Polish Journal of Microbiology
2008, Vol. 57, No 4, 281–287

ORIGINAL PAPER

Exopolysaccharide Production by *Bacillus* Strains Colonizing Packaging Foils

JOANNA SZUMIGAJ[1,2*], ZOFIA ŻAKOWSKA[1] and LESZEK KLIMEK[3]

[1] Institute of Fermentation Technology and Microbiology, Technical University of Łódź, Poland
[2] Laboratory of Mushroom Cultivation, Research Institute of Vegetable Crops, Skierniewice, Poland
[3] Institute of Materials Science and Technology, Technical University of Łódź, Poland
[3] Department of Basic and Pre-Clinical Science, Medical University of Łódź, Poland

Received 3 June 2008, revised 25 August 2008, accepted 10 September 2008

Abstract

The influence of the chemical composition of medium, availability of glucose and pH on the production of exopolysaccharides (EPS) by different *Bacillus* strains were investigated. *Bacillus* strains were isolated from the surface of polyethylene foils modified with mineral compounds after their biodegradation in compost soil. Moreover, the effect of EPS production on bacterial adhesion onto the surface of the materials was examined. The enhanced synthesis of exopolysaccharides in nutrient-starved conditions was revealed. The most effective synthesis of polymers was observed during the logarithmic phase of culture growth. The increased amount of EPS facilitated bacterial adhesion to material surfaces. It was determined that the biofilm on the material surface positively affects its biodegradation. Based on the results, we conclude that the biodegradation of polymers may be accelerated in low-nutrient environment.

Key words: biodegradation, biofilm, extracellular polymeric substances, polyethylene foils

Introduction

In recent years the ecological hazard resulting from the large number of used plastic materials has increased. The development of new biodegradable materials that are resistant to biological disintegration can be an interesting alternative for packaging. These materials can resolve the problem of packaging waste.

Many authors indicate the relevance between the biological disintegration of polymer materials and microbial adhesion onto solid surface of various materials. Therefore, the biodegradation of packaging materials is relevant with adhesion phenomena and biofilm formation on the solid surface of materials (Beech, 2006; Flemming, 1998; Ford and Mitchell, 1990; Tsuneda *et al.*, 2003). This process results in a complex microbial structure (microbial aggregate) consisting of bacteria and fungi and their metabolic products, as well as various inorganic substances (Characklis and Cooksey, 1983; Costerton *et al*., 1987). The attachment of bacteria to a solid surface is the first and essential stage in the formation of a biofilm. It seems that there is two-step process, reversible and irreversible attachment (Allison and Sutherland, 1987; Marshall *et al*., 1971). In the first step, the microorganisms come close to the material's surface to be weakly held by electrostatic forces. In the second, the attached microorganisms are more difficult to remove from the surface, as the bacteria produce extracellular polymeric substances (EPS) that finally form the biofilm matrix (Czaczyk, 2004; Hall-Stoodley and Stoodley, 2002; Sutherland, 1982). It has been reported that EPS play a significant role in cell adhesion and biofilm formation onto the surface of a material (Olofsson *et al.*, 2003; Rijnaarts *et al*., 1995; Sutherland, 1980; Sutherland, 2001).

Extracellular polymeric substances, which are secreted by microorganisms during growth, consist of various organic substances such as polysaccharides, proteins, nucleic acids and lipids (Horan and Eccles, 1986). The exact functions of EPS are not completely known because of their heterogeneous nature. They protect cells from harsh external environments and provide energy and carbon when nutrients are in short supply (Sutherland, 1999; Wang *et al*., 2007). They also play an important role in the flocculation of bacterial cells (Morikawa, 2006; Pratt and Kolter, 1999). Many researchers have investigated the factors

* Corresponding author: J. Szumigaj, Laboratory of Mushroom Cultivation, Research Institute of Vegetable Crops, Skierniewice, Rybickiego 15/17, 96-100 Skierniewice, Poland; **e-mail: joanna.szumigaj@inwarz.skierniewice.pl**

influencing the EPS production by microorganism and the role of EPS in the bacterial adhesion onto various surfaces (Allison and Sutherland, 1987; Gu *et al*., 1998; Langille *et al*., 2000; Parkar *et al.*, 2001).

