5 \times 10^{-4}$ M FeNTA. Metallic or metallic oxide nanoparticles (NPs) represent a new generation of compounds to improve environmental remediation and biological processes [10, 32, 33]. They could provide solutions to some of the most challenging environmental clean-up problems. The use of iron and/or palladium $NP_S$ is very effective for the biodegradation of a wide variety of common environmental contaminants, such as chlorinated organic solvents and polychlorinated biphenyls or PCBs [33, 34]. However, some environmental issues still need to be addressed, for instance in relation with the potential internalization and bioaccumulation or NPs in organisms cells, particularly increased due to their very small size [35]. In order to prevent these mechanisms and also to avoid NPs agglomeration reducing their specific area, different methods have been developed such as the sol–gel process. It is based on the encapsulation of metallic or metallic oxide NPs inside a porous matrix like silica by the cogelation method [36–40]. Using this process, Mahy et al. [10] showed that the presence of $10^{-4}$ M of iron $NP_S$ anchored inside porous silica matrix, with UV light/$H_2O_2$ system was able to degrade $10^{-4}$ M of *p*-nitrophenol after 24-h incubation in aqueous medium.

This sol–gel chemical synthesis method is based on the concomitant hydrolysis and condensation of tetraethoxysilane [TEOS, $Si(OC_2H_5)_4$] with a modified alkoxide of formula $(RO)_3Si$-XL, in which the ligand L is able to form a complex $(LM)_n^{m+}$, with a metallic cation $M^{m+}$ (such as $Pd^{2+}$, $Ag^+$, $Pt^{2+}$,…) and that is connected to the alkoxide $(RO)_3Si$- moiety

through an inert and hydrolytically stable organic group, X. After heat treatments of texture of xerogel (drying, calcination and reduction), metallic nanoparticles (2–3 nm) are homogeneously dispersed through the silica matrix. [36–40]. The experiments reported in this paper were carried out with different metallic NPs anchored inside porous silica synthesized using TEOS as silica precursor and 3-(2-aminoethylamino) propyltrimethoxysilane [EDAS, $(CH_3O)_3Si(CH_2)_3NH(CH_2)_2NH_2$] as the modified alkoxide. According Alié et al. [41, 42], the ligand EDAS initiated a nucleation process as its hydrolysis and condensation reactions are faster than that of TEOS. So hydrolyzed EDAS molecules can act as a nucleation agent leading to silica particles with hydrolyzed EDAS core and principally made of hydrolyzed TEOS. The proof of anchored metallic particles into the silica matrix was obtained by electron tomography and rotating transmission electron microscopy [43–46]. These methods applied on $Pd/SiO_2$ samples demonstrate that the palladium particles are localized deep inside the silica skeleton. The metallic particles are regularly spaced in the middle of the silica skeleton, with a distance between them comparable to the diameter of the struts of silica.

Therefore, the goal of this work was to investigate the inhibitory or stimulating effect of metal NPs anchored in silica matrix ($Cu/SiO_2$, $Ag/SiO_2$, $Pd/SiO_2$ or $Co/SiO_2$) or metal ions ($Co^{2+}$, $Cu^{2+}$ or $Ag^{+}$) at concentrations ranging from 0 (positive control) to $10^{-4}$ M of metal element on the rate of biphenyl degradation by *R. erythropolis* T902.1.

## 2 Materials and methods

### 2.1 Preparation of concentrated suspensions of metal nanoparticles and concentrated solutions of metal ions

The samples $Co/SiO_2$, $Pd/SiO_2$, $Cu/SiO_2$ and $Ag/SiO_2$ are prepared by the sol–gel method, as described by Lambert et al. [47]. This method consists of (1) the preparation in one step (the cogelation method) of the porous silica from TEOS, in which metallic complexes $Mn^{+}[NH_2–CH_2–CH_2–NH–(–(CH_2)_3–Si\ (OCH_3)_3]n$ are homogeneously dispersed at molecular level; (2) the drying under vacuum to remove solvent; and (3) the thermal treatments like calcination to remove organic moieties and reduction to reduce metallic ions into the corresponding metals. After the different steps of preparation, the samples are characterized by using the methods described by Lambert et al. [38, 47, 48] and Heinrichs et al. [43, 44]. For the clarity of this study, $Co/SiO_2$, $Pd/SiO_2$, $Cu/SiO_2$ and $Ag/SiO_2$ samples are used as notations and are defined as corresponding metallic NPs anchored inside porous silica after vacuum drying, calcination and reduction steps.

For all metallic NP, a $10^{-3}$ M concentrated suspension in ultrapure water was prepared in 50-mL bottles by finely pounding (at micrometre size) and weighing some $Cu/SiO_2$, $Ag/SiO_2$, $Pd/SiO_2$ and $Co/SiO_2$ sample, i.e., 0.079, 0.316, 0.160 or 0.147 g, respectively. According to the experimental conditions, a defined volume of homogenized suspension was transferred in the culture medium prior to sterilization in order to reach a final concentration of Cu, Ag, Pd or Co equal to $10^{-4}$, $10^{-5}$ or $10^{-6}$ M. An equivalent $SiO_2$ suspension without metal was also prepared in 50 mL ultrapure water using 0.0030 g of $SiO_2$ sample synthesized by the sol–gel process [47] in order to check whether silica plays a significant role in the biodegradation of biphenyl. In the same time, 0012 g of $CoCl_2{\cdot}6H_2O$, 0009 g of $CuCl_2{\cdot}2H_2O$ or 0008 g of $AgNO_3$ were added under stirring in 50 mL ultrapure water in order to obtain concentrated solutions of $10^{-3}$ M of metal ions ($Co^{2+}$, $Cu^{2+}$ or $Ag^{+}$).

