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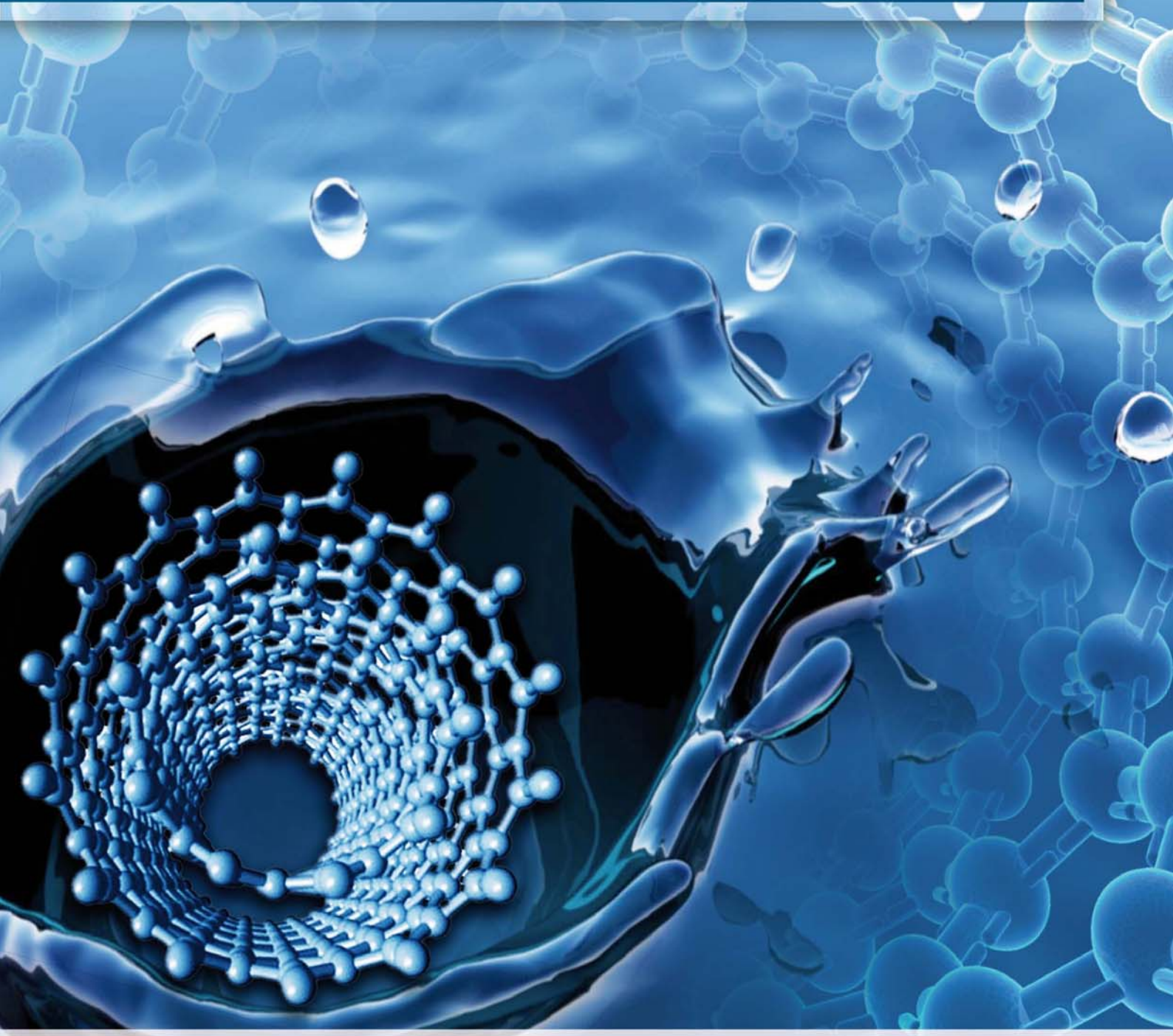

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Effects of rhamnolipid biosurfactant JBR425 and synthetic surfactant Surfynol465 on the peroxidase-catalyzed oxidation of 2-naphthol

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Abstract

The kinetics of the recombinant *Coprinus cinereus* peroxidase-catalyzed 2-naphthol oxidation was investigated in the presence of rhamnolipid biosurfactant JBR425 and synthetic surfactant Surfynol465 at pH 5.5 and 25°C, with concentrations of (bio)surfactants both less than critical micelle concentrations (CMC) and larger than CMC. It was shown that monomers of JBR425 as well as monomers of Surfynol465 had an enhancing effect on the conversion of 2-naphthol in dose response manner and did not influence the initial rate of 2-naphthol oxidation. The results were accounted by a scheme, which contains a stadium of enzyme inhibition by oligomeric 2-naphthol oxidation products. The action of the biosurfactant's (or synthetic surfactant's) monomers was explained by avoidance of the enzyme active center clothing with oligomers. Similar results have demonstrated the potential of rhamnolipid biosurfactant JBR425 due to its biodegradability. When biosurfactants' concentrations are larger than CMC, (bio)surfactants have an opposite effect on the oxidation of 2-naphthol by peroxidase.

Key words: oxidation; peroxidase; biosurfactant; critical micelle concentration

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Introduction

2-Naphthol, containing hydroxyl group at beta position and depending on phenolic compounds, is widely used for dyes, medicinal organics and synthetic perfumes production (Gosselin et al., 1976; Roch and Alexander, 1995). Therefore, 2-naphthol as most of phenolic compounds is toxic and classified as dangerous pollutants (Croera et al., 2008; Puy-Azurmendi et al., 2010). If released into the environment, it may accumulate in ground water, surface water, in sediments, in the fat of exposed animals and remain in the environment in contaminating levels (Puy-Azurmendi et al., 2010; Zang et al., 2010). Peroxidases and other phenol-oxidizing enzymes might be useful for removing phenolic contaminants from water or wastewater as an alternative to other methods of treatment (Ghioureliotis and Nicell, 1999; Klibanov et al., 1983; Masuda et al., 2001). Also, the compounds produced during oxidative coupling of 2-naphthol may be useful as a new polymeric materials (**Fig. 1**) as an alternative method used instead of harmful phenol-phormaldehyde method (Reihmann and Ritter, 2006). However, some actions, such as the interactions of phenoxyl radicals with the active center of enzymes and the clothing of active center of enzymes with the oligomeric products formed during the reaction, can lead to the enzyme inactivation during the oxidation of phenolic compounds (Ji et al., 2009; Kulys and Ivanec-Goranina, 2009; Ziemys and Kulys, 2005; Wu et al., 1998). In an attempt to decrease the inhibition rate, some additives were used (Bratkovskaja et al., 2004; Kinsley and Nicell, 2000; Kulys et al., 2007; Modaressi et al., 2005). It was shown that the yield of phenol derivatives oxidation catalyzed by *Coprinus cinereus* peroxidase significantly increased if albumins or non-ionic polymeric compounds were used (Bratkovskaja et al., 2004; Kulys et al., 2007). Several studies have shown that the addition of surfactants can prevent enzyme inhibitions (Ji et al., 2009; Kulys and Ivanec-Goranina, 2009; Liu et al., 2008). However, these investigations mainly focus on chemical additives.