Tsuneda *et al*. (2003) investigated bacterial adhesion onto glass in connection with the amounts and composition of the EPS produced by bacterial strains. It was shown that the enhanced synthesis of extracellular polymers has a significant effect on the adhesion phenomena. Moreover, he indicated that proteins and polysaccharides accounted for 75–80% of the EPS composition. Sheng *et al.* (2006) showed that the quantity and composition of exopolysaccharides is influenced by cell growth phase, the C/N ratio and the concentration of carbon and nitrogen sources as well as of NaCl in the medium. It was documented that degradation of metal surface, termed microbially influenced corrosion or biocorrosion, occurs when contact between microbial cells, products of their metabolism such as EPS, and the surface is established. The microorganisms, which are involved in the polymers biodegradation, then produce exopolysaccharides (EPS) (Beech, 2006).

The previous research on the biodegradation of packaging foils indicated that polyethylene materials modified by mineral compounds were not easily degraded. It was found that there were no changes on the foil surface even after 8 months of composting (Szumigaj *et al*., 2005). The aim of the current studies was to determine the conditions that could positively affect EPS production by *Bacillus* strains participating in the degradation of plastic. Information dealing with the conditions influencing exopolysaccharide production could prove useful for evolving strategies for the regulation of polymer degradation.

The influence of the chemical composition of the medium, availability of glucose and pH of the medium on the production of exopolysaccharides (EPS) by *Bacillus* spp. strains was investigated. Strains of *Bacillus* sp. were isolated from the surface of polyethylene foils modified with mineral compounds. Moreover, the influence of EPS synthesis on bacterial adhesion onto the surface of the materials was examined.

Experimental

Materials and Methods

Bacterial strains and cultivation. Five strains of *Bacillus* species, *i.e*. *Bacillus coagulans*, *Bacillus firmus*, *Bacillus subtilis*, *Bacillus megaterium* and *Bacillus* sp. were isolated from the surface of foil during biodegradation in compost. Bacterial growth and EPS production were monitored at 4, 8, 18, 24, 48 and 72 hour of cultivation. The process was carried out at 30ºC on a rotary shaker at 85 rpm in broth medium (pH 7.4) composed of the following: meat extract 2g; yeast extract 2g; bactopeptone 5g; NaCl 4g; distilled water 1l and mineral growth medium (pH 5.0) composed of the following: yeast extract 5g; $MgSO_4 \times 7H_2O$ 5g; $(NH_4)_2SO_4$ 3g; KH_2PO_4 1g; distilled water 1 l. The media contained various concentrations of glucose (0%, 1% or 5%) and 0.1% polyethylene foil modified mineral compounds. The foils were sterilised by immersing them for 1 minute in a 70% solution of ethyl alcohol and then each side was exposed to UV rays for a period of 15 minutes. The results given below are the arithmetic mean of three series of experiments.

Extraction of EPS. The quantity of EPS produced by bacteria was determined by a modified acid hydrolysis method using dextran (Mp. 50 000, Fluka) as a standard (Parkar *et al*., 2001; Czaczyk and Myszka, 2004). The vegetative cells were suspended in distilled water to 1.0×10^8 cfu/ml, sonicated and centrifuged at $10\,000 \times g$ for 10 min. Then 1 ml supernatant was precipitated with 8 ml 95% ethyl alcohol and kept at 4°C for 24 hours. Next, the probes were centrifuged at 10 000×g for 20 min and the pellet was suspended in 1 ml water, then 7 ml 77% sulphuric acid and 1 ml 1% tryptophan was added. Afterwards, the probes were kept in boiling water for 20 min and absorbance was measured at wavelength $\chi = 500$ nm. A standard curve using dextran (Mp. 50 000, Fluka) was prepared to estimate the amount of EPS. The result are given in μm EPS per 10^8 cells (Parkar *et al*., 2001; Czaczyk and Myszka, 2004).

Evaluation of bacterial adhesion onto foil's surface. The bacterial adhesion onto foil's surface and biofilm formation was monitored by using scanning electron microscope (Hitachi 3000N) (SEM). The material before analysis was covered with a thin layer of gold.

Results

Lack of carbon source and EPS synthesis. The production of extracellular polysaccharides in media without carbon source was examined. The results showed that the average proportion of EPS produced by *Bacillus* spp., except for *B. megaterium*, was higher in the mineral growth medium (pH 5.0) than in the broth medium (pH 7.4) (Fig. 1A). The highest amount of EPS was noticed for *B. coagulans*, *B. firmus* and *B. subtilis* strains. These strains were characterised by significantly enhanced exopolysaccharides synthesis in starvation medium.