### 2.2 Culture

A preculture was prepared in 250-mL baffled flasks containing 50 mL of M284 minimal medium [49] complemented by 5 g/L of glucose. The medium was sterilized for 20 min at 121 °C and cooled down to room temperature before inoculation at an initial cell density of about $10^9$ cell/mL by transfer of colonies of *R. erythropolis* T902.1 developed on M284 + agar medium. The microbial suspension was incubated for 3 days at 30 °C and 150 rpm orbital agitation. The glucose was totally consumed after 3 days of incubation (assayed by the Kit RTU glucose, BioMérieux, F). For the biphenyl biodegradation experiments, the culture medium was prepared similarly in 100-mL flasks or 250-mL (in triplicates) baffled flasks with 20 mL of M284 supplemented with dried, calcined or reduced $Ag/SiO_2$, $Cu/SiO_2$, $Pd/SiO_2$, $Co/SiO_2$ and $SiO_2$ samples or $Ag^{+}$, $Cu^{2+}$, $Co^{2+}$, $Pd^{2+}$ ions at concentrations ranging from 0 (positive control) to $10^{-4}$ M of metal element. After sterilization, 1 mL of a 10 g/L biphenyl solution in *n*-hexane was added on the culture medium to achieve a biphenyl initial concentration of 500 ppm as source of carbon and energy. The evaporation of *n*-hexane was allowed overnight under a ventilated hood leaving biphenyl crystals suspended in the M284 minimal medium. A relatively homogenous suspension of biphenyl crystals in aqueous phase was achieved by vigorous mixing for 2 min using a mixer (POLYTRON® PT 1200 E, KINEMATICA AG, CH) sterilized by immersion in three successive solutions: SDS 5 %, sodium hypochlorite 4 % and norvanol 9 %. The resulting suspension was inoculated with 3 mL of preculture and incubated at 30 °C (150 rpm orbital agitation). Test controls were also prepared in the same conditions (1) without added $NP_S$ nor $SiO_2$ (positive control), (2)

with the sole $SiO_2$ matrix (positive control + $SiO_2$), and (3) without inoculum (negative control with or without NPs anchored in $SiO_2$). The negative controls were carried out in triplicates to examine the abiotic removal/evaporation of biphenyl. The optical density was measured at 600 nm (Ultrospec III, Pharmacia LKB) at different time points of incubation (after 3, 8 and 18 days). The interference of nanoparticles on absorbance was corrected with the control samples that contained only nanoparticles to confirm the bacterial growth.

### 2.3 Biphenyl analysis

Biphenyl residual concentration in the culture medium was measured by HPLC at different time points of incubation (after 3, 8 and 18 days) after liquid–liquid extraction in organic solvent. Glass tubes of a total volume of 10 mL (culture Tube 16 × 100 SVL SCRE, Pyrex®, UK) with Teflon seal and containing 2 mL sample and 4 mL *n*-hexane were mixed for 24 h at 30 °C. After centrifugation at 7000 rpm (SLA-1500 rotor in Sorvall® RC5B+ centrifuge) for 15 min, the organic phase was transferred in glass tubes for overnight evaporation of solvent under a constant ventilated hood. Crystals of biphenyl were resuspended in 10 mL methanol before analysis by HPLC.

### 2.4 HPLC analyses

HPLC analyses were performed using an Agilent 1100 Series equipment and a C18 column (LiChroCART® 250 4.6HPLC-cartridge Purospher® STAR RP -18 endcapped 5 µm Merck, D) at 30 °C. The mobile phase contained acetonitrile and Milli-Qwater (resistivity = 18.2 MΩ cm) in the ratio 70/30 in order to determine the biphenyl concentration. Its pH was adjusted to 2.75 with 1.5 M phosphoric acid. The flow rate was 0.8 mL/min, and 10 µL of the sample was injected. Biphenyl was detected at 254 nm, and the concentration in samples was calculated from a standard graph determined using pure compound.

### 2.5 Enzyme assays

For the enzyme assays needing larger biomass material, the cultures were carried out in 500-mL flasks (in triplicates) with 100 mL of M284 minimal medium complemented by $Ag/SiO_2$, $Cu/SiO_2$, $Pd/SiO_2$ and $Co/SiO_2$ samples or $Ag^+$, $Cu^{2+}$, $Co^{2+}$, $Pd^{2+}$ ions at concentrations of 0 (positive control) or $10^{-4}$ M of metal element. The cultures were sterilized for 20 min at 121 °C. After sterilization, 5 mL of 10 g/L biphenyl solution in *n*-hexane was added as the sole carbon and energy source. The other steps were as described above (Sect. 2.2). After 18 days of incubation, the cells were centrifuged for 20 min at 13,000 rpm at 4 °C and washed twice with a potassium phosphate buffer 0.05 M ($K_2HPO_4$, $KH_2PO_4$, pH 7.5). The pellets were resuspended in a sufficient volume of the same buffer to obtain a cellular concentration of 0.05 g of wet cells per mL. The cell suspension was then sonicated at a frequency of 10 kHz for 2 min and centrifuged at 13,000 rpm for 40 min. The supernatant containing crude cell-free extracts was used to determine the biphenyl dioxygenase activity and catechol-1,2-dioxygenase activity. During all these operations, the extracts were maintained at 4 °C. For the determination of catechol-1,2-dioxygenase specific activity, a 100 µL volume of concentrated cell-free extracts was added to 900 µL of 0.05 M phosphate buffer ($K_2HPO_4$, $KH_2PO_4$, pH 7.5) and 20 µL of 10 mM catechol. The specific activity was monitored at 260 nm ($\varepsilon = 16.8$ mM) [50]. The amount of cis, cis-muconic acid formed in the samples was calculated from a standard graph determined using pure compound of cis, cis-muconic acid. One unit of enzyme activity was defined as the amount of enzyme catalyzing the production of 1 µmol cis, cis-muconic acid per min at 30 °C.