Recently, biosurfactants have gained more and more attentions because of their special characteristics, such as biodegradability, low toxity and safety (Fu et al., 2007; Kitamoto et al., 2002; Zeng et al., 2005). One of the most extensively studied biosurfactants is rhamnolipids (Liu et al., 2012; Noordman et al., 2000; York and Firoozabadi,

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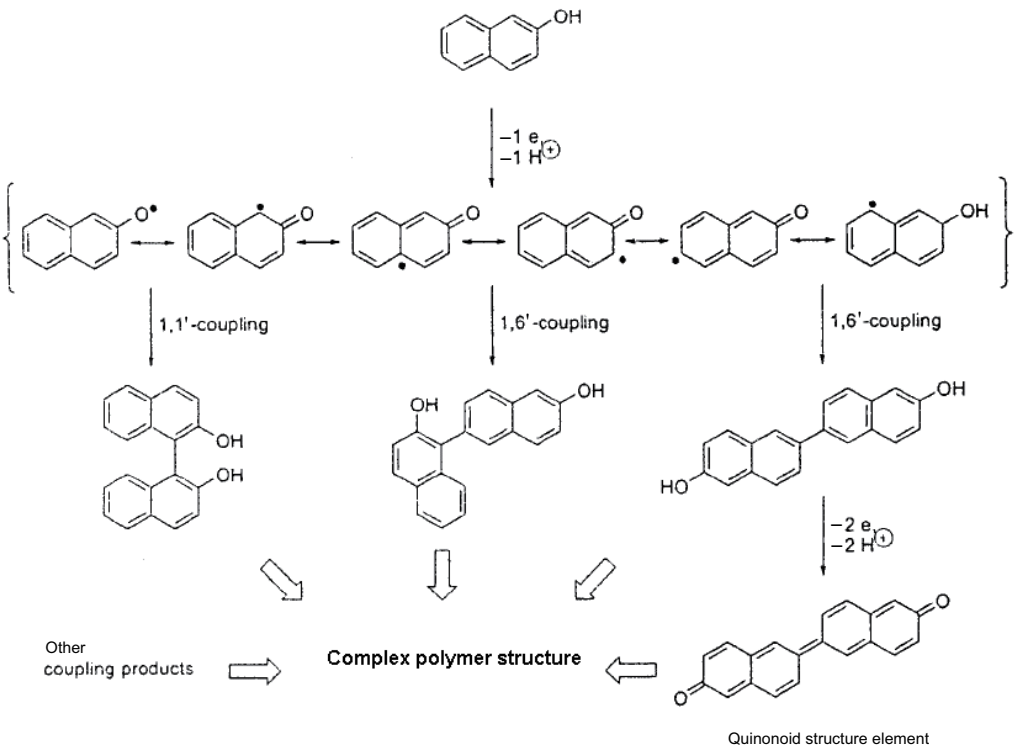

Fig. 1 Polymerization of 2-naphthol. Various radical resonance structures resulting from one-electron oxidation of 2-naphthol in the presence of peroxidase and H_2O_2 (Reihmann and Ritter, 2006).

2008). Chemically, the major rhamnolipids are glycosides of rhamnose (6-deoxymannose) and B-hydroxydecanoic acid. Typical commercial products consist of both the monorhamnolipid and dirhamnolipid forms (**Fig. 2**).

The objective of this study was to investigate the effect of rhamnolipid biosurfactant JBR425 on 2-naphthol oxidation catalyzed by recombinant *Coprinus cinereus* peroxidase. The effect of rhamnolipid was also compared with the synthetic nonionic gemini surfactant Surfynol465 (ethoxylated 2,4,7,9-tetramethyl-5-decyne-4,7-diol, **Fig. 3**). Surfynol465 is not related to JBR425, but compared to other synthetic surfactants is less toxic. The producer of Surfynol465 Air Products states in its technical documentation, that Surfynol465 demonstrates very low aquatic toxicity (Air Products, 2001). On the other hand such synthetic surfactants as SDS, CTAB and Triton X-100 are very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment (Dayeh et al., 2004; Li, 2008). Also, a number of academic studies have demonstrated the potential usefulness of Surfynol family surfactants, e.g. for the synthesis of silica-bound stationary phases for applications in gas and liquid chromatography (Wickramanayake and Aue, 1986); for complex formation with a variety of polymers in order to improve the quality of water-based printing inks (Krishnan and Sprycha, 1999); for their reducing and stabilizing abilities in the preparation of gold nanoparticles (Sato et al., 1999); for

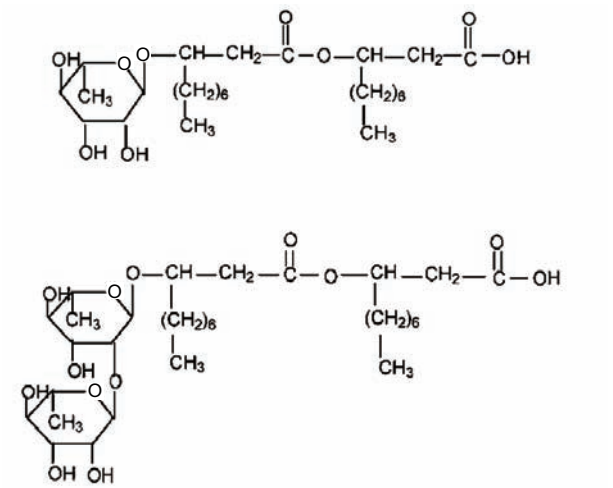

Fig. 2 Structure of JBR425 (York and Firoozabadi, 2008). Two forms of rhamnolipid (monorhamnolipid and dirhamnolipid) are present.

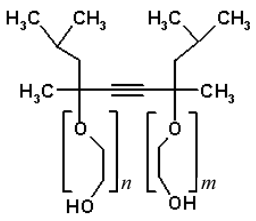

Fig. 3 Structure of Surfynol465 (Páhi et al., 2009).

their wetting efficiency in the aqueous colloidal processing of ceramics (Navarro et al., 2004). Therefore Surfynol465 was selected for comparison due to its wide usage and low aquatic toxicity despite the fact that it is not a green chemical and its usage may be undesirable.

1 Materials and methods

1.1 Reagents

Recombinant *Coprinus cinereus* peroxidase (rCiP) was received from Novozymes A/S (Denmark). 2-Naphthol was from Aldrich (Germany). Hydrogen peroxide (30%) was obtained from Polskie Odczynniki Chemiczne S.A. (Poland). Surfactant Surfynol465 was kindly donated by Air Products Nederland BV (the Netherlands). Rhamnolipid biosurfactant JBR425 was received from Jeneil Biosurfactant Co. (USA). It is a mixture of two forms, shown in **Fig. 2**, at 25 wt.% in water.

Sodium acetate was a product of Chempur (Poland). The rCiP and hydrogen peroxide solutions were prepared in deionized water and concentrations of these substances were determined spectrophotometrically by a computer-controlled Nicolet evolution 300 spectrophotometer (Thermo Electron Corporation, USA). For rCiP and hydrogen peroxide, the extinction coefficients are ε_{405} = 109 L/(mmol·cm) (Andersen et al., 1991) and ε_{240} = 39.4 L/(mol·cm) (Nelson and Kiesow, 1972), respectively. Sample of 2-naphthol was weighted and dissolved in deionized water. Surfynol465 and JBR425 solutions were prepared in 50 mmol/L acetate buffer pH 5.5.

