ENVIRONMENTAL CONSEQUENCES OF EMULSIFYING PROPERTIES OF BIOSURFACTANT PRODUCED BY *PSEUDOMONAS* sp.

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ABSTRACT:
Biosurfactants are natural surfactants produced by variety of microorganisms. They are very prospective product due to good biodegradability, low toxicity, good physicochemical properties and high efficiency in many applications. Moreover, they are efficient in wide range of pH, temperatures and salinity. Thus, they have potential to be used in many applications. However, their later presence in municipal and industrial wastewater can pose many problems due to emulsification. Thus, the emulsifying properties of crude biosurfactant produced by *Pseudomonas* sp. and its single species were investigated. Paraffin and rapeseed oils were used as oil phase.

Keywords: biosurfactant, rhamnolipid, alginate, paraffin oil, rapeseed oil, stability of emulsion

INTRODUCTION

Biosurfactants are natural surfactants produced by variety of microorganisms (Kitamoto et al., 2002; Banat et al., 2000; Desai et al., 1997). They are environmentally sustainable (green) chemicals due to low toxicity (Hwang et al., 2009; Edwards et al., 2003; Kolwzan et al., 2008) and high susceptibility to biodegradation (Cappello et al., 2011; Lima et al., 2011). Thus, biosurfactants find practical applications in cosmetics (Gumienna et al., 2010; Domańska et al., 1996) and food industry (Shepherd et al., 1995; Kosaric, 2001; Van Haesendonck et al., 2004), physical (flooding with surfactant solution) (Kuyukina et al., 2005; Urum et al., 2006) and biological soil remediation (enhancing biodegradation by surfactant solutions addition) (Hua et al., 2003; Wong et al., 2003; Makkar et al., 2003), removal of oil spills from water (Edwards et al., 2003; Saeki et al., 2009), ecological agriculture (Anderson, 2006; Karpenko E. et al., 2005; Karpenko et al., 2007) etc. One of the properties of biosurfactants that respond for their wide application is the ability to stabilize emulsions. Stable emulsions are preferable in biodegradation of oil pollutants where they increase bioavailability of oil to microorganisms (Volkering et al., 1998). In turn, high stability of emulsions in undesirable in the case of soil remediation by flooding with surfactant solution, where used solution with solubilized and emulsified oil has to be pumped out for recovering. At this point very stable and viscous emulsions can block up the soil pores hindering the solution movement and later the oil separation from used surfactant solution. The presence of very stable emulsions is also unwelcome in wastewater treatment, where emulsified or free oil has to be removed before biological treatment in order to not to glue over the activated sludge. However, the oil in free form can be easily removed by clarification whereas the presence of oil in emulsified form requires application of more advanced deoiling methods (emulsions have to be destabilized first). Therefore, the industrial processes including surfactants application have to be designed carefully, taking into account minimization or recycling of surfactant rich wastewaters. On the other hand, municipal wastewaters may contain significant amounts of emulsions stabilized not only by surfactants from detergents but also by protein or lecithin present in food products.

The aim of this work was to investigate the emulsifying properties of biosurfactant produced by *Pseudomonas* sp. The product of these bacteria is not pure rhamnolipid but its mixture or even complex with alginate. The behavior of its single components during emulsification process may differ substantially. Therefore, the emulsification properties of rhamnolipids and alginate were also investigated. The emulsifying properties of lecithin and the effect of its presence in oil and water system was also investigated. The potential sources of lecithin in wastewater are *e.g.* wastes from degumming of vegetable oils (degumming of vegetable oils with biosurfactants solutions is also our interest), wastes from households containing margarine, *etc*. According to potential applications of biosurfactants paraffin and rapeseed oils were used as oil phases.

CHEMICALS AND METHODS

Two oil phases were used: paraffin oil and rapeseed oil (Rapso, VOG Poland). As the emulsifier crude biosurfactant produced by *Pseudomonas* sp. (Sotirova et al., 2008), rhamnolipids mixture (JBR 425, Jeneil Biosurfactant CO., LLC), alginate (POCh Gliwice, Poland) and lecithin (POCh Gliwice, Poland) were used. As oil phases paraffin (ONDINA 934, Brenntag Poland) and rapeseed (VOG Poland) oils were used.