Availability of glucose and EPS synthesis. Addition of glucose to the mineral medium resulted in decreased production by *B. coagulans*, *B. subtilis* and *Bacillus* sp., while in case of *B. firmus* and

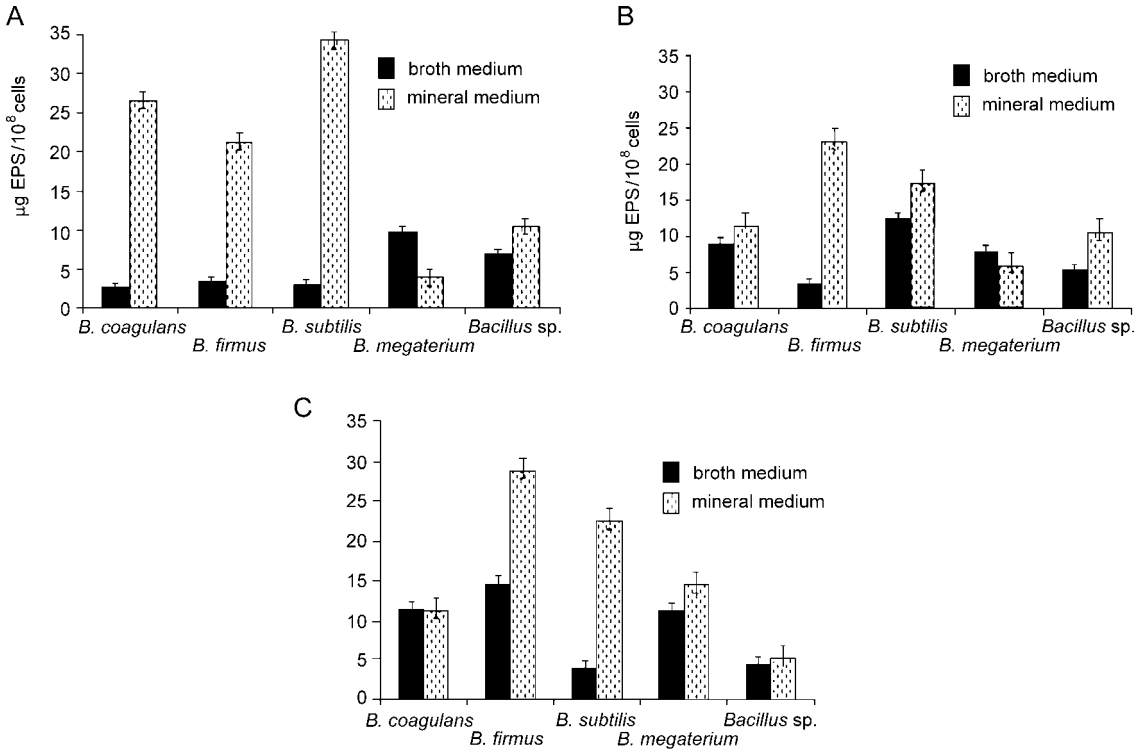

Fig. 1. The average amount of EPS (µg EPS/10^8 cells) produced by *Bacillus* strains in mineral and broth medium A – without glucose, B – with 1% glucose concentration, C – with 5% glucose concentration

B. megaterium strains the quantity of EPS increased (Fig. 1B and C).

The increase of glucose concentration to 5% (Fig. 1C) in broth medium affected the increase of polysaccharides production by 3 strains, *i.e. B. coagulans*, *B. firmus* and *B. megaterium* strains as compared to the medium containing 1% of glucose, while EPS synthesis was decreased in the case of *B. subtilis* and did not change for *Bacillus* sp. (Fig. 1B). The exopolysaccharides production by *Bacillus* spp. was higher in the mineral medium than in the broth medium. Only the *B. megaterium* and *B. coagulans* strains were characterised by increased EPS synthesis in the broth medium, at 1% and 5% glucose concentration, respectively (Fig. 1B and C).

Dynamics of EPS production. Growth dynamics and EPS production in the broth and the mineral medium at various glucose concentrations are presented on the example of the *B. firmus* strain. Figure 2A shows strain growth dynamics in the broth medium. The stationary phase started latest and the biomass increase was the lowest in the glucose-free medium. Until the 24th hour, an increase of the number of cells, reaching 6.2×10^7 cfu/ml, was observed. At 1% glucose concentration, the strain attained the stationary phase faster, at the 18th hour, and the number of cells