1.2 Critical micelle concentration determination

The critical micelle concentration (CMC) of surfactants in 50 mmol/L sodium acetate buffer at pH 5.5 and 25 ± 0.1°C in 1-cm thermostated plastic cuvette was measured by using a computer-controlled Zetasizer Nano-ZS analyzer (Malvern Instruments, UK). This analyzer contains a 4-mW He-Ne laser operating at a wavelength of 633 nm and an avalanche photodiode detector. The scattered light is detected at an angle of 173° and optics arrangement maximizes the detection of scattered light while maintaining signal quality. For dynamic light scattering measurements 50 mmol/L acetate buffer solution (pH 5.5), the Surfynol465 and JBR425 solutions were filtered (pore size = 200 nm). The Surfynol465 concentration in the solutions was varied up to 24 mmol/L with an increment of 1 mmol/L. The JBR425 concentration in the solutions was varied up to 0.1 mmol/L with an increment of 0.02 mmol/L and up to 1.2 mmol/L with an increment of 0.1 mmol/L. All prepared solutions were allowed to equilibrate for 15 to 20 min and hydrodynamic diameters were measured.

1.3 Kinetic measurements

The kinetic measurements were performed at (25 ± 0.1)°C in 1-cm thermostated quartz cuvette by using a computer-controlled Aminco Bowman luminescence spectrometer (Thermo Electron Corporation, USA). The fluorescence of 2-naphthol was measured at 460 nm and at excitation 328 nm. The kinetic measurements were performed in 50 mmol/L sodium acetate buffer solution at pH 5.5 and 25°C and the reaction mixture contained 25 μmol/L 2-naphthol, 1 nmol/L rCiP, 100 μmol/L of hydrogen peroxide, 0–30 mmol/L of Surfynol465, 0–3 mmol/L of JBR425. The reactions started with addition of the enzyme solution.

1.4 Calculations

The fluorescence intensity of 2-naphthol was standardized by the initial concentration of 2-naphthol, when concentrations of (bio)surfactant were less than CMC. When concentrations of biosurfactant are larger than CMC, micelles of JBR425 and Surfynol465 show little fluorescence. Fluorescence intensity of biosurfactants' micelles was calibrated using (bio)surfactants' solutions of various concentrations at the same conditions. Fluorescence intensity of 2-naphthol was received by removing fluorescence intensity of (bio)surfactants' micelles from common fluorescence intensity. Then fluorescence intensity of 2-naphthol was normalized in proportion to concentration of 2-naphthol. The initial reaction rate (V) was calculated as a slope of substrate concentration change in 50 sec time interval – the initial linear portion of each recording.

Computer programs OriginPro and Mathcad 2001 Professional were employed for data processing. The kinetic parameters (k_1, k_2, k_3, k_4, $k_5 = k_6 = k_{in}$) were calculated using KinFitSim 2.1 program (Svir et al., 2004). KinFitSim uses the summation of squared differences between calculated and experimental data points (SSQ) to determine the quality of fit between simulated and experimental progress curves. The program uses non-linear least squares regression to find the best fit between experimental and calculated curves. The search technique was used for parameter optimization, or for finding the minimum of the SSQ function.

2 Results and discussion

2.1 Critical micelle concentration determination of JBR425/Surfynol465

JBR425 and Surfynol465 are the amphiphilic molecules. At low concentration, they can exist in a monomer. At high concentration, they can aggregate forming a micelle. There is a particular concentration characteristic of the (bio)surfactant (in a given solvent) at or above which these will form micelles and this is called the critical micelle concentration (CMC). We determined the CMC of JBR425 and Surfynol465 at 25°C in 50 mmol/L sodium acetate buffer (pH 5.5) using the dynamic light scattering technique (**Fig. 4**).

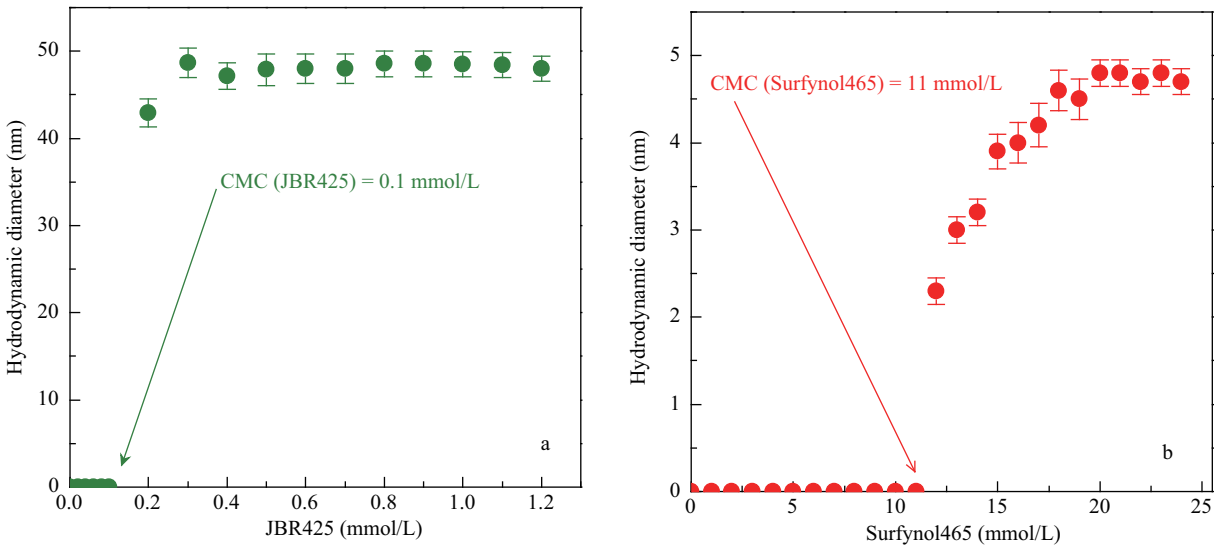

Fig. 4 Dependence of hydrodynamic diameter on various concentrations of JBR425 (a) and on various concentrations of Surfynol465 (b) prepared in 50 mmol/L acetate buffer solution pH 5.5, 25°C with defined CMC. Vertical lines with cap indicate error bars.

Figure 4a shows the variation of the hydrodynamic diameter with the JBR425 concentration at 25°C. It indicates that the CMC of JBR425 was 0.1 mmol/L in the buffer solution system. **Figure 4b** shows the variation of the hydrodynamic diameter with the Surfynol465 concentration at 25°C, where the CMC of surfactant was 11 mmol/L in the buffer solution system. Further studies showed that influence of monomers and micelles of (bio)surfactants on peroxidase-catalyzed 2-naphthol oxidation differ. The influence difference is presented and discussed in further sub-sections.