Emulsions of oil and water were prepared with YellowLine DI 25 Basic homogenizer (8000 rpm, 10 min.) at room temperature. The samples of emulsion were placed in glass tubes and scanned along all its height (55 mm) in Turbiscan Lab apparatus at programmed time and at room temperature. The intensities of backscattered light (BS) were analysed. As the result the curves representing the percentage of backscattered light were obtained. The instabilities of emulsions (creaming, clarification and coalescence) were recognized as it is presented in the figure 1. The higher the backscattering value the smaller the particles of dispersed phase. The more constant the BS value in time the more stable emulsion (Turbiscan La*b* – User Guide).

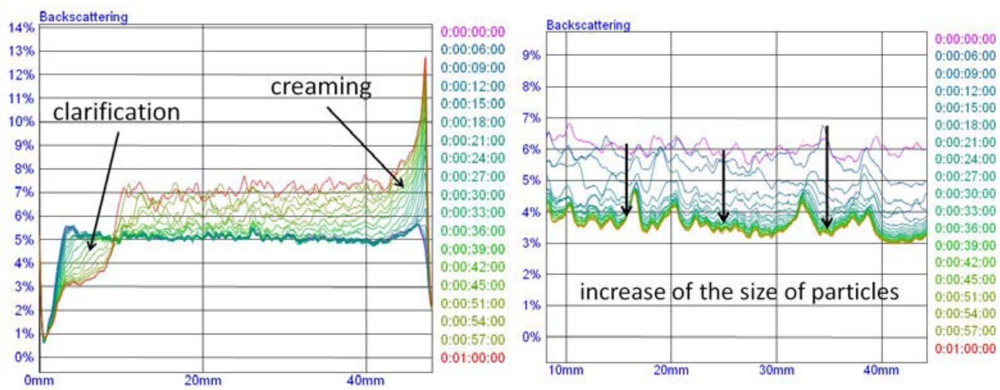

Figure 1. Instabilities of emulsions observed in Turbiscan apparatus: creaming and clarification (left) and coalescence (right) (Turbiscan La*b* – User Guide).

RESULTS AND DISCUSSION

The effect of oil content

The backscattering profiles for emulsions of paraffin oil stabilized with biosurfactant are presented in figure 2. The emulsions containing low amounts of oil (up to 70%) were not stable and two separate phases (the lower phase was aqueous and the upper phase was the cream phase) were observed almost immediately after preparation (Fig. 2 left). The highest and backscattering value on every height of sample was observed for the emulsion containing 90% of paraffin oil but after one day only emulsion containing 80% of oil remained stable (Fig. 2 right).

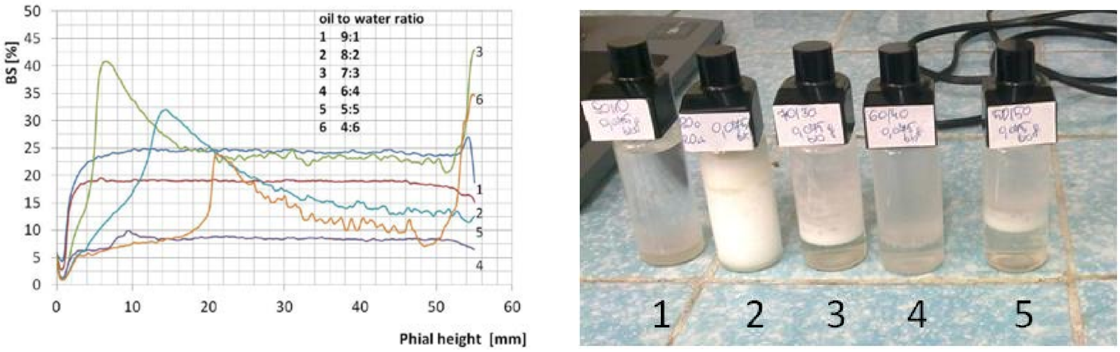

Figure 2. Emulsions of paraffin oil with biosurfactant solutions. Initial backscattering profiles (top) and a photography of emulsions after 1 day (bottom).

The backscattering profiles in time for emulsions of rapeseed oil stabilized with biosurfactant are presented in Fig. 3. The emulsions containing low amounts of oil were not stable. The BS value decreased from 52% to about 20% in lower part of phial within 35 minutes and to about 10% within one day (Fig. 3 left). The creaming in upper and clarification in lower parts of phials were observed. The processes have led to the formation of two separate phases (aqueous and cream phase) The stability of emulsions was increasing with increasing amount of oil phase. The most stable were emulsions containing 80% of rapeseed oil (Fig. 3 right). The maximal BS value was 75% and no formation of two separate layers was observed in this case.