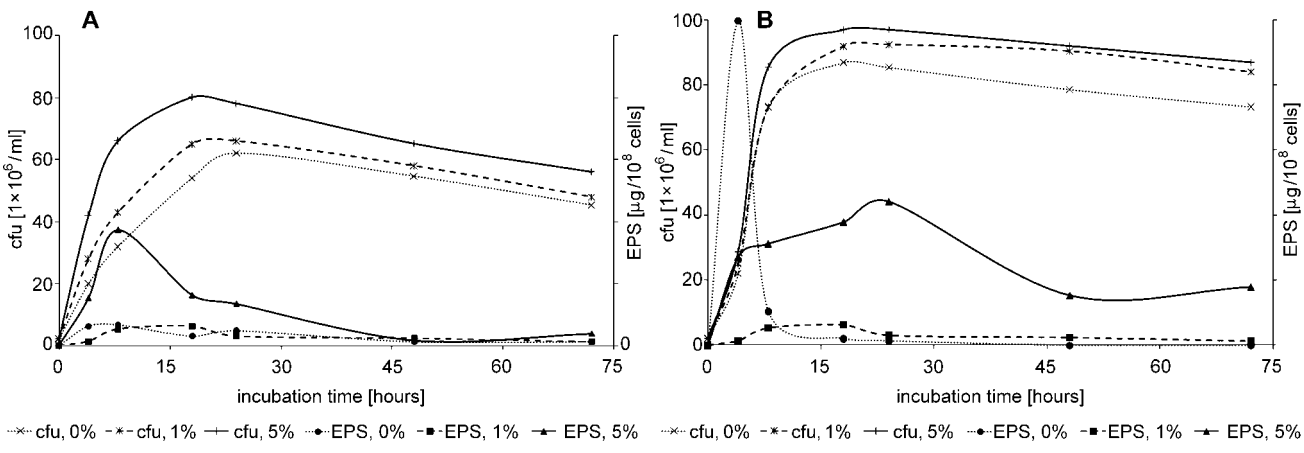

Fig. 2. Growth dynamic of *Bacillus firmus* and exopolysaccharides production A – in broth medium, B – in mineral medium

was 6.6×10^7 cfu/ml, *i.e.* approx. 20% higher than in the glucose-free medium.

The growth of the EPS production to the value of 6.6 μg/10^8 cells was in the first few hours of cultivation in the glucose-free medium. Following the 8th hour, a decrease of extracellular polymers synthesis was noted. In the medium containing 1% of glucose, in the first hours of cultivation, the strain produced fewer EPS than in the glucose-free medium, but an increase of these compounds was revealed at the 18th hour of cultivation (Fig. 2A).

The greatest biomass growth was observed in the medium containing 5% of glucose, *i.e.* 8.2×10^7 cfu/ml at the 18th hour of cultivation. At that hour, the strain also achieved the stationary phase of growth. Addition of 5% of glucose also effectively influenced exopolysaccharides production. After 8 hours of cultivation, the amount of synthesised compounds was the highest, *i.e.* 37.3 μg/10^8 cells. It was shown that the fastest polymer growth was during the logarithmic growth phase. The stationary phase started at the 18th hour of cultivation and the quantity of EPS started to decrease significantly. After the 48th hour, inhibition of EPS synthesis was detected in all the variants (Fig. 2A).

Analysing dynamics of cells growth in the mineral medium without source of carbon, it was observed that the strain reached the stationary phase at the 18th hour of cultivation, and the number of cells amounted to 8.7×10^7 cfu/ml. In the medium containing 1% of glucose, the stationary phase was also at the 18th hour, but the number of cells was higher, *i.e.* 9.2×10^7 cfu/ml (Fig. 2B).

In the carbon-free medium, a dynamic growth of the extracellular polysaccharides synthesis started at within the first hours of cultivation and at the 4th hour, the EPS content achieved the value of 99.7 μg/10^8 cells. In the next hours of cultivation, synthesis of the extracellular polymers started to decrease to an undetectable quantity after 48 hours. Addition of 1% of glucose did not affect EPS synthesis effectiveness and was lower than in the glucose-free medium. The content of extracellular polysaccharides at the 18th hour reached the value of 6.5 μg/10^8 cells, and within the next hours of cultivation it decreased below 3.0 μg/10^8 cells.

Dynamics of cells growth in the medium with 5% glucose concentration indicated that the strain reached the stationary phase at the 8th hour of cultivation already, and the number of cells was highest, *i.e.* 9.6×10^7 cfu/ml. EPS synthesis in these conditions remained high until the 24th hour of cultivation and decrease of production of the compounds was noted in the consecutive hours (Fig. 2B).

Bacterial adhesion to foil surface. Following incubation, the foil surface was analysed by the SEM method for the *B. firmus* strain, which in all the variants was characterised by significantly enhanced EPS synthesis in the mineral medium. In order to compare the level of bacteria adhesion depending on the quantity of the extracellular polymers, an image of the foil surface after cultivation was shown, in which the strain was characterised by effective EPS production and the synthesis was inhibited.