2.2 Kinetics of 2-naphthol oxidation without (bio)surfactants

Figure 5 shows the kinetics of peroxidase-catalyzed 2-naphthol oxidation in the absence surfactants. Enzyme, hydrogen peroxide and the products of the reaction do not show any fluorescence, therefore decrease of fluorescence intensity corresponds only to 2-naphthols concentration decrease. During oxidation of 2-naphthol in the absence surfactants kinetic curves have saturated. The addition of new portion of hydrogen peroxide did not affect the reaction. On the other hand, the addition of a new portion of enzyme caused further 2-naphthol oxidation. This meant the enzyme inhibition during the reaction.

2.3 Effect of JBR425/Surfynol465 monomers on 2-naphthol oxidation

Figure 6a shows the kinetics of peroxidase-catalyzed 2-naphthol oxidation in absence and presence of rhamnolipid biosurfactant JBR425. With a small addition (up to 3 μmol/L) of biosurfactant the conversion of 2-naphthol increased gradually. It completely retarded the enzyme inhibition in presence of 6 μmol/L–0.1 mmol/L concentration of biosurfactant. As shown in **Fig. 4a**, within this concentration range JBR425 exists in a monomer (up to

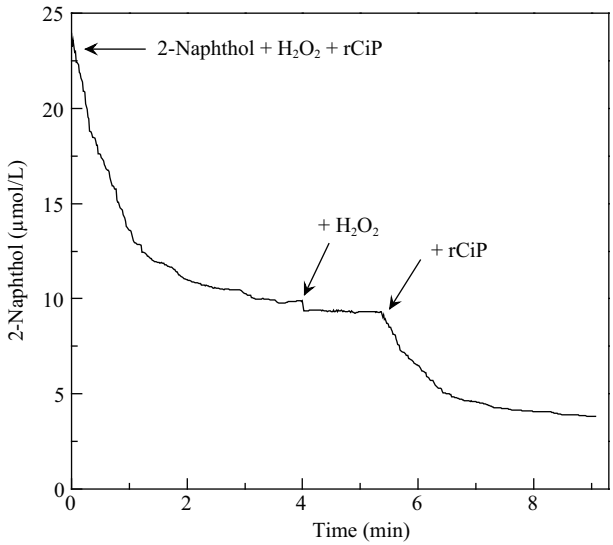

Fig. 5 Influence of additives (hydrogen peroxide (H_2O_2) and enzyme (rCiP)) on the peroxidase-catalyzed oxidation of 2-naphthol. Conditions: 50 mmol/L acetate buffer solution pH 5.5, 25°C. Concentrations: 25 μmol/L 2-naphthol, 1 nmol/L rCiP, 100 μmol/L H_2O_2.

CMC). Also, JBR425 (up to CMC) did not influence the initial rate of 2-naphthol oxidation.

The rhamnolipid biosurfactant JBR425 based system was compared with the system based on synthetic non-ionic surfactant Surfynol465 under the same conditions. **Figure 6b** shows the kinetics of peroxidase-catalyzed 2-naphthol oxidation in absence and presence of synthetic surfactant Surfynol465. Surfactant enhanced the conversion of 2-naphthol in presence of small concentration (up to 2 μmol/L) of surfactant in dose response manner and had completely prevented enzyme inhibition in presence of 3 μmol/L –11 mmol/L concentration of surfactant. As shown in **Fig. 4b**, within this concentration range Surfynol465 also exists in a monomer (up to CMC). Also, Surfynol465

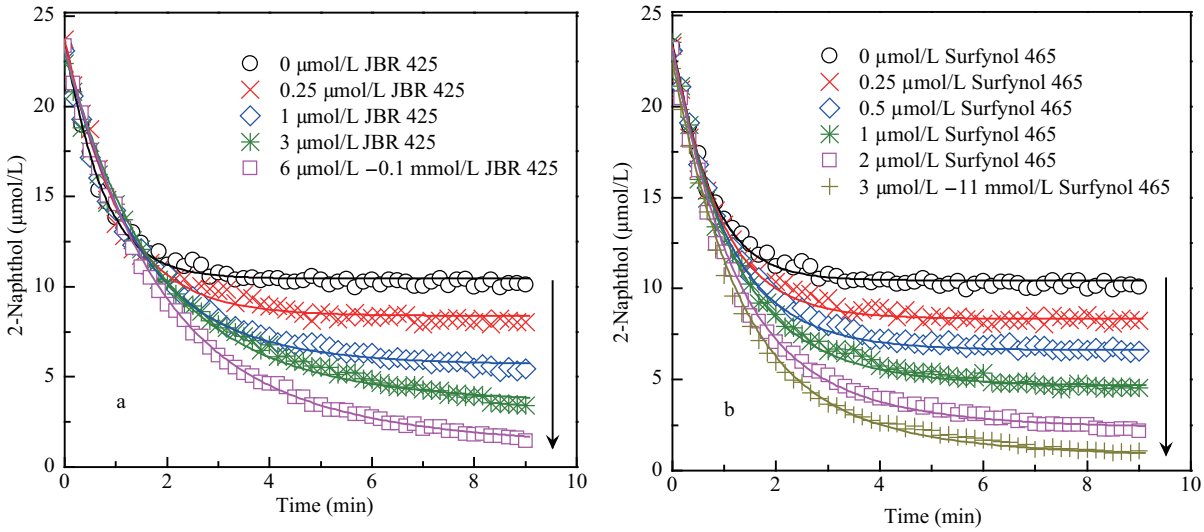

Fig. 6 Kinetics of peroxidase-catalyzed 2-naphthol oxidation in presence rhamnolipid (bio)surfactant JBR425 (a) and synthetic surfactant Surfynol465 (b), when concentrations of (bio)surfactants are less than CMC. The reaction mixture contained 25 μmol/L 2-naphthol, 1 nmol/L rCiP, 100 μmol/L H_2O_2, 0–0.1 mmol/L JBR425 (a); 0–11 mmol/L Surfynol465 (b), in 50 mmol/L acetate buffer pH 5.5, 25°C. Curves were drawn following a model (Reactions (1)–(6)).

(up to CMC) did not influence initial rate of 2-naphthol oxidation. In summary, JBR425 as well as Surfynol465 has an enhancing effect on the oxidation of 2-naphthol by peroxidase when their concentrations are less than CMC.

The saturation of reaction rate during 2-naphthol oxidation shows that peroxidase is inhibited during the reaction (**Fig. 5**). The prevention of inhibition by addition of (bio)surfactants' monomers indicates that inhibition proceeds by enzyme interaction with oxidation products. The enzymatic reaction could be considered as a modified type of ping-pong kinetics. This is referred to as peroxidase ping-pong by Dunford (1990), which can be represented by the following reaction scheme:

$$E + H_2O_2 \xrightarrow{k_1} cpd\text{I} + H_2O \quad (1)$$

$$cpd\text{I} + Naph \xrightarrow{k_2} cpd\text{II} + Naph\cdot \quad (2)$$

$$cpd\text{II} + Naph \xrightarrow{k_3} E + Naph\cdot + H_2O \quad (3)$$

$$Naph\cdot + Naph\cdot \xrightarrow{k_4} \text{Interim} \xrightarrow{\text{Fast}} ... \longrightarrow Olig \quad (4)$$

Native peroxidase (*E*) is oxidized by hydrogen peroxide (H_2O_2) with compound I (*cpd*I) formation. The *cpd*I in its turn oxidizes 2-naphthol (*Naph*) *via* the formation of radical (*Naph*·) and compound II (*cpd*II) (Reactions (1)–(3)). The resulting radicals can reacts chemically forming intermediates (Interim, Reaction (4)) that is a subject to further oxidation by peroxidase to constitute oligomers (*Olig*) and higher polymers until the solubility limit is reached.