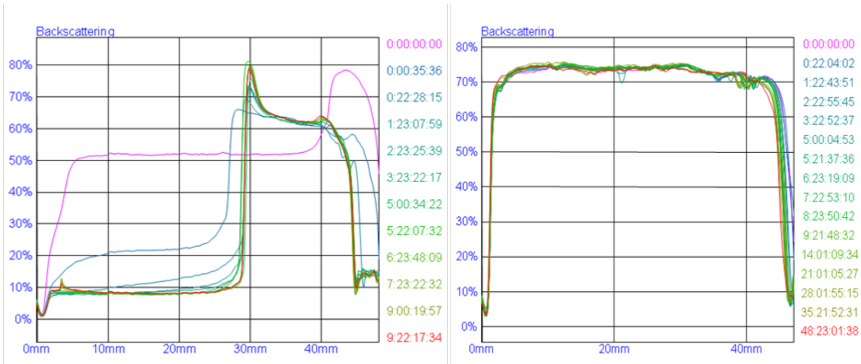

Figure 3. Backscattering profiles for emulsions of rapeseed oil with biosurfactant solutions. Oil phase content: 20% (left) and 80% (right).

The effect of emulsifying agent

Considering the composition of non-purified biosurfactants, where rhamnolipids are present next to an exopolysaccharide alginate it was essential for this work to investigate emulsification properties of its single components. Thus, the series of emulsions stabilized with rhamnolipids (JBR 425) or alginate were prepared. The backscattering profiles in time for emulsions of rapeseed oil stabilized by the presence of rhamnolipids and alginate are presented in figure 4.

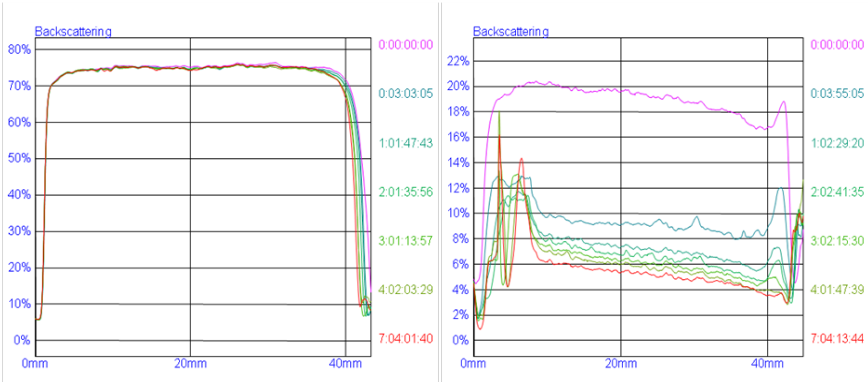

Figure 4. Backscattering profiles for emulsions of rapeseed oil with rhamnolipids (left) and alginate (right) solutions. Oil phase content 80%.

Emulsions stabilized by rhamnolipid and containing less than 70% of oil phase (rapeseed oil) were unstable and in a short period of time after their preparation two separate phases were observed. The most stable emulsion was the one which contained 80% of oil phase (BS 75%, Fig. 4, left). On the contrary, the alginate turned out to have little tendency to stabilize emulsions at all range of oil phase ratios, when applied alone (Fig. 4, right). The initial BS value was about 20% and within 4 hours it decreased to less than 10%.

Wastewaters often contains some wastes of food products. They may be a source of surface active compounds, e.g. lecithin, which affect the emulsification process. Therefore, the emulsifying properties of lecithin were also examined. The backscattering profiles for emulsions of rapeseed oil containing 1% of lecithin are presented in figure 5.

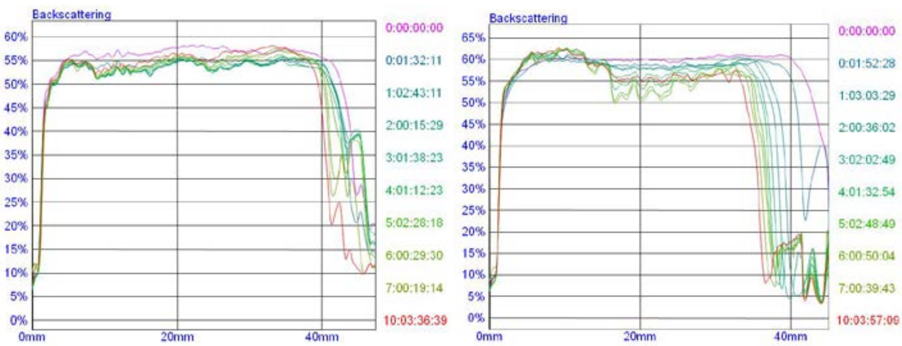

Figure 5. Backscattering profiles for emulsions of rapeseed oil with lecithin (1% w/w). Oil phase content: 70% (left) and 80% (right).