Figure 3 shows the foil surface following cultivation in glucose-free mineral medium, in which the strain was characterised by effective exopolysaccharides production. After the 24th hour of incubation, only single cells on the foil surface were observed, while after 72 hours large bacteria concentrations were detected. Moreover, the bacterial morphology had changed, cell dimensions were reduced compared to the cells incubated in optimal growth conditions (Fig. 3).

After incubation in the glucose-free broth medium, where *B. firmus* strain produced much fewer EPS than in the case described above, single cells or insignificant concentrations of them on the material surface were noted. In broth medium containing 5% of glucose, synthesis of the extracellular polysaccharides produced by *B. firmus* significantly increased compared to the glucose-free broth medium. Furthermore,

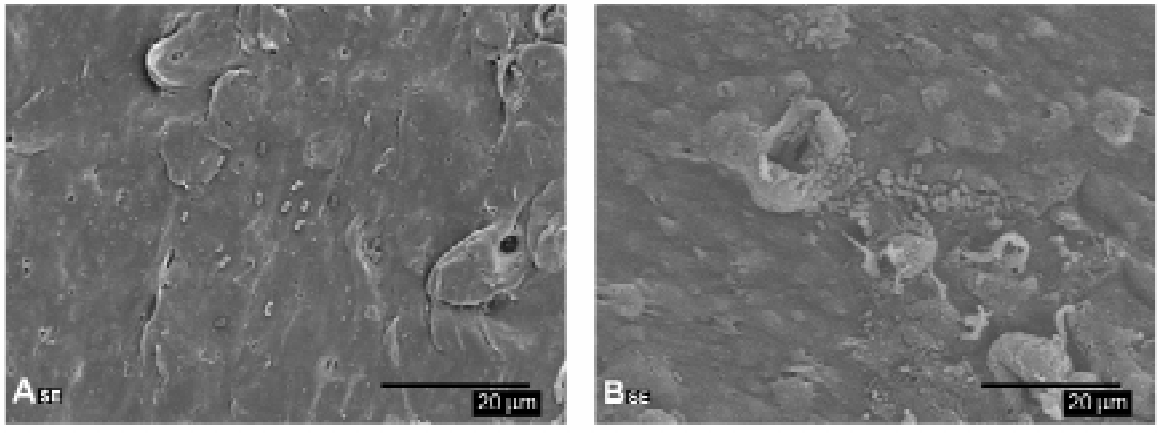

Fig. 3. SEM microphotographs of foils surface after incubation with *Bacillus firmus* in mineral medium without glucose A – after 24 h, B – after 72 h

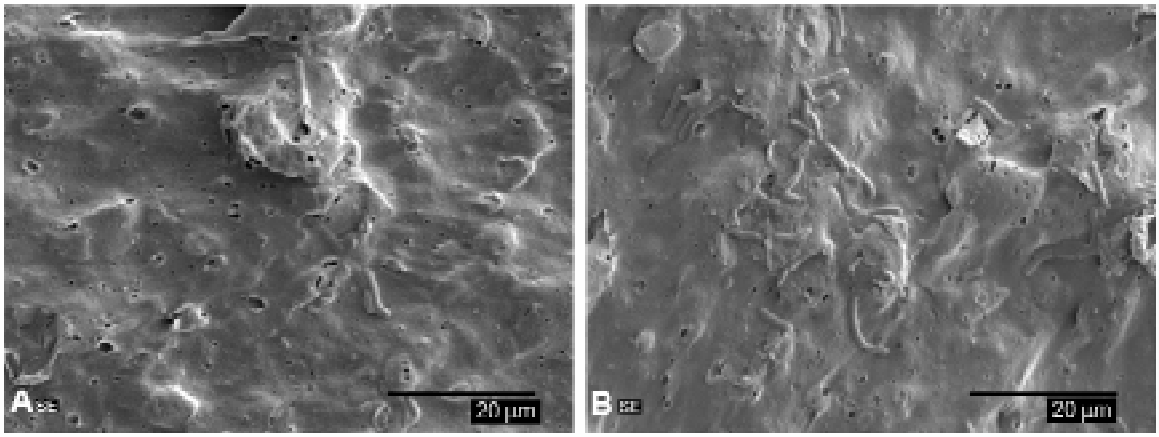

Fig. 4. SEM microphotographs of foils surface after incubation with *Bacillus firmus* in broth medium containing 5% of glucose A – after 24 h, B – after 72 h

after 72 hours of incubation large concentrations of bacteria on the foil surface were noticed. The morphology of the bacterial cells did not change (Fig. 4).