To calculate kinetic parameters of inhibition the "classical" scheme of peroxidase action was complemented by bimolecular reaction of native enzyme and *cpd*I interaction with the oligomers (Reactions (5)–(6)) according to the scheme described in our previous article (Kulys and Ivanec-Goranina, 2009):

$$\text{E} + Olig \xrightarrow{k_5} E_{\text{in}} \quad (5)$$

$$cpd\text{I} + Olig \xrightarrow{k_6} E_{\text{in}} \quad (6)$$

The rate of these reactions is related to unspecific native peroxidase and *cpd*I interaction with oligomers therefore k_5 is assumed similar to k_6 and these constants were named as k_{in}. The *cpd*II can also contribute to enzyme inhibition, but this process was not included into the scheme due to low *cpd*II concentration and symmetry of *cpd*I inhibition. If the rate of monomers oligomerization is high, the limiting step of oligomers formation is the single oxidized product (radical, *Naph*·) production. Therefore, the kinetic scheme of the enzyme inhibition can be simplified by Reactions (5) and (6). Reactions (5) and (6) were assumed as irreversible since peroxidase inactivation was irreversible (Baynton, 1994).

The solution of the system of differential equations describing the kinetic scheme (Reactions (1)–(6)) was done using KinFitSim program (Svir et al., 2004). The second order constant of hydrogen peroxide reaction with the native enzyme (Reaction (1)) was taken as $k_1 = 7.1 \times 10^6$ L/(mol·sec) at 25°C (Andersen et al., 1991). The fitting of data gave the same value of k_1 at different JBR425 as well as Surfynol465 concentrations. This means that neither JBR425 monomers nor Surfynol465 monomers does not interact with hydrogen peroxide and native peroxidase. The fitting of data gave constants of 2-naphthol reactivity with compound I (k_2, Reaction (2)) and with compound

II (k_3, Reaction (3)), which were similar with different biosurfactants as well as surfactants concentrations. In case of JBR425, a mean value of k_2 was (1.4 ± 0.1) × 10^7 L/(mol·sec) and k_3 was (0.8 ± 0.2) × 10^7 L/(mol·sec) if biosurfactant concentration have changed between 0 and 0.1 mmol/L (**Table 1**). In case of Surfynol465, a mean value of k_2 was (1.5 ± 0.2) × 10^7 L/(mol·sec) and k_3 was (1.1 ± 0.1) × 10^7 L/(mol·sec) if surfactant concentration have changed between 0 and 11 mmol/L (**Table 1**).

The results have shown that k_2 and k_3 values did not practically change at different (bio)surfactant as well as surfactant concentrations. This also means that neither JBR465 monomers nor Surfynol465 monomers does not interact with peroxidase. While the inhibition constant k_{in} value permanently decreased if (bio)surfactants' concentration has increased (**Table 1**). Such the decrease of k_{in} value when (bio)surfactants' concentration has increased could be explained by reversible oligomers interaction with monomers of (bio)surfactant (M):

$$Olig + M \longleftrightarrow Olig\_M \tag{7}$$

where, association constant K_{ass} can be expressed as:

$$K_{ass} = \frac{[Olig\_M]}{[Olig] \times [M]} \tag{8}$$

At equilibrium the concentration of (bio)surfactants' monomers (Eq. (9)) and of oligomers (Eq. (10)) is:

$$[M] = [M]_t - [Olig\_M] \tag{9}$$

$$[Olig] = [Olig]_t - [Olig\_M] \tag{10}$$

Solving Eqs. (8)–(10) produces expression of oligomers concentration:

$$[Olig] = \frac{1}{2K_{ass}}\left[(K_{ass} \times [Olig]_t - K_{ass} \times [M]_t - 1 + \sqrt{K_{ass}^2 \times [M]_t^2 - 2K_{ass}^2 \times [M]_t \times [Olig]_t + 2K_{ass} \times [M]_t + K_{ass}^2 \times [Olig]_t^2 + 2K_{ass} \times [Olig]_t + 1}\right] \tag{11}$$

where, $[Olig]_t$ and $[M]_t$ are the total concentration of oligomers of 2-naphthol oxidation and monomers of (bio)surfactant, respectively.

The last equation shows that concentration of uncomplexed oligomer decreases when concentration of biosurfactant increases. The decrease of the oligomer concentration will decrease the calculated value of k_{in} (**Table 1**). Explanation of the mechanism of peroxidase inhibition the molecular clothing of active center by oligomeric compounds has been suggested recently (Wu et al., 1998; Ziemys and Kulys, 2005). Docking and molecular dynamics calculations have showed that oligomeric naphthols interacted with the enzyme stronger than the substrates. In contrast to the substrate, the binding of oligomers did not form productive complexes and blocked the active center (**Fig. 7a**). With a small addition of (bio)surfactants (less than CMC) the enzyme inhibition was prevented. This can be explained by the fact that monomers of (bio)surfactants join with oligomeric naphthols, which show strong hydrophobicity (Kulys and Ivanec-Goranina, 2009) and interact with biosurfactant's monomers (**Fig. 7b**). Similar results have demonstrated the potential of rhamnolipid biosurfactant JBR425 due to its biodegradability.

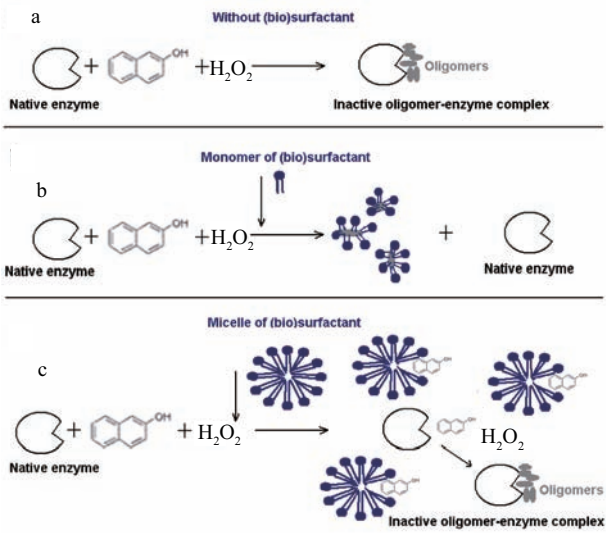

Fig. 7 Schematic representation of the peroxidase-catalyzed oxidation of 2-naphthol without (bio)surfactant (a), with addition of (bio)surfactant in a monomer form (b), with addition of (bio)surfactant in a micelle form (c).