Emulsions stabilized by lecithin containing less than 50% of oil phase were undergoing rapid stratification (two separate phases were observed). In turn, emulsions containing 50% and more of oil phase were quite stable. However, the clarification of upper part of emulsions was observed in time (Fig. 5). The clarification occurred the most slowly in the emulsions containing 70% of oil phase (Fig. 5, left). This means, that part of emulsified oil droplets underwent the coalescence and moved up as a free oil phase. All of the emulsions looked “curdy” what was observed visually and it has been reflected in the middle part of backscattering profiles (variations of backscattering values along the height of phial) (Fig. 5).

In order to investigate emulsifying properties of crude biosurfactant at conditions referring to those in municipal wastewaters (containing *e.g.* wastes of food products) the experiments in the presence of lecithin were run. The backscattering profiles for emulsions of rapeseed oil containing 1% of lecithin and stabilized by crude biosurfactants are presented in figure 6.

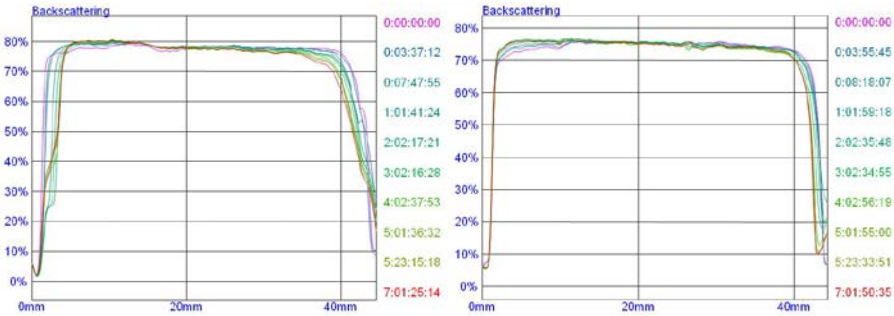

Figure 6. Backscattering profiles for emulsions of rapeseed oil with lecithin (1% w/w) and with crude biosurfactant solutions. Oil phase content: 70% (left) and 80% (right).

The most stable emulsion of rapeseed oil with lecithin stabilized by crude biosurfactants was the one containing 80% of oil phase and the backscattering value was equal to 75% (Fig 6 right). Although the highest backscattering value (78%) was observed for the emulsions containing 70% of oil phase, they turned out to be unstable and the clarification in lower part was observed after 1 day of incubation (Fig. 6 left). Moreover, the addition of lecithin turned out to slightly decrease the stability of emulsions with biosurfactant.

CONCLUSIONS

The crude biosurfactant produced by *Pseudomonas* sp. stabilizes the most the emulsions of paraffin and rapeseed oil with or without lecithin containing 80% of oil phase (BS 18%, 75% and 75%, respectively). Emulsions containing different amounts of oil phases are not stable due to clarification in lower and creaming in upper parts of emulsions in time (what leads to formation of two separate phases – aqueous phase and cream).

The emulsifying properties of crude biosurfactant and the mixture of rhamnolipids do not differ, although the biosurfactant contains alginate – an exopolysaccharide which poorly emulsified rapeseed oil (initial BS 20%). Moreover, the presence of lecithin in the system did not affect the stability of emulsions stabilized by biosurfactant examined.

Summarizing, the presence of biosurfactants in municipal and industrial wastewater may cause some problem with its treatment. Biosurfactant examined forms very stable emulsions of paraffin and rapeseed oils. Therefore, regardless of the oil content in wastewater there is a possibility to form higher or lower amounts of stable emulsion. The emulsions formed in wastewater on one hand hinder the wastewater treatment processes (coating of the activated sludge, necessity of application of different destabilization methods, etc.) on the other hand they increase the bioavailability of oils (especially mineral ones) to microorganisms and therefore enhance their biodegradation by microorganisms.

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