Discussion

The effect of the environmental conditions on the production of extracellular polysaccharides as well as the role of EPS in bacterial adhesion to plastic surfaces was investigated.

Data showed that EPS synthesis by indicated *Bacillus* genus representatives was more effective in carbon-free environment than in the environment optimal for growth. The highest EPS values were recorded for *B. coagulans* and *B. subtilis* strains, which produced exopolysaccharides more efficiently in low nutrition environment. Similar observations were described by Czaczyk (2004) and Czaczyk *et al.* (2005). Liu *et al*. (2004) also reported that EPS production is positively affected by low-carbon conditions. Moreover, according to Christensen (1989) and Tsuneda *et al.* (2003) exopolysaccharides biosynthesis is initiated by conditions unfavourable for growth.

The obtained results regarding EPS synthesis in conditions unfavourable for growth suggest that exopolysaccharides may constitute an auxiliary source of carbon and energy for microorganisms. The literature data indicate that extracellular polysaccharides, in carbon-free environmental conditions, may be biodegraded by the same strain and used as a source of carbon and energy (Frølund *et al*., 1996; Sutherland, 1999; Zhank and Bishop, 2003; Wang *et al.*, 2007). Moreover, according to Frølund *et al*. (1996), exopolymers constitute a film protecting cells against the external environment.

The influence of glucose concentration on exopolysaccharides production was also examined. Addition of carbon source to the mineral medium resulted in a decrease of EPS production in the case of 3 strains; *B. coagulans*, *B. subtilis* and *Bacillus* sp., whereas in the case of other strains, it increased at the 5% glucose concentration. In the case of most of the strains increased polysaccharides production in the broth medium with the addition of 5% glucose was observed. Majumdar *et al.* (1999) also showed favourable impact of carbon source availability on the EPS synthesis. Along the growth of carbon source concentration in the medium, increased polysaccharides production was stated.

The results demonstrated that EPS synthesis by *Bacillus* spp. strains depends on the medium chemical composition and nutrient availability. Significant impact of the environmental conditions on exopolysaccharides production was also described in studies by Gandhi *et al*. (1997) and Sheng *et al.* (2006). According to Gandhi *et al.* (1997), the increase of the carbon source concentration affects the increase of EPS production, whereas the increase of nitrogen concentration unfavourably affects the production of these substances. Similar conclusions may be drawn from these findings, because EPS production in the broth medium rich in nitrogen compounds was reduced.

Moreover, synthesis of extracellular substances also depends on the type of microorganisms (Czaczyk, 2004; Czaczyk *et al*., 2005). Studies proved that certain strains produce greater EPS quantities in the mineral medium, and others are characterised by an increased synthesis in the nutritive medium (*B. megaterium*). In this case, EPS synthesis may also be affected by the pH of the medium. In the study by Gandhi *et al*. (1997), it was discovered that pH close to the neutral is favourable for the process. Similar results were obtained in this work, because the EPS production by *B. megaterium* in the broth medium at pH 7.0 was the most effective.

Assessment of growth dynamics and EPS production suggested that the process depends on the

microorganism growth phase. Polysaccharides synthesis was the most effective within the first few hours of cultivation, that is during the logarithmic growth phase of the culture of the strain. Similar conclusions were drawn by Sutherland (1982), who followed EPS synthesis by *P. aeuroginosa*. He showed results which indicated greater production of polysaccharides in the first hours of incubation. In the study by Boza *et al.* (2004), it was also noted that polysaccharides production by *Beijerinckia* increases until the 12th hour of cultivation. The findings in this study confirmed the observations of the authors cited. Exopolysaccharides may be a source of carbon for microorganisms and may be used by them in conditions of insufficient glucose availability, as was mentioned and described previously (Frølund *et al*., 1996; Sutherland, 1999; Zhank and Bishop, 2003)

The studies were also aimed at indicating correlations between exopolysaccharides production and bacterial adhesion to foil surface. Literature data imply that exopolysaccharides play a fundamental role in biofilm formation (Allison, 1998; Majumdar *et al.*, 1999; Rijnaarts *et al*., 1995; Sivan *et al*., 2006). Similar conclusions may be drawn from these experiments. SEM observations confirmed that *B. firmus*, which produced large amounts of exopolysaccharides, effectively adhered to the foil surface, whereas in the event in which EPS production was low, only insignificant cell concentrations could be observed on the foil surface. The key role of EPS in cell aggregate and biofilm formation was observed by Beech (2006), Czaczyk *et al*. (2005), Liu *et al*. (2004) and Yeo *et al.* (2007). Moreover, in the experiments conducted in the mineral environment, a decrease of *Bacillus* sp. cell dimensions was observed. Cell morphology change may also be a form of adaptation to the conditions and, consequently, affect faster adhesion to the surface of solids. Cell morphology change in low-nutrient conditions was also observed by Humphrey *et al*. (1983) and Stretton *et al*. (1997).