### 2.6 Protein concentration

Protein concentration was measured by the Bradford method [51]. Bovine Serum Albumin (Sigma, USA) was used as a standard.

### 2.7 Statistical analysis

The SAS software (SAS Institute 2001) was used for all statistical analyses. The general linear model (GLM) was used to determine whether the effect of metal nanoparticles or metal ions was significant on the biodegradation potential of *R. erythropolis* T902.1. Least square means and standard errors were calculated. A $p$ value of $<0.05$ was chosen as the threshold for significance of all statistical comparisons.

## 3 Results and discussion

### 3.1 Effects of metal nanoparticles (Ag, Cu, Pd and Co) on biphenyl biodegradation

The present study investigates the effect of $NP_S$ of about 2–3 nm in diameter of four metals (cobalt, palladium, silver and copper) at a concentration of $10^{-4}$ M on biphenyl degradation by *R. erythropolis* T902.1. These $NP_S$ were encapsulated in a porous silica ($SiO_2$) matrix [36–39, 46–48]. Figure 1 shows that the presence of $10^{-4}$ M of cobalt $NP_S$, added as $Co/SiO_2$ sample in the culture medium without inoculum, did not exhibit any effect on degradation of biphenyl when compared to the negative control without

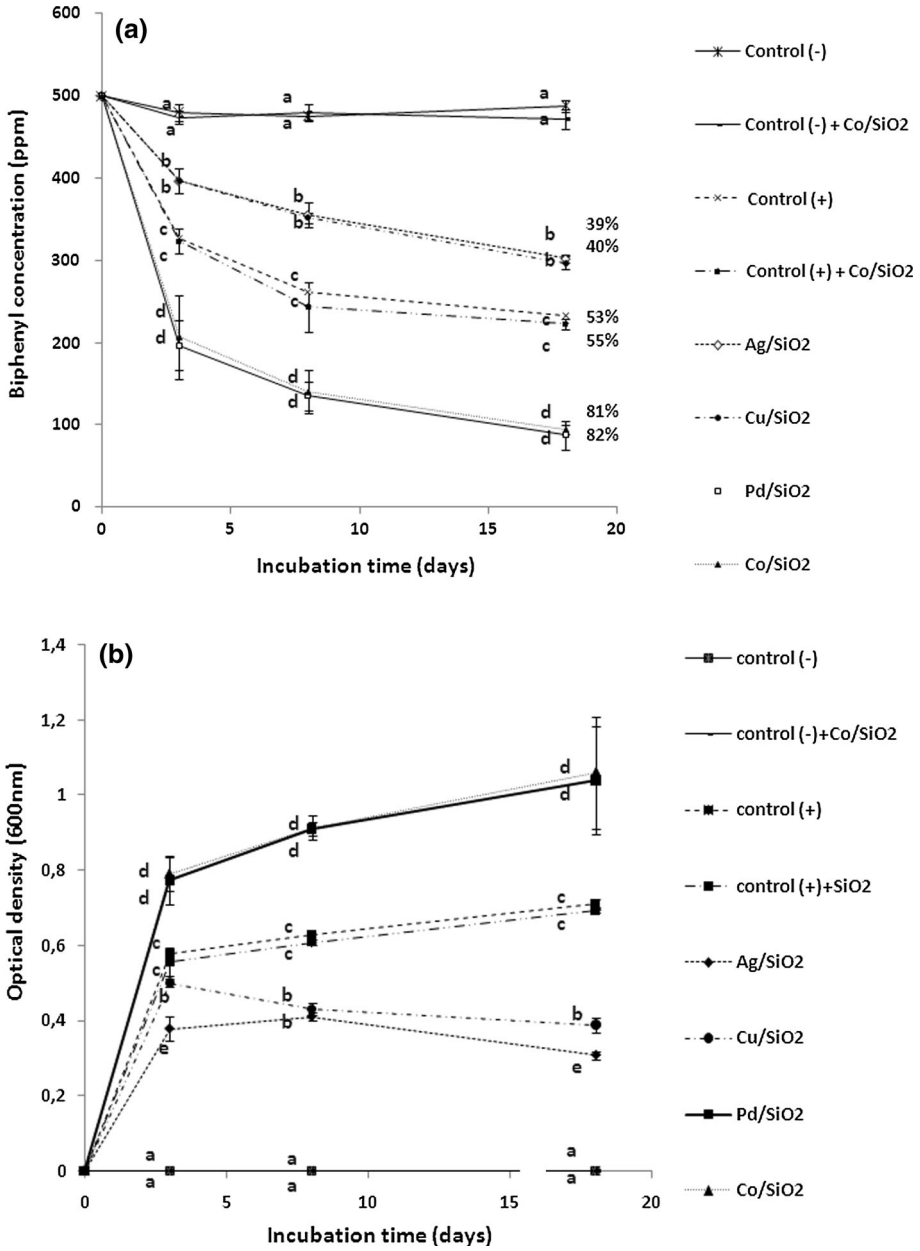


**Fig. 1** Evolution of **a** biphenyl concentration and **b** growth measured by optical density of *Rhodococcus erythropolis* T902.1 in 100-mL flasks containing 20 mL of M284 culture medium with 500 ppm biphenyl and $10^{-4}$ M of calcined (cobalt, palladium, copper or silver) nanoparticles encapsulated in $SiO_2$ matrix by sol–gel method: control (−) and control (−) + $Co/SiO_2$ without microorganisms, control (+) without NP nor $SiO_2$, control (+) + $SiO_2$ without $NP_S$ (the % indicated on the figure refer to % of biphenyl degraded). The *similar letters* (aa, bb, cc and dd) indicate that in the presence of calcined (cobalt, palladium, copper or silver) nanoparticles, no significant statistical differences were observed ($p > 0.05$) at different time points of biphenyl biodegradation. The *error bars* means standard deviation on triplicates

$Co/SiO_2$ at all time points ($p > 0.05$). Similar results were recorded with $Cu/SiO_2$, $Pd/SiO_2$ and $Ag/SiO_2$ samples (not shown). Moreover, the presence of the sole $SiO_2$ with inoculum in the culture medium (positive control + $SiO_2$) has no significant inhibitory or stimulating effect on the biphenyl biodegradation by *R. erythropolis* T902.1 compared to the positive control without $SiO_2$ ($p > 0.05$) (Fig. 1). Biphenyl was degraded up to 53 ± 1 and at 55 ± 1 %, respectively after 18 days of incubation. These results are in accordance with Mu et al. [52] who reported that $SiO_2$ NPs with sizes of 10–20 nm up to 150 mg/g total suspended solids (estimated at $3.75 \times 10^{-3}$ M) showed no inhibitory effect on methane generation by anaerobic digestion.