2.4 Influence of JBR425/Surfynol465 micelles on 2-naphthol oxidation

In order to demonstrate an influence of (bio)surfactants' micelles on 2-naphthol oxidation, the kinetics of peroxidase-catalyzed 2-naphthol oxidation in presence of JBR425 (**Fig. 8a**) and Surfynol465 (**Fig. 8b**) with concentrations of (bio)surfactants larger than CMC were studied. With concentration of JBR425/Surfynol465 exceeding the CMC, the initial fluorescence of 2-naphthol (before introducing the enzyme) was gradually decreasing with the increase of biosurfactant concentration. After normalization of 2-naphthol fluorescence to concentration of 2-naphthol as it was described in Calculations sub-section, it can be seen that initial concentrations of 2-naphthol also decreased with the increase of (bio)surfactants' concentrations (before introducing the enzyme) (**Fig. 8**), although the same initial fixed 25 μmol/L concentration of 2-naphthol was used. It could be concluded that 2-naphthol molecules bind to micelles, forming non fluorescent associates, while unbound 2-naphthol shows fluorescence and the reaction of peroxidase-catalyzed 2-naphthol oxidation takes place in the buffer solution.

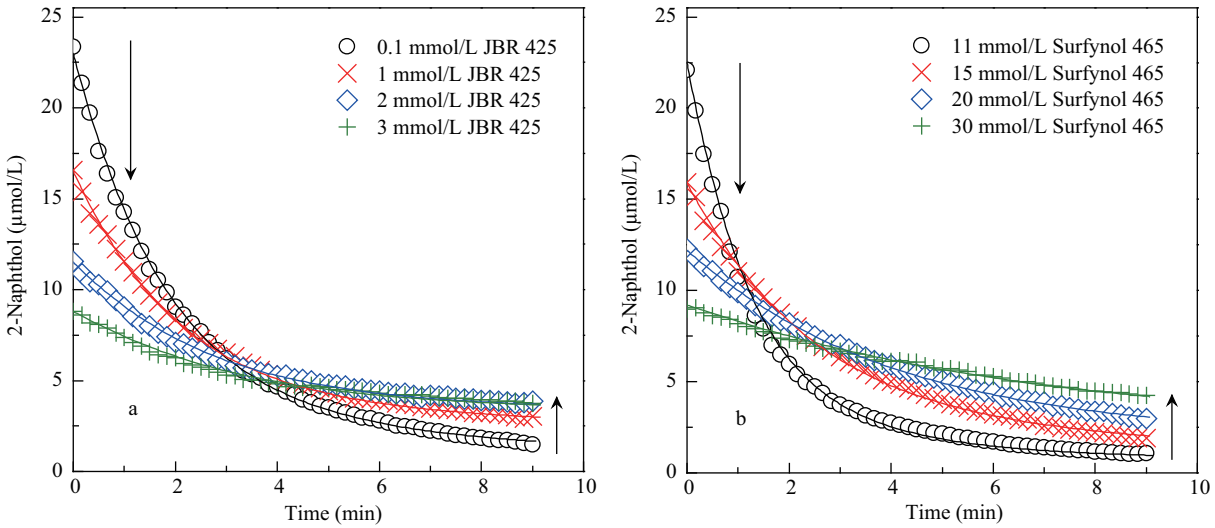

Fig. 8 Kinetics of peroxidase-catalyzed 2-naphthol oxidation in the presence of rhamnolipid biosurfactant JBR425 (a) and synthetic surfactant Surfynol465 (b), when concentrations of (bio)surfactants are larger than CMC. The reaction mixture contained 25 μmol/L 2-naphthol, 1 nmol/L rCiP, 100 μmol/L H_2O_2, JBR425, Surfynol465 in 50 mmol/L acetate buffer pH 5.5, 25°C. Curves were drawn following a model (Reactions (1)–(6)).

As in the monomers of (bio)surfactants case, the fitting of data gave constants of 2-naphthol reactivity with compound I (k_2, Reaction (2)), with compound II (k_3, Reaction (3)) and the inhibition constant k_{in}. In contrast to the monomers of (bio)surfactants case k_2 and k_3 values permanently decreased with the increase of (bio)surfactants concentration (**Table 2**). This means that JBR465 and Surfynol465 micelles influence the peroxidase. While rCiP is a hydrophilic enzyme. The isoelectric point of rCiP is approximately 3.5 and rCiP contains, on average, two glycosamines and 10–12 mannoses per molecule (Tams et al., 1999). That means that rCiP should not get into micelles. On the other hand the decreasing k_2 and k_3 values show that the reaction of peroxidase-catalyzed 2-naphthol oxidation in the buffer solution is slowing down. We can make an assumption that the reaction of peroxidase-catalyzed 2-naphthol oxidation in the buffer solution is slowing down due to the encirclement of enzyme by micelles (**Fig. 7c**). Increasing k_{in} values show that inhibition of enzyme increases gradually.

The calculated initial rate values of 2-naphthol oxidation (V) have also shown that the increase of both JBR425 and Surfynol465 concentrations decreased the oxidation rate (**Fig. 9**).

The quantitative treatment of data in terms of the Berezin "phseudo-phase model" of micellar catalysis (Berezin et al., 1973) is based on the assumption that a bimolecular interaction between reactants may occur both in the "micellar" pseudo-phase and the aqueous phase. As it has been already mentioned, rCiP is a hydrophilic enzyme. Therefore, to examine the kinetics data it was assumed that the enzyme is dissolved in water phase (in our case there is a buffer solution), and that the 2-naphthol distributes between water and the micellar phase, after that rCiP catalyzes the oxidation of the 2-naphthol that is dissolved in water solution. Following these assumptions the dependence of the calculated initial rate on the (bio)surfactants concentrations should be parabolic as expressed by Eq. (12) (Kulys and Vidziunaite, 2000):

$$V = \frac{V_w}{1 + P_{naph} \times V_m \times (c - CMC)} \quad (12)$$

Table 1 Kinetic parameters of peroxidase-catalyzed oxidation of 2-naphthol in the presence of JBR425 and Surfynol465

(Bio)surfactant	Concentration (μmol/L)	k_2 (L/(mol·sec))	k_3 (L/(mol·sec))	k_{in} (L/(mol·sec))
JBR425	0	1.6×10^7	1.0×10^7	8.0×10^3
	0.25	1.3×10^7	9.0×10^6	4.5×10^3
	1	1.3×10^7	7.0×10^6	2.9×10^3
	3	1.3×10^7	6.0×10^6	1.9×10^3
	6 μmol/L–0.1 mmol/L	1.4×10^7	6.5×10^6	1.0×10^3
Surfynol465	0	1.6×10^7	1.0×10^7	8.0×10^3
	0.25	1.6×10^7	1.0×10^7	5.5×10^3
	0.5	1.3×10^7	1.1×10^7	3.5×10^3
	1	1.3×10^7	1.1×10^7	2.4×10^3
	2	1.6×10^7	1.1×10^7	1.7×10^3
	3 μmol/L–11 mmol/L	1.6×10^7	1.2×10^7	9.0×10^2

The concentrations of (bio)surfactants are less than CMC, in 50 mmol/L acetate buffer pH 5.5, 25°C.