Literature data imply that the biofilm produced on the foil surface may accelerate the process of their biodegradation. Such favourable effect of biofilm on the material surface was described, among others, by Gilan *et al.* (2004) and Sivan *et al*. (2006). Increased synthesis of extracellular polysaccharides constituting the first biofilm production stage, in unfavourable conditions, may affect faster degradation of the polymer materials. The current date and represented surveys quite unambiguously suggest the hypothesis that the biodegradation of polymer foils in soil rich in nutritive components does not always effectively influence the process of its degradation.

Acknowledgements

The work was supported by the Polish Ministry of Science and Higher Education (MNiSW), grant number N N508 0880 33.

Literature

Allison D.G. 1998. Exopolysaccharides production in bacterial biofilm. *Biofilm* 3: BF98002

Allison D.G. and I.W. Sutherland. 1987. The role of exopolysaccharides in adhesion of freshwater bacteria. *J. Gen. Microbiol*. 133: 1319–1327.

Beech I.B. 2006. Corrosion of technical materials in the presence of biofilms. *Inter. Biodeter. Biodegrad*. 58: 89–93.

Boza Y., L.P. Neto, F.A.A. Costa and A.R.P. Scamparini. 2004. Exopolysaccharides production by encapsulated *Beijerinckia* cultures. *Process Biochem*. 39: 1201–1209.

Characklis W.G. and K.E. Cooksey. 1983. Biofilms and microbial fouling. In Laskin A.I. (eds.) *Applied Microbiology*, vol. 29. Academic Press, New York, p. 93–138.

Christensen B.E. 1989. The role of extracellular polysaccharides in biofilms. *J. Biotechnol.* 10: 181–202.

Costerton J.W., K.J. Cheng, G.G. Geesey, T.I. Ladd, J.C. Nickel, M. Dasgupta and T.J. Marrie. 1987. Bacterial biofilms in nature and disease. *Ann. Rev. Microbiol*. 41: 435–464.

Czaczyk K. 2004. Factors affecting the adhesion of microorganisms to solid surface (in Polish). *Post. Mikrobiol*. 43: 267–283.

Czaczyk K. and K. Myszka. 2004. Biosynthesis of exopolysaccharides and their role in the adhesion of *Bacillus megaterium* to a stainless steel surface. (in Polish). *Żywn. Technol. Jakoś.* 3: 27–36.

Czaczyk K., **K. Myszka and A. Mueller**. 2005. The effect of nutrient availability on biosynthesis of extracellular polysaccharides by *Bacillus* spp. and its role in adhesion. *Folia Univ. Agric. Stetin*. 246: 33–46.

Ford T. and R. Mitchell. 1990. The ecology of microbial corrosion. *Adv. Microb. Ecol*. 11: 231–261.

Flemming H.C. 1998. Relevance of biofilms for the biodegradation of surfaces of polymeric materials. *Polymer Degrad. Stabil*. 59: 309–315.

Frřlund, B., R. Palmgren, K. Keiding and P.H. Nielsen. 1996. Extraction of extracellular polymers from activated sludge using a cation exchange resin. *Water Res*. 30: 1749–1758.

Gandhi H.P., R.M. Ray and R.M. Patel. 1997. Exopolymer production by *Bacillus* species. *Carbohydr. Polymer*. 34: 323–327.

Gilan I., Y. Hadar and A. Sivan. 2004. Colonization, biofilm formation and biodegradation of polyethylene by a strain of *Rhodococcus ruber*. *Appl. Microbiol. Biotechnol*. 65: 97–104.

Gu J.D., M. Roman, T. Esselman and R. Mitchell. 1998. The role of microbial biofilms in deterioration of space station candidate materials. *Inter. Biodeter. Biodegrad*. 41: 25–33.

Hall-Stoodley L. and P. Stoodley. 2002. Developmental regulation of microbial biofilms. *Curr. Opin. Biotechnol*. 13: 228–233.

Horan N.J. and C.R. Eccles. 1986. Purification and characterization of extracellular polysaccharide from activated sludge. *Water Res*. 20: 1427–1432.

Humphrey B., Kjelleberg S., Marshall K.C. 1983. Responses of marine bacteria under starvation conditions at a solid-water interface. *Appl. Environ. Microbiol*. 45: 43–47.