Figure 1 indicates also that $Ag/SiO_2$ and $Cu/SiO_2$ samples containing the metallic NPs at $10^{-4}$ M effectively reduced the rate of biphenyl degradation as compared to the positive control ($p < 0.05$). Indeed, silver and copper are widely used as antibacterial agents since they destruct the cell membrane by close contact between the bacteria and these agents (silver and copper) [4, 53–57]. Shahverdi et al. [53] reported that silver and copper bound to cell membranes leading to an increase of permeability and cell death. Other studies [54, 57] indicated that toxicity depends on the particle size. Luna-del Risco et al. [57] tested the influence of particle size of ZnO and CuO on methane production during anaerobic digestion of cattle manure and reported that the inhibitory effects of ZnO and CuO NPs

(about 60 and 30 nm size respectively) were much greater than the bulk ZnO and CuO (1 and 5 μm size, respectively). The highest inhibition was recorded for methanogenic microorganisms in presence of NPs with half effective concentration EC50 of about $0.9 \times 10^{-4}$ and $1.7 \times 10^{-4}$ M for Zn and Cu NPs, respectively. By comparison, the EC50 were about twofold and tenfold higher for bulk ZnO and CuO particles, respectively, and additionally twofold higher for the other microorganisms involved in the anaerobic digestion process regarding ZnO. Their colleagues [54] confirmed the effect on the yeast strain *Saccharomyces cerevisiae*. Mu et al. [52] studied the influence of dissolved $Zn^{2+}$ from ZnO NPs on methane production during waste activated sludge anaerobic digestion and found that the release of $Zn^{2+}$ from ZnO NPs was an important cause of inhibition of methane production.

In the present study, the metallic Pd, Ag, Cu and Co nanoparticles, with sizes of around 2–3 nm are embedded in inorganic microporous silica particles of around 10–20 nm diameter with pores not exceeding 0.8 nm diameter [38, 46, 47]. Therefore, *R. erythropolis* cells cannot enter into the $SiO_2$ matrix nor have a direct contact with the metallic NP anchored inside the $SiO_2$ network. Furthermore, the metallic nanoparticles could not release spontaneously outside the $SiO_2$ crystallites as suggested by ICP-AES measurements [10] with $Fe_2O_3/SiO_2$ samples synthesized by the cogelation method. Indeed, in this study [10], it appears that the amount of $Fe^{3+}$ ions in an aqueous medium stabilize at around $8 \times 10^{-7}$ M after 28 days. However, while there is not a full inhibitory effect, the results indicate that $Ag/SiO_2$ and $Cu/SiO_2$ samples with metallic NPs at $10^{-4}$ M slow down the biodegradation of biphenyl and the growth of *R. erythropolis* T902.1 compared to the positive control ($p < 0.05$) (Fig. 1). However, this bacteria strain is known to produce siderophore compounds in order to complex the metal ions that are essential for its metabolism [58–60]. Our results suggest that these siderophores would not be selective enough and therefore would be able to progressively attract metal ions from the inside of the microporous $SiO_2$ structure. These results should also be related with those reported by Kasemets et al. [54] about copper NPs toxicity on *S. cerevisiae*, the NPs being directly in suspension in the culture medium and not anchored in silica matrix as in our experiments. They mentioned a 60-fold higher toxicity for CuO nanoparticles (30 nm), i.e., EC50 of about $2 \times 10^{-4}$ M, than for the bulk copper oxide powder, assuming a cumulative effect of copper solubility and oxidative stress mediated by NPs, since in the normal conditions of viable cells, NPS cannot enter the yeast cells.