Table 2 Kinetic parameters of peroxidase-catalyzed oxidation of 2-naphthol in the presence of JBR425 and Surfynol465

Biosurfactant	Concentration (mmol/L)	k_2 (L/(mol·sec))	k_3 (L/(mol·sec))	k_{in} (L/(mol·sec))
JBR425	0.1	1.4×10^7	6.5×10^6	1.0×10^3
	1	8.0×10^6	5.5×10^6	1.8×10^3
	2	5.0×10^6	3.5×10^6	3.2×10^3
	3	3.5×10^6	2.5×10^6	4.5×10^3
Surfynol465	11	1.6×10^7	1.2×10^7	9.0×10^2
	15	7.2×10^6	5.0×10^6	1.0×10^3
	20	4.0×10^6	3.0×10^6	1.1×10^3
	30	2.6×10^6	1.3×10^6	1.8×10^3

The concentrations of (bio)surfactants were above CMC, in 50 mmol/L acetate buffer pH 5.5, 25°C.

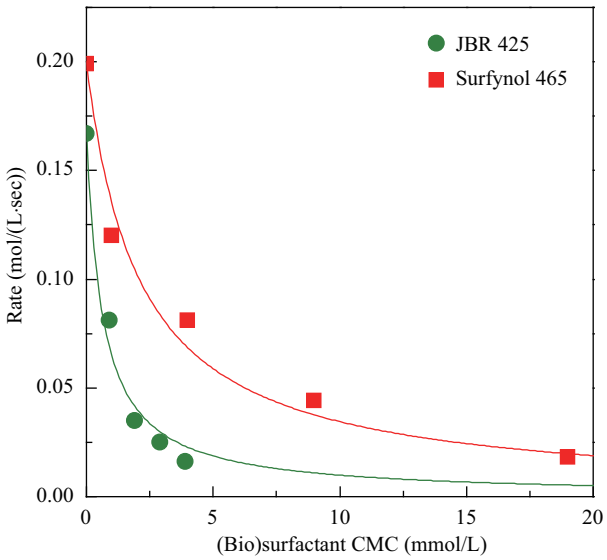

Fig. 9 Dependence of 2-naphthol oxidation rate on JBR425/Surfyno465 concentrations. Concentrations: 25 μmol/L 2-naphthol, 1 nmol/L rCiP, 100 μmol/L H_2O_2 in 50 mmol/L acetate buffer pH 5.5, 25°C. The lines are theoretical curves calculated according to Eq. (12).

Table 3 Best-fit values of the distribution coefficients P_{naph} calculated according to Eq. (12).

Substrate	Micellar phase	P_{naph}
2-Naphthol	JBR425	5400 ± 684
2-Naphthol	Surfynol465	1600 ± 180

Conditions: 50 mmol/L acetate buffer pH 5.5, 25°C.

where, V_w is the reaction rate in water solution (in our case in 50 mmol/L acetate buffer pH 5.5), P_{naph} is the distribution coefficient of the 2-naphthol, v_m is the molar volume of the micelles (0.3 L/mol, Ryabov and Goral, 1997), c is the total concentration of (bio)surfactant and CMC is the critical micelle concentration. The data presented in **Fig. 9** shows rather good correspondence between the experiment and the model.

The results presented in **Table 3** show that in JBR425 micelles the distribution coefficient of 2-naphthol is 3.4 times larger than that in Surfynol465 micelles. This result can be explained by different sizes of the micelles. As shown in **Fig. 4a**, the hydrodynamic diameter of JBR425 micelles is about 50 nm, while the hydrodynamic diameter of Surfynol465 micelles is about 5 nm (**Fig. 4b**). This means that JBR425 micelle is 10 times larger than Surfynol465 micelle. Despite the different P_{naph} values, JBR425 as well as Surfynol465 (with their concentrations larger than CMC) demonstrate an opposite effect (**Fig. 7c**) on the oxidation of 2-naphthol by peroxidase compared with the case when they exist in monomer's form (**Fig. 7b**).

3 Conclusions

In the present study, the kinetics of peroxidase-catalyzed 2-naphthol oxidation in the presence of rhamnolipid biosurfactant JBR425 and synthetic surfactant Surfynol465, with concentrations of (bio)surfactants both less than CMC and larger than CMC, was investigated. JBR425 as well as Surfynol465 has an enhancing effect on the oxidation of 2-naphthol by peroxidase when their concentrations are less than CMC. In order to explain the achieved results, the scheme which contains a stadium of enzyme inhibition by oligomeric 2-naphthol oxidation products was applied for peroxidase-catalyzed 2-naphthol oxidation in presence of (bio)surfactants monomers. Similar results have demonstrated the potential of rhamnolipid biosurfactant JBR425 due to its biodegradability. When (bio)surfactants concentrations are larger than CMC (bio)surfactants have an opposite effect on the oxidation of 2-naphthol by peroxidase.

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References

Air Products, 2001. SURFYNOL®SURFACTANTS Biodegradation and Toxicity Information. http://www.airproducts.com/~/media/Files/PDF/industries/

graphics-arts-biodegradation-and-toxicity-information.pdf.

Andersen M B, Hsuanyu Y, Welinder K G, Schneider P, Dunford H B, 1991. Spectral and kinetic properties of oxidized intermediates of *Coprinus cinereus* peroxidase. *Acta Chemica Scandinavica*, 45: 1080–1086.

Baynton K J, Bewtra J K, Biswas N, Taylor K E, 1994. Inactivation of horseradish peroxidase by phenol and hydrogen peroxide: a kinetic investigation. *Biochimica et Biophysica Acta*, 1206(2): 272–278.

Berezin I V, Martinek K, Yatsimirsky A K, 1973. Physicochemical foundations of micellar catalysis. *Russian Chemical Reviews Uspekhi Khimii*, 42(10): 787–802.

Bratkovskaja I, Vidziunaite R, Kulys J, 2004. Oxidation of phenolic compounds by peroxidase in the presence of soluble polymers. *Biochemistry (Moscow)*, 69(9): 985–992.

Croera C, Ferrario D, Gribaldo L, 2008. *In vitro* toxicity of naphthalene, 1-naphthol, 2-naphthol and 1, 4-naphthoquinone on human CFU-GM from female and male cord blood donors. *Toxicology in Vitro*, 22(6): 1555–1561.

Dayeh V R, Chow S L, Schirmer K, Lynn D H, Bols N C, 2004. Evaluating the toxicity of Triton X-100 to protozoan, fish, and mammalian cells using fluorescent dyes as indicators of cell viability. *Ecotoxicology and Environmental Safety*, 57(3): 375–382.

Dunford H B, 1990. Horseradish peroxidase: Structure and kinetic properties. In: Peroxidases in Chemistry and Biology (Everse J E, Everse K E, Grisham M B, eds.). CRC Press, Boca Raton. 2: 1–24.

Fu H Y, Zeng G M, Zhong H, Yuan X Z, Wang W, Huang G H et al., 2007. Effects of rhamnolipid on degradation of granular organic substrate from kitchen waste by a *Pseudomonas aeruginosa* strain. *Colloids and Surfaces B: Biointerfaces*, 58(2): 91–97.

Ghioureliotis M, Nicell J A, 1999. Assessment of soluble products of peroxidase catalyzed polymerization of aqueous phenol. *Enzyme and Microbial Technology*, 25(3-5): 185–193.