Langille S.E., G.G. Geesey and R.M. Weiner. 2000. Polysaccharide-specific probes inhibit adhesion of *Hyphomonas rosenbergii* strain VP-6 to hydrophilic surfaces. *J. Ind. Microbiol. Biotechnol.* 25: 81–85

Liu Y.Q., Y. Liu and J.H. Tay. 2004. The effects of extracellular polymeric substances on the formation and stability of biogranules. *Appl. Microbiol*. 54: 46–62.

Majumdar I., F. D'Souza and N.B. **Bhosle.** 1999. Microbial exopolysaccharides: Effects on corrosion and partial chemical characterization. *J. Indian Inst. Sci.* 79: 539–550.

Marshall K.C., R. Stout and R. Mitchell. 1971. Mechanism of the initial events in the sorption of marine bacteria to surfaces. *J. Gen. Microbiol*. 68: 337–348.

Morikawa M. 2006. Beneficial biofilm formation by industrial bacteria *Bacillus subtilis* and related species. *J. Biosci. Bioeng.* 101: 1–8.

Olofsson A.C., M. Hermansson and H. Elwing. 2003. N-Acetyl-L-cysteine affects growth, extracellular polysaccharides production, and bacterial biofilm formation on solid surfaces. *Appl. Environ. Microbiol*. 69: 4814–4822.

Parkar S.G., S.H. Flint, J.S. Palmer and J.D. Brooks. 2001. Factors influencing attachment of thermophilic bacilli to stainless steel. *J. Appl. Microbiol*. 90: 901–908.

Pratt L.A. and R. Kolter 1999. Genetic analyses of bacterial biofilm formation. *Curr. Opin. Microbiol.* 2: 598–603.

Rijnaarts H.H.M., W. Norde, E.J. Bouwer, J. Lyklema and A.J.B. Zehnder. 1995. Reversibility and mechanism of bacterial adhesion. *Colloid. Surface* 4: 5–22.

Sivan A., M. Szanto and V. Pavlov. 2006. Biofilm development of the polyethylene-degrading bacterium *Rhodococcus ruber*. *Appl. Microbiol. Biotechnol*. 72: 346–352.

Sheng G.P., H.Q. Yu and Z. Yue. 2006. Factors influencing the production of extracellular polymeric substances by *Rhodopseudomonas acidophila*. *Inter. Biodeter. Biodegrad*. 58: 89–93.

Stretton S., S.J. Danon, S.M. Kjelleberg, and A.E. Goodman. 1997. Changes in cell morphology and motility in the marine *Vibrio* spp. Strain S14 during conditions of starvation and recovery. *FEMS Microbiol. Lett.*146: 23–29.

Sutherland I.W. 1980. Polysaccharides in the adhesion of marine and freshwater bacteria. In: *Microbial Adhesion to Surfaces*, Berkeley R. C. W., Lynch J. M., Melling J., Rutter P. R. and Vincent B. (eds), Ellis Horwood Publ., Chichester, UK, p. 329–338.

Sutherland I.W. *1982. Biosynthesis of microbial exopolysaccharides. Adv. Microb. Physiol.* 23: 79–100.

Sutherland I.W. 1999. Polysaccharases for microbial exopolysaccharides. *Carbohydr. Polym*. 38: 319–328.

Sutherland I.W. 2001. Biofilm exopolysaccharides: a strong and sticky framework. *Microbiology* 147: 3–9.

Szumigaj J., Z. Żakowska, L. Klimek and A. Bartkowiak. 2005. Biodegradation of polymer packaging foils. *Ann. Pol. Chem. Soc*. 1: 520–524

Tsuneda S., H. Aikawa, H. Hayashi, A. Yuasa and A. Hirata. 2003. Extracellular polymeric substances responsible for bacterial adhesion onto solid surface. *FEMS Microbiol. Lett*. 223: 287–292.

Wang Z.W., Y. Liu and J.H. Tay. 2007. Biodegradability of extracellular polymeric substances produced by aerobic granules. *Appl. Microbiol. Biotechnol*. 74: 462–466.

Zhang X. and P.L. Bishop. 2003. Biodegradability of biofilm extracellular polymeric substances. *Chemosphere* 50: 63–69.

Yeo Y., **N. Jang, J. Cho, K.S. Kim and I.S. Kim**. 2007. Identification of the effect of extracellular polymeric substances on bacterial adhesion to the membrane surface in a membrane bioreactor using *Pseudomonas fluorescens*. *Water Sci. Tech*. 55: 35–42.