By contrast, these siderophores would be favorable in the other experiments reported here since the addition of $Co/SiO_2$ or $Pd/SiO_2$ samples at a metal concentration of

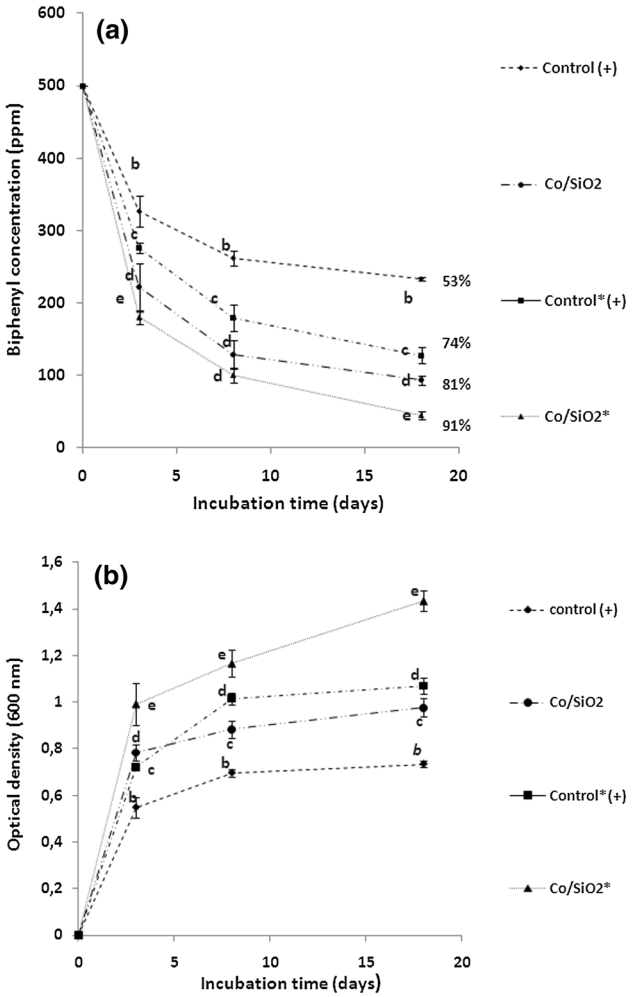


**Fig. 2** Evolution of **a** biphenyl concentration and **b** growth measured by optical density of *Rhodococcus erythropolis* T902.1 in 100-mL flasks or 250-mL baffled flasks (*) containing 20 mL of M284 culture medium with 500 ppm biphenyl and $10^{-4}$ M of calcined cobalt nanoparticles encapsulated in $SiO_2$ matrix by sol–gel method. The *similar letters* (aa, bb, cc and dd) indicate that in the presence of nanoparticles, no significant statistical differences were observed ($p > 0.05$) at different time points of biphenyl biodegradation. The *error bars* means standard deviation on triplicates

$10^{-4}$ M induced a significant increase of the biphenyl biodegradation and growth of *Rhodococcus* strain compared to the positive control ($p > 0.05$) (Fig. 1). Biphenyl was, respectively, degraded up to 81 ± 1 and 82 ± 4 % after 18 days of incubation. This is in agreement with the results of Sridevi et al. [31] and Kasemets et al. [54] who described the bioavailability of cobalt or palladium as an important factor for enhancement of organic pollutant biodegradation. Sridevi et al. [31] reported that the presence of cobalt up to $1.7 \times 10^{-4}$ M improves the degradation of phenol. Murugesan et al. [34] showed that the presence of palladium–iron bimetallic NPS up to 0.1 g/L (estimated at $2 \times 10^{-3}$–$2 \times 10^{-6}$ M, respectively) in minimal salt medium is effective on the complete

**Table 1** Physico-chemical properties of the used (Cu, Co, Ag, Pd) nanoparticles encapsulated in $SiO_2$ matrix by sol–gel method: diameters of metallic ($d_{MX+}$) and $SiO_2$ ($d_{SiO_2}$) particles measured by TEM and specific surface area obtained by BET method

| Sample | Reference sample | $d_{MX+}$ (nm) | $d_{SiO_2}$ (nm) | $S_{BET}$ ($m^2/g$) ±5 |
|---|---|---|---|---|
| $Co/SiO_2$ calcined | – | 3 | 25 | 350 |
| $Co/SiO_2$ dried | – | 3 | 25 | 75 |
| $Co/SiO_2$ reduced | – | 3 | 25 | 350 |
| $Ag/SiO_2$ | Ag0.1 [47] | 3 | 20 | 280 |
| $Cu/SiO_2$ | Cu1.5 [47] | 3 | 35 | 395 |
| $Pd/SiO_2$ | Pd3.1 [47] | 2.5 | 13 | 495 |

**Table 2** Biphenyl biodegradation yields (and standard deviation on triplicates) after 18 days of incubation with *R. erythropolis* T902.1 in 100-mL flasks containing 20 mL of M284 minimal medium with 500 ppm biphenyl and different concentrations $10^{-6}$, $10^{-5}$, $10^{-4}$ M of (dried, calcined or reduced) cobalt nanoparticles encapsulated in $SiO_2$ matrix by sol–gel method or cobalt ions

| Conditions | Biphenyl biodegradation yield | | |
|---|---|---|---|
| Control (+) | 53 ± 1 % | | |
| Metal concentration | $10^{-6}$ M (%) | $10^{-5}$ M (%) | $10^{-4}$ M (%) |
| $Co/SiO_2$ dried | 68 ± 2 | 81 ± 1 | 83 ± 1 |
| $Co/SiO_2$ calcined | 66 ± 4 | 78 ± 2 | 81 ± 1 |
| $Co/SiO_2$ reduced | 62 ± 3 | 74 ± 5 | 77 ± 2 |
| $Co^{2+}$ | 75 ± 2 | 85 ± 1 | 90 ± 1 |

dechlorination of triclosan (2,4,40-trichloro-20-hydroxydiphenyl ether; up to 27 ppm initial concentration) and biodegradation of the intermediates metabolites by a strain of *Sphingomonas* genus.