Gosselin R E, Hodge H C, Smith R P, Gleason M N, 1976. Clinical Toxicology of Commercial Products (4th ed.). Williams & Wilkins, Baltimore.

Ji G L, Zhang H B, Huang F, Huang X, 2009. Effects of nonionic surfactant Triton X-100 on the laccase-catalyzed conversion of bisphenol A. *Journal of Environmental Sciences*, 21(11): 1486–1490.

Kinsley Ch, Nicell J A, 2000. Treatment of aqueous phenol with soybean peroxidase in the presence of polyethylene glycol. *Bioresource Technology*, 73(2): 139–146.

Kitamoto D, Isoda H, Nakahara T, 2002. Functions and potential applications of glycolipid biosurfactants–from energy-saving materials to gene delivery carriers. *Journal of Bioscience and Bioengineering*, 94(3): 187–201.

Klibanov A M, Tu T M, Scott K P, 1983. Peroxidase-catalyzed removal of phenols from coal-conversion waste waters. *Science*, 221(4607): 259–261.

Krishnan R, Sprycha R, 1999. Interactions of acetylenic diol surfactants with polymers: part 1. maleic anhydride copolymers. *Colloids and Surfaces A-Physicochemical and Engineering Aspects*, 149(1-3): 355–366.

Kulys J, Ivanec-Goranina R, 2009. Peroxidase catalyzed phenolic compounds oxidation in presence of surfactant Dynol 604: A kinetic investigation. *Enzyme and Microbial Technology*, 44(6-7): 368–372.

Kulys J, Vidžiünaite R, 2000. The role of micelles in mediator-assisted peroxidase catalysis. *Progress in Colloid and Polymer Science*, 116: 137–142.

Kulys J, Vidžiünaite R, Ziemys A, Bratkovskaja I, 2007. Peculiarities of hydroxyaryl oxidation by fungal peroxidase: reactivity and inhibition of the enzyme. *Biologija*, 53(3): 40–44.

Li M H, 2008. Effects of nonionic and ionic surfactants on survival, oxidative stress, and cholinesterase activity of planarian. *Chemosphere*, 70(10): 1796–1803.

Liu X L, Zeng G M, Tang L, Zhong H, Wang R Y, Fu H Y et al., 2008. Effects of dirhamnolipid and SDS on enzyme production from *Phanerochaete chrysosporium* in submerged fermentation. *Process Biochemistry*, 43(11): 1300–1303.

Liu Z F, Zeng G M, Zhong H, Yuan X Z, Fu H Y, Zhou M F et al., 2012. Effect of dirhamnolipid on the removal of phenol catalyzed by laccase in aqueous solution. *World Journal of Microbiology and Biotechnology*, 28(1): 175–181.

Masuda M, Sakurai A, Sakakibara M, 2001. Effect of enzyme impurities on phenol removal by the method of polymerization and precipitation catalyzed by *Coprinus cinereus* peroxidase. *Applied Microbiology and Biotechnology*, 57(4): 494–499.

Modaressi K, Taylor K E, Bewtra J K, Biswas N, 2005. Laccase catalyzed removal of bisphenol-A from water: Protective effect of PEG on enzyme activity. *Water Research*, 39(18): 4309–4316.

Navarro A, Alcock J R, Whatmore R W, 2004. Aqueous colloidal processing and green sheet properties of lead zirconate titanate (PZT) ceramics made by tape casting. *Journal of the European Ceramic Society*, 24(6): 1073–1076.

Nelson D P, Kiesow L, 1972. Enthalpy of decomposition of hydrogen peroxide by catalase at 25°C with molar extinction coefficients of H_2O_2 solution in the UV. *Analytical Biochemistry*, 49(2): 474–478.

Noordman W H, Brusseau M L, Janssen D B, 2000. Adsorption of a multicomponent rhamnolipid surfactant to soil, *Environmental Science and Technology*, 34(5): 832–838.

Páhi A B, Királya Z, Puskásb S, 2009. Mass spectrometric characterization of the non-ionic gemini surfactant Surfynol465 and a microcalorimetric study of its micelle formation in water. *Colloids and Surfaces A-Physicochemical and Engineering Aspects*, 345(1-3): 13–17.

Puy-Azurmendi E, Navarro A, Olivares A, Fernandes D, Martínez E, de Alda M L et al., 2010. Origin and distribution of polycyclic aromatic hydrocarbon pollution in sediment and fish from the biosphere reserve of Urdaibai (Bay of Biscay, Basque Country, Spain). *Marine Environmental Research*, 70(2): 142–149.

Reihmann M, Ritter H, 2006. Synthesis of phenol polymers using peroxidases. *Advances in Polymer Science*, 194: 1–49.

Roch F, Alexander M, 1995. Biodegradation of hydrophobic compounds in the presence of surfactants. *Environmental Toxicology and Chemistry*, 14(7): 1151–1158.

Ryabov A, Goral V N, 1997. Steady-state kinetics, micellar effects, and the mechanism of peroxidase-catalyzed oxidation of n-alkylferrocenes by hydrogen peroxide. *Journal of Biological Inorganic Chemistry*, 2(2): 182–190.

Sato S, Toda K, Oniki S, 1999. Kinetic study on the formation of colloidal gold in the presence of acetylenic glycol non-ionic surfactant. *Journal of Colloid and Interface Science*, 218(2): 504–510.

Svir I, Klymenko O V, Oleinick A I et al., 2004. KinFitSim (Version 2.1) – a powerful tool for kinetic simulation of any reaction mechanism and fitting of any number of pairs of theoretical and experimental data sets. *Radioelektronics and Informatics*, 4: 21–24.

Tams J W, Vind J, Welinder K G, 1999. Adapting protein solubility by glycosylation. N-glycosylation mutants of *Coprinus cinereus* peroxidase in salt and organic solutions. *Biochimica et Biophysica Acta*, 1432(2): 214–221.

Wickramanayake P P, Aue W A, 1986. A bonded polyoxyethylene phase for gas and liquid chromatography. *Canadian Journal of Chemistry*, 64(3): 470–476.

Wu Y M, Taylor K E, Biswas N, Bewtra J K, 1998. A model for the protective effect of additives on the activity of horseradish peroxidase in the removal of phenol. *Enzyme and Microbial Technology*, 22(5): 315–322.

York J D, Firoozabadi A, 2008. Comparing effectiveness of rhamnolipid biosurfactant with a quaternary ammonium salt surfactant for hydrate anti-agglomeration. *The Journal of Physical Chemistry B*, 112(3): 845–851.

Zang S Y, Lian B, Wang J, Yang Y J, 2010. Biodegradation of 2-naphthol and its metabolites by coupling *Aspergillus niger* with *Bacillus subtilis*. *Journal of Environmental Sciences*, 22(5): 669–674.

Zeng G M, Zhong H, Huang G H, Fu H I, 2005. Physico-chemical and microbiological effects of biosurfactant on the remediation of HOC-contaminated soil. *Progress in Natural Science*, 5(7): 577–585.

Ziemys A, Kulys J, 2005. Heme peroxidase clothing and inhibition with polyphenolic substances revealed by molecular modeling. *Computational Biology and Chemistry*, 29(2): 83–90.

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