Moreover, our study showed (Fig. 2) that the presence of $Co/SiO_2$ sample at a metal concentration of $10^{-4}$ M has a cumulative effect with the improvement of oxygen transfer from ambient air to the liquid culture medium. This is confirmed by the degradation rate achieved during the first 3 days of culture in 250-mL baffled flasks (i.e., 107 ± 3 ppm/day) that was around 15 and 85 % higher than the 93 ± 11 and 58 ± 7 ppm/day achieved in classical culture conditions (100 mL flasks) with or without $NP_S$ ($p < 0.05$). Similar trends were recorded for the biodegradation yields achieved after 18 days of incubation (i.e., 91 ± 1, 81 ± 1 and 53 ± 1 % of biphenyl conversion). However, the biodegradation rate decreased after 3 days. This decrease in the kinetic pattern coincides with the release of soluble metabolites, which gives a yellow color to the medium, indicating that the biphenyl is partially metabolized by *R. erythropolis* T902.1 [61].

The physico-chemical properties, such as the specific surface area, $S_{BET}$, the size of silica particles, $d_{SiO_2}$, the size of metallic nanoparticles, $d_{MX+}$, (Table 1) of $Cu/SiO_2$, $Co/SiO_2$, $Ag/SiO_2$ and $Pd/SiO_2$ samples could have an important impact on the biphenyl degradation by *Rhodococcus*. $Pd/SiO_2$ sample has the smallest $d_{SiO_2}$ but the greatest $S_{BET}$ (i.e., 13 nm and 495 $m^2/g$, respectively). By comparison, $Co/SiO_2$ sample presents the smallest $S_{BET}$ (i.e., 75 $m^2/g$), while $d_{SiO_2}$ is equal to 25 nm. These significant differences and the similar $d_{MX+}$ of all the tested NPs suggest that no real impact of $d_{MX+}$, $d_{SiO_2}$ or the $S_{BET}$ might be related to the biphenyl degradation rate by *Rhodococcus* in presence of $Co/SiO_2$ and $Pd/SiO_2$ samples. Moreover, these properties could not explain the lower biphenyl degradation rate recorded in presence of $Ag/SiO_2$ and $Cu/SiO_2$ samples since they are of the same order than those of $Pd/SiO_2$ sample.

### 3.2 Effect of different concentrations of $Co^{2+}$ ions and $Co/SiO_2$ sample on biphenyl biodegradation

The improvement effect of different forms of $Co/SiO_2$ sample (i.e., dried, calcined and reduced) and $Co^{2+}$ ions at different cobalt concentrations, i.e., $10^{-6}$, $10^{-5}$ or $10^{-4}$ M was studied. The results show that the biphenyl biodegradation yields increased with the concentration of $Co/SiO_2$ sample present in the vessels, whatever $Co/SiO_2$ sample is dried, calcined and/or reduced (Table 2). Significantly different yields were observed when the cobalt concentration is decreased from $10^{-5}$ to $10^{-6}$ M. However, the yields were similar in the presence of $Co/SiO_2$ sample at $10^{-5}$ and $10^{-4}$ M of cobalt, biphenyl being degraded up to about 80 % after 18 days of incubation. This suggests that an excess of cobalt up to $10^{-4}$ M has no real impact on the efficiency of biphenyl bioconversion except with the reduced cobalt $NP_S$ yielding at 77 ± 2 % instead of about 82 % with both the other calcined or dried forms (Table 2). Indeed, in dried $Co/SiO_2$ sample, the oxidation state of cobalt is 2+ and in calcined $Co/SiO_2$ sample, the oxidation states can be 2+ or 3+ because these nanoparticles are present as metallic oxide in the form $Co_3O_4$ [62]. In contrast, the oxidation state of cobalt is zero in reduced $Co/SiO_2$ sample. The effect of these differences in cobalt oxidation state on biphenyl biodegradation should be further investigated and also in relation with the availability of the cobalt-containing compounds as highlighted by Santos et al. [63] demonstrated that the presence of different forms of solubles iron at the concentrations of $10^{-4}$ M ($FeCl_3$, $Fe(NO_3)_3$, $Fe_2O_3$, $FeSO_4$) had a positive impact on biodegradation of anthracene by *pseudomonas* sp with up to 25 % higher biodegradation yield than in the iron nitrate.

**Table 3** Biphenyl biodegradation yields (and standard deviation on triplicates) after 18 days of incubation with *R. erythropolis* T902.1 in 100-mL flasks containing 20 mL of M284 minimal medium with 500 ppm biphenyl and different concentrations $10^{-6}$, $10^{-5}$, $10^{-4}$ M of calcined (copper or silver) nanoparticles encapsulated in $SiO_2$ matrix by sol–gel method or (copper or silver) ions

| Conditions | Biphenyl biodegradation yield | | |
|---|---|---|---|
| Control (+) | 53 ± 1 % | | |
| Metal concentration | $10^{-6}$ M (%) | $10^{-5}$ M (%) | $10^{-4}$ M (%) |
| $Cu/SiO_2$ calcined | 53 ± 1 | 43 ± 2 | 40 ± 1 |
| $Cu^{2+}$ | 51 ± 1 | 38 ± 2 | 35 ± 1 |
| $Ag/SiO_2$ calcined | 50 ± 1 | 43 ± 1 | 39 ± 1 |
| $Ag^{+}$ | 33 ± 1 | 29 ± 1 | 20 ± 3 |

The effect of $CoCl_2$ was also studied using the same concentrations tested for $Co/SiO_2$ sample. The results show that $CoCl_2$ has a strong positive effect on the efficiency of biphenyl biodegradation at different concentrations ranging from $10^{-6}$ to $10^{-4}$ M (Table 2) compared to the cultures with $Co/SiO_2$ sample. This is consistent with an improvement effect due to a higher solubility of the benefic metallic element as reported by Santos et al. [63]. By contrast, Yeom and Yoo [64] achieved a complete inhibition of benzene and toluene biodegradation by *Alcaligenes xylosoxipans* Y234 in presence of cobalt at a concentration of $2.5 \times 10^{-4}$ M. Kotresha and Vidyasagar [29] also reported a twofold decrease of phenol degradation rate by *Pseudomonas aeruginosa* MTCC 4996 in the presence of $2 \times 10^{-4}$ M of cobalt compared to $2.5 \times 10^{-5}$ M.

### 3.3 Effect of different concentrations of silver and copper ions ($Ag^{+}$ or $Cu^{2+}$) and $Ag/SiO_2$ or $Cu/SiO_2$ samples on biphenyl biodegradation

Table 3 shows that the presence of $Cu/SiO_2$ or $Ag/SiO_2$ sample in the culture medium at a metal concentration from $10^{-5}$ to $10^{-4}$ M leads to about 20 % lower biphenyl degradation yields compared to the positive control ($p < 0.05$), while no significant effect was recorded at a concentration of $10^{-6}$ M. In addition, Table 3 shows significantly higher negative impact in presence of silver and copper ions at the same concentrations. Furthermore, the effect was already measurable at $10^{-6}$ M of $Ag^{+}$ ions. Therefore, it can be assumed that, since the metallic NPs were anchored in silica, the $Ag/SiO_2$ and $Cu/SiO_2$ samples were less toxic toward bacteria at similar concentrations than the tested copper chloride or the silver nitrate. These results are consistent with those of the literature attributing metal toxicity to the dissolved ions [65] and reporting inhibition of different bacterial metabolisms at concentrations of about $2 \times 10^{-5}$ M of soluble copper [59, 66]. Additionally, the encapsulation of the metal NPs inside the porous silica matrix would limit the metal availability in the bacterial environment even when influenced by the suggested siderophore mechanism (Sect. 3.1).

On the other hand, Table 3 shows that the $Ag^{+}$ ions effectively reduced the biphenyl degradation yields at all tested concentrations ($10^{-6}$, $10^{-5}$ or $10^{-4}$ M) as compared to the $Cu^{2+}$ ions, whereas, at a same concentration, similar yields were recorded for both anchored NPs in silica. Moreover, the lowest degradation yield recorded in

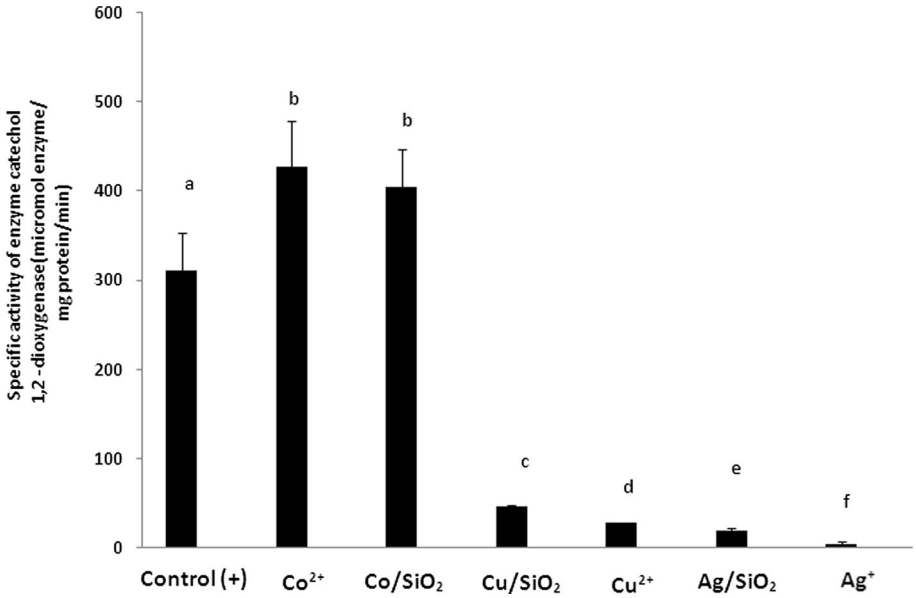


**Fig. 3** Effect of $10^{-4}$ M of calcined (cobalt, copper or silver) nanoparticles encapsulated in $SiO_2$ matrix by sol–gel method or (cobalt, copper or silver) ions on catechol 1,2-dioxygenase activity. *Different letters* above histograms means that the results are significantly different from the control ($p < 0.05$). The *error bars* means standard deviation on triplicates

presence of $Ag/SiO_2$ (40 ± 1 % at $10^{-4}$ M Ag) was still higher than the 33 ± 1 % measured at $10^{-6}$ M $Ag^+$. These results highlight the tremendous toxicity of $Ag^+$ ions on the potential of the *R. erythropolis* T902.1 for biphenyl degradation compared to the $Cu^{2+}$ ions and the encapsulated NPs. Furthermore, it is to mention that Kuo and Sharak Genthner [65] reported a positive effect of $Cu^{2+}$ ions at 0.01 ppm (i.e., about $10^{-7}$ M) on the degradation of benzoate, 2-chlorophenol and both phenol and benzoate by anaerobic bacterial consortia.

Despite low concentrations of $Cu^{2+}$ ions are essential for bacteria since they provide vital cofactors for metalloproteins and enzymes [67], these ions have an inhibitory action on bacteria by blocking essential functional groups, replacing other essential metal ions, or modifying the active conformation of biological molecules [68]. However, the bacteria have different strategies to deal with toxic metal concentration in the environment [69]. These strategies can be divided into two classes: (1) prevent entry of the metal into the cell and (2) actively pump the toxic metal out of the cell [70].

### 3.4 Effect of metallic ions ($Co^{2+}$, $Ag^+$ or $Cu^{2+}$) and the nature of $Co/SiO_2$, $Ag/SiO_2$ or $Cu/SiO_2$ samples on catechol 1,2-dioxygenase activity

Various aerobic bacteria catabolism of PAH involve two key steps that are (1) the activation of the aromatic ring and (2) its subsequent cleavage [71]. Dioxygenase enzymes are responsible for the conversion of these compounds by adding molecular oxygen to the ring. In the case of biphenyl metabolism, these enzymes are called biphenyl dioxygenases [72]. In the second step, catechol dioxygenases are responsible to open the ring through ortho or meta oxidation, i.e., the catechol 1,2-dioxygenase plays a central role in the cleavage of the aromatic ring of catechol to cis, cis-muconic acid in presence of 1 mol of oxygen [50, 73]. The biodegradation mechanism of biphenyl is well characterized in several bacterial strains such as *Burkholderia xenovorans* LB400 [74], *Achromobacter* sp. BP3 [61] and *Rhodococcus* sp. [25, 67, 75]. Takeda et al. [25] proposed the major pathway for the *Rhodococcus* sp. strain RHA1 as follows: biphenyl oxidation to 2,3-dihydroxybiphenyl by 1,2-dioxygenase, followed ring cleavage to form 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate, which is then cleaved to benzoate and 2-hydroxypenta-2,4-dienoate. The effects of dissolved metallic ions or metal $NP_S$ encapsulated in porous silica matrix were investigated on the activity of catechol 1,2-dioxygenase. The results are shown in Fig. 3. The $Co^{2+}$ ions or $Co/SiO_2$ sample at $10^{-4}$ M of metal has a slight positive effect on the efficiency of enzyme activity ($p < 0.05$). By contrast, the results indicated that the addition of $10^{-4}$ M of $Ag^+$ or $Cu^{2+}$ ions or $Ag/SiO_2$ or $Cu/SiO_2$ samples inhibited the enzymatic reaction as compared to the positive control ($p < 0.05$). These trends are in accordance with the results discussed in the former sections regarding the biphenyl biodegradation yields. They confirm some experimental data reported in the literature. Nadaf and Ghosh [50] showed that the activity of catechol 1,2-dioxygenase was minimally affected by $Co^{2+}$ ions for concentrations lower than $10^{-3}$ M, whereas the enzymatic reaction was completely inhibited by the addition of $10^{-3}$ and $10^{-2}$ M of $Co^{2+}$ ions. Similarly, Yeom and Yoo [64] reported that silver ions at low concentrations have a strong inhibitory effect on the catechol 1,2-dioxygenase.

## 4 Conclusions

The present study investigates the potential of the *R. erythropolis* T902.1 to biodegrade biphenyl in the presence of encapsulated metals ($Co/SiO_2$, $Pd/SiO_2$, $Ag/SiO_2$ or $Cu/SiO_2$) inside a porous silica matrix and metallic ions ($Co^{2+}$, $Cu^{2+}$ or $Ag^+$) at different concentrations $10^{-6}$, $10^{-5}$ or $10^{-4}$ M. Biodegradation experiments found that silver ions are the most toxic to the *R. erythropolis* T902.1, already at a concentration of $10^{-6}$ M. The presence of $10^{-5}$ or $10^{-4}$ M of $Cu^{2+}$ ions has also an inhibitory effect on the microbial metabolism and particularly on the activity of catechol 1,2-dioxygenase, a major enzyme involved in the biodegradative pathways of biphenyl.

By contrast, the results show a strong positive effect of $Co^{2+}$ ions on biphenyl biodegradation at different concentrations with a 70 % higher degradation yield recorded at $10^{-4}$ M of $Co^{2+}$ compared with the control conditions without metal ions of cobalt. On the other hand, the results indicate that the addition of $Co/SiO_2$ or $Pd/SiO_2$ samples at the same $10^{-4}$ M of metal concentration also stimulates biphenyl biodegradation with around 30 % higher degradation yield than in control conditions. The highest biphenyl biodegradation rate and yield, i.e., 107 ± 3 and 91 ± 1 % ppm/day, respectively, were recorded in 250-mL baffled flasks stirred at 150 rpm with $10^{-4}$ M of $Co/SiO_2$ sample. They are about 85 and 70 % higher than in control conditions without $NP_S$. As a consequence, two advantages are suggested for the use of metallic NPs encapsulated inside silica matrix in order to improve *R. erythropolys* activity: the encapsulation by sol–gel method (1) prevents agglomeration of the active sites, maintaining a high specific surface area and (2) limits the availability of the anchored elements to the microorganisms able to produce siderophore-like molecules for catching them